The hydrostatic gradient, not light availability, drives height-related variation in *Sequoia sempervirens* (Cupressaceae) leaf anatomy[1]

Alana R. Oldham[2,5], Stephen C. Sillett[3], Alexandru M. F. Tomescu[2], and George W. Koch[4]

[2]Department of Biological Sciences, Humboldt State University, Arcata, California 95521 USA; [3]Department of Forestry and Wildland Resources, Humboldt State University, Arcata, California 95521 USA; and [4]Department of Biological Sciences and the Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, Arizona 86011 USA

- *Premise of the study*: Leaves at the tops of most trees are smaller, thicker, and in many other ways different from leaves on the lowermost branches. This height-related variation in leaf structure has been explained as acclimation to differing light environments and, alternatively, as a consequence of hydrostatic, gravitational constraints on turgor pressure that reduce leaf expansion.
- *Methods*: To separate hydrostatic effects from those of light availability, we used anatomical analysis of height-paired samples from the inner and outer tree crowns of tall redwoods (*Sequoia sempervirens*).
- *Key results*: Height above the ground correlates much more strongly with leaf anatomy than does light availability. Leaf length, width, and mesophyll porosity all decrease linearly with height and help explain increases in leaf-mass-to-area ratio and decreases in both photosynthetic capacity and internal gas-phase conductance with increasing height. Two functional traits—leaf thickness and transfusion tissue—also increase with height and may improve water-stress tolerance. Transfusion tissue area increases enough that whole-leaf vascular volume does not change significantly with height in most trees. Transfusion tracheids become deformed with height, suggesting they may collapse under water stress and act as a hydraulic buffer that improves leaf water status and reduces the likelihood of xylem dysfunction.
- *Conclusions*: That such variation in leaf structure may be caused more by gravity than by light calls into question use of the terms "sun" and "shade" to describe leaves at the tops and bottoms of tall tree crowns.

Key words: Cupressaceae; hydrostatic gradient; leaf expansion; mesophyll porosity; *Sequoia sempervirens*; sun leaves; tracheid collapse; transfusion tissue; water stress.

Leaves at the tops and bottoms of many tree crowns differ morphologically and anatomically from each other. Whether this variation among genetically identical leaves is caused more by biophysical constraints or by environmentally induced investment in functional traits remains unclear. As the plant species with the tallest individuals, redwood (*Sequoia sempervirens* D. Don) provides an unparalleled opportunity to investigate and separate potential effects of water stress and light availability on structure and growth in an individual plant. The species varies dramatically in leaf morphology with height (Koch et al., 2004), but the extent of corresponding anatomical variation, as well as its causes and tree-level consequences, are poorly understood.

Height increases the influence of gravity on water potential (Ψ), which decreases by 0.0098 MPa per meter above the ground (Zimmermann, 1983). The gravitational component of pressure potential (hydrostatic tension) interacts with hydraulic path-length resistance (hydrodynamic tension) to further lower Ψ during transpiration. Trees can compensate for this reduction in Ψ and maintain turgor pressure by decreasing osmotic potential in upper crown leaves, but this involves carbon-costly solute use and may be limited in its effectiveness (Woodruff et al., 2004). A fundamental factor limiting maximum tree height thus may be a reduction in photosynthetic efficiency caused by lower water potentials at the treetop. As trees grow taller, an increase in leaf-level water stress leads to decreased photosynthesis and carbon uptake as a direct result of reduced stomatal aperture and early closure in the tree tops (Ryan and Yoder, 1997). There is a delicate balance between maintaining photosynthesis and avoiding xylem cavitation due to increasingly negative Ψ at the tops of tall trees (Tyree and Sperry, 1988).

Changes in leaf structure within tall tree crowns are caused, in part, by height-associated reductions in turgor pressure (Jennings, 2002; Boyer and Silk, 2004; Koch et al., 2004; Woodruff et al., 2004, 2009; Zwieniecki et al., 2004a, b; Ishii et al., 2008; Mullin et al., 2009). Adequate guard cell turgor keeps stomata open and is therefore required for CO_2 assimilation. Turgor pressure also drives cell expansion and thus leaf expansion or growth in length and width (Cosgrove, 1993, 2000). In fact, a branch cut from the upper crown of a tall redwood showed lateral leaf expansion like that of lower crown branches when grown in a high light environment with unlimited water (Koch et al., 2004). This result contrasts with the classical view that within-crown foliar variation, especially leaf

expansion, is caused primarily by light acclimation, with broad "shade" leaves in the lower crown and small, thick "sun" leaves in the upper crown (Ellsworth and Reich, 1993; Niinemets and Kull, 1995; Bond et al., 1999; Han et al., 2003; Niinemets et al., 1998).

Like the degree of leaf expansion, the vascular architecture of a tree also changes with height. The total tracheid area (xylem plus transfusion tissue) of redwood leaf cross sections in the upper crown is more than double that of leaves from the lower crown (Jennings, 2002), which indicates a substantial investment in nonphotosynthetic tissue in an area of limited photosynthetic capacity. Variation in redwood leaf anatomy also includes an increased leaf-mass-to-area ratio (LMA) and thus higher tissue density with greater height (Jennings, 2002; Koch et al., 2004; Ishii et al., 2008; Ambrose et al., 2009). The potential impacts of these changes on the whole tree are unknown, but such tissue investments may provide functional advantages, perhaps by mitigating negative effects of low water potential. As redwood height increases, foliar mitochondrial respiration rate rises and net photosynthesis declines at the treetop (Koch et al., 2004; Ishii et al., 2008; Mullin et al., 2009). Leaf-level anatomical variation may control, or be reflected in, these physiological changes.

The height-associated increase in LMA suggests that leaf mesophyll porosity (hereafter "mesoporosity") may decrease with height in *S. sempervirens* (Jennings, 2002). Mesoporosity, defined here as the proportion of a leaf cross section devoted to air space, is an index of relative tissue density or "sponginess" and so has a strong influence on leaf and shoot mass, as well as on gas exchange capacity. The volume of air in a leaf, including substomatal chambers, is positively related to the degree of leaf expansion and should be closely tied to turgor during leaf development. A loss of intercellular air space lowers the internal conductance of CO_2 by reducing the distance traveled in the gas phase and forcing absorbed gas to pass through more diffusion-resistant mesophyll cells before reaching chloroplasts (Flexas et al., 2008). Thus, low mesoporosity limits the photosynthetic capacity of leaves (Parkhurst, 1994; Hanba et al., 1999).

Approaching existing height gradients within tall redwood crowns as continuous manipulations in a natural experiment in this study, we sought to clarify the relative effects of water and light availability on foliar anatomy by quantifying expansion, mesoporosity, and distribution of leaf vascular tissues within crowns. The broad morphological plasticity of leaves and the deep crowns of tall redwoods permitted exploration of the biophysical circumstances favoring certain leaf designs in conifers. To separate the effects of gradients in Ψ and light, we used height-paired samples from dark inner and bright outer crowns. Data obtained from anatomical analyses of transverse leaf sections were used to estimate proportions of midleaf cross-sectional area devoted to transfusion tissue, xylem, and air space in leaves from different heights and degrees of light availability. To target leaf-level features most closely associated with impacts on tree-level carbon assimilation and water-stress tolerance, we focused on vascular tissues and mesoporosity. These measurements from across broad natural gradients in water potential and light environment helped decouple the effects of light and water availability on leaf anatomy with increasing height.

MATERIALS AND METHODS

The largest remaining old-growth redwood forest occurs on the alluvial terraces of Bull Creek in Humboldt Redwoods State Park, California, USA (40.3°N, 124.0°W). From this forest, we selected five trees, 108 to 113 m tall,

that have been the focus of ongoing research (i.e., the five tallest primary study trees in Sillett et al., 2010). Although these five trees are quite tall by modern standards, they should not be viewed as an extreme fringe of the redwood population but rather as representatives of a once widespread size-class virtually removed from the landscape by logging. Fewer than 150 living trees this tall or taller remain (S. C. Sillett, unpublished data).

Leaves were collected from both inner and outer crown positions at ~10-m height intervals, starting with the lowest branches at ~50–60 m and continuing to 110 m ($N = 12–16$ samples per tree representing inner and outer crowns at six to eight heights). Inner crown samples came from near the main trunk, and outer crown samples came from as far from the trunk as could be accessed using arborist-style rope-climbing techniques. Hemispherical photographs taken directly above each sampling location with a digital camera on a self-leveling mount were used to calculate light availability expressed as canopy openness (% sky), direct site factor (proportion of direct light at a site relative to that in the open), and indirect site factor (proportion of diffuse light at a site relative to that in the open) with WinScanopy (Régents Instruments, Nepean, Ontario, Canada). At each sampling location, 10 leaves from the midsection of second-year and mature, first-year annual shoots, excluding those with any visible physical damage, were selected for preservation. We sectioned 114 leaves (57 samples × two leaves each) for anatomical analyses.

Midsections of leaves (a 2-mm segment from half way between leaf tip and its attachment to stem) were removed with a razor blade, fixed in FPA (10% formalin-propionic acid in 50% ethanol), and dehydrated in stages with isopropyl alcohol before being embedded in Paraplast (McCormick Scientific, Maryland Heights, Missouri, USA) at 58°C. Each leaf sample was transversely sectioned at 10 μm thickness with a microtome, and sections were mounted on glass slides. To differentiate all tissue types, we quadruple-stained sections with Weigert's iron hematoxylin, Bismark brown, phloxine, and fast green-orange G (D. K. Walker, Humboldt State University, unpublished data). The first high-quality section from the top left corner of a given slide was selected to represent each leaf and photographed with a Canon PowerShot digital camera (Canon USA, Lake Success, New York, USA) mounted on a compound microscope (Leitz 020-441.004, Wetzlar, Germany). Each leaf was photographed twice: at 40× magnification to capture the whole cross section and at 400× magnification to obtain an image of the vascular tissue.

Fifteen anatomical traits of leaves were selected based on the probability they may be affected by either water stress, light availability, or both (Table 1). Seven of the 15 traits should respond in the same way to both water stress and light, albeit for different reasons. These traits were used not only to compare inner and outer crowns and to assess strength of correlations with height and light, but also to serve as an internal control to rule out the possibility that neither Ψ nor light availability control redwood leaf anatomy. The remaining eight traits should respond differently to water stress than to light availability. Traits potentially influenced by water stress include those that can be controlled by low Ψ through reduced leaf expansion such as the length, width, perimeter, and mesoporosity as well as those promoting water-stress tolerance such as cross-sectional circularity and features of transfusion tissue. Traits potentially controlled by light availability include those that influence self-shading such as length and width of leaves as well as traits that influence gas exchange such as mesoporosity, leaf thickness, and cross-sectional area of xylem. An effort was made to consider all leaf traits that were not redundant. For example, while both the perimeter and circularity of a cross section can be considered representative of its shape, the perimeter includes variation in leaf texture and size, and the interaction of these two traits has a curved relationship to height (data not shown), suggesting different responses to water stress.

Photographs of all leaf cross-sections were analyzed with the program ImageJ (National Institute of Mental Health, Bethesda, Maryland, USA). Each image was converted to 32-bit gray scale and then made binary with a threshold value individually selected for each leaf at the point where the color histogram began to grow steep. The entire cross section was then selected, and the image was cleared outside the selected area to remove any nonleaf artifacts. This resulted in an isolated binary image of the section allowing for automated measurement of leaf area, perimeter, width, thickness, and circularity (0–1 index).

To create an index of mesoporosity, we added black to any cellular (not air) space that was not already black, including the vascular bundles, transfusion tissue, resin ducts, and any pale mesophyll cell lumens. All the now-black cellular material in the section was selected, excluding intercellular (air) space within the mesophyll. This cellular area was then subtracted from the total cross-sectional area to quantify the amount of air space in the leaf section. This empty space, expressed as a proportion of the total area (A_{leaf}/A_{air}) was used as an index of leaf mesoporosity. An index of mesoporosity was preferred here to the classically used "mesophyll surface area per unit leaf area" (A_{mes}/A; Nobel

TABLE 1. Anatomical traits of leaves of *Sequoia sempervirens* considered in this study and their predicted responses if variation is driven by either water stress or light availability. The first seven of the 15 traits should respond in the same way to both water stress and light, while the last eight traits we predicted to respond differently. All traits measured from midleaf cross-sections, except length.

| | Predicted response to increasing | |
Leaf trait (unit)	Water stress	Light
Length (mm)	decrease with reduced leaf expansion	decrease to avoid self-shading
Width (mm)	decrease with reduced leaf expansion	decrease to avoid self-shading
Perimeter (mm)	decrease with reduced leaf expansion	decrease to avoid self-shading
Area of transfusion tissue (mm^2)	increase to improve water-stress tolerance	increase to improve hydraulic capacity
Maximum lumen area of a transfusion tracheid (mm^2)	increase to enhance collapsibility	increase to lower hydraulic resistance
Circularity (0–1 index)	increase to minimize water loss	increase to avoid self-shading
No. tracheids in xylem	decrease with reduced leaf expansion	decrease as cell size increases
Thickness (mm)	unclear	increase to maximize photosynthetic yield
Width of xylem	decrease with reduced leaf expansion	increase to maximize photosynthetic yield
Total area (mm^2)	decrease with reduced leaf width	unclear
Cellular area (mm^2)	no change	increase to maximize photosynthetic yield
Area of xylem (mm^2)	decrease with reduced leaf expansion	increase to improve hydraulic capacity
Area of phloem (mm^2)	decrease with lower photosynthetic yield	increase with higher photosynthetic yield
Mean circularity of transfusion tracheids (0–1 index)	decrease with deformation/collapse	no change
Mesoporosity (A_{leaf}/A_{air})	decrease with reduced leaf expansion	increase to maximize photosynthetic yield

et al., 1975) because it better represents both CO_2 storage capacity and internal gas-phase conductance.

In higher magnification, photographs of the vascular system, area and width of xylem as well as area of phloem and transfusion tissue were measured by outlining the boundaries of each tissue type. Xylem tracheids were counted for use in estimating cell size, and then the original image was cleared of everything but transfusion tracheids. Cell walls of each tracheid ($N = 5499$) were individually traced in black matching the wall thickness. The resulting image was converted to 32-bit gray scale and then to binary with threshold adjusted until all cell lumens were shown in white and all cell walls were still black. After smoothing, the image was reset to binary and inverted so cell lumens appeared black on a white background, which permitted us to simultaneously obtain all transfusion tracheid areas (to determine mean and maximum lumen areas) and circularity values (0–1 index) as well as to count cells. Values obtained for the two leaves from each crown position in a given tree were averaged to create a single representation of leaf anatomy at that site ($N = 57$). Whole leaves were also digitally scanned (Epson Expression 10000XL, Long Beach, California, USA) to quantify average leaf length, which was multiplied by perimeter to calculate leaf area and by cross-sectional areas to calculate tissue volumes.

Data analyses—Anatomical traits of redwood leaves were compared among the five trees with multiple response permutation procedures (MRPP). Among-tree differences in each trait were assessed with Kruskal–Wallis tests using the Bonferroni correction for multiple comparisons. This nonparametric test was selected because not all variables were normally distributed within all trees. Principal components analysis (PCA) was used to illuminate the dominant patterns of variation among leaf traits. This process reduces the dimensionality of normally distributed multivariate data to a smaller number of orthogonal axes (principal components) that represent the strongest patterns of linear covariation in the primary data matrix. Our goal in using PCA was to uncover the dominant factor controlling variation in leaf anatomy, whether it be height-induced water stress or light availability. To better isolate these factors, we removed noise from variation among trees using only the nine of 15 traits that did not vary significantly among the five trees. Correlation coefficients were used to create the cross-products matrix, and the solution was not rotated. All nine traits in the matrix used for PCA met our criteria for a normal distribution by having a skewness of <1 and kurtosis of <3 (mean skewness = 0.32, mean kurtosis = 0.09). Relationships of the resulting principal component to height and light availability were assessed by linear regression of PCA axis scores against these independent variables. The Mann–Whitney U test was used to compare leaves of the inner and outer tree crowns. This nonparametric test was used because not all variables met our criteria for normality when separated by crown position. To fully remove height from this analysis, we excluded treetops as well as the lowermost inner-crown samples so that only height-paired inner and outer crown leaves were considered. The program PC-ORD (McCune and Mefford, 2006) was used for all multivariate analyses, and the program NCSS (Hintze, 2002) was used for all univariate analyses. Results were considered significant at α = 0.05.

RESULTS

There was more within-tree leaf anatomical homogeneity than expected by chance in the 15-trait data set (MRPP: $A = 0.12$, $t = -5.49$, $P < 0.01$). This separation of trees remained significant when individuals were removed from the analysis, suggesting that MRPP results were not caused by a single unusual tree. There were significant differences among trees in six of the 15 anatomical traits of redwood leaves (Table 2). When the data set was reduced to the nine traits not differing among trees, there was no more within-tree leaf anatomical homogeneity than expected by chance (MRPP: $A = 0.01$, $t = -0.45$, $P = 0.27$).

Principal components analysis revealed a single statistically significant axis of variation ($P < 0.00001$) (Peres-Neto et al., 2005) that explained 75.3% of the variation among nine anatomical traits of redwood leaves (Table 3). Directions of the variation in anatomical traits associated with this axis matched the predicted responses to increasing water stress highlighted in

TABLE 2. Variation among five redwood trees on the basis of anatomical traits of leaves. All traits measured from midleaf cross-sections, except length. Traits significantly different among trees marked with asterisk ($P < 0.05$ with Bonferroni correction for multiple comparisons). Statistic H derived from Kruskal–Wallis tests (df = 4).

Leaf trait	H
Width	3.34
Mean circularity of transfusion tracheids	4.24
Perimeter	4.33
Circularity	4.75
Length	5.59
Mesoporosity	6.24
Thickness	9.15
Area of xylem	10.18
Area of transfusion tissue	11.05
Total area	11.64*
Maximum lumen area of a transfusion tracheid	11.83*
Width of xylem	12.06*
Area of phloem	12.75*
Tracheids in xylem	13.95*
Cellular area	16.36*

TABLE 3. Linear correlations between traits of leaves of *Sequoia sempervirens* and sample scores along the first principal component axis (PC1). All relationships are statistically significant ($P < 0.01$).

| Trait	PC1	
	R^2	Direction
Circularity	0.93	+
Width	0.90	−
Length	0.87	−
Perimeter	0.85	−
Thickness	0.74	+
Mean circularity of transfusion tracheids	0.70	−
Area of xylem	0.63	−
Area of transfusion tissue	0.61	+
Mesoporosity	0.55	−

Fig. 1. Samples scores along the dominant axis from principal components analysis of nine leaf anatomical traits increase with height in *Sequoia sempervirens* ($N = 5$ trees indicated by different symbols).

Table 1. Ordination scores along the first principal component correlated much more strongly with height ($R^2 = 0.78$, $P < 0.01$) than with any of the variables describing light environment (percentage sky had the closest correlation: $R^2 = 0.47$, $P < 0.01$), so we interpreted this axis as a gradient reflecting anatomical response of redwood leaves to increasing water stress (Fig. 1). Ordination scores did not vary significantly among trees or between inner and outer crowns.

All nine anatomical traits in which the five trees could be pooled had significant linear relationships to height (Table 4). Again, directions of the variation in anatomical traits with height were consistent with the predicted responses to increasing water stress highlighted in Table 1. Mesoporosity of redwood leaves decreased with increasing height in the crown (Fig. 2). The circularity of leaf cross-sections more than doubled with height due to increasing thickness accompanied by decreasing width. Leaves were just over three times shorter at the tops of the trees than in the lowermost crown. The cross-sectional area of the transfusion tissue increased almost 300% along the vertical gradient (Fig. 3), while tracheids in the transfusion tissue became less circular. The cross-sectional area of xylem became smaller with increasing height. The vascular volume of leaves decreased slightly overall ($R^2 = 0.15$, $P < 0.01$) but did not change significantly with height in three of the five study trees despite drastic shortening of leaves. Also, leaf surface area decreased strongly with height ($R^2 = 0.68$, $P < 0.01$) as a consequence of reduced perimeter and length, so that the ratio of vascular volume to leaf surface area increased along the vertical gradient ($R^2 = 0.51$, $P < 0.01$).

On an individual tree basis, most but not all of the 15 anatomical traits varied significantly with height, but responsiveness to Ψ varied among trees (Table 5). The maximum area of transfusion tracheid lumens increased with height in four of five trees. The number of tracheids in the xylem decreased in four trees and the width of the xylem decreased in three trees as height increased. The area of the phloem correlated with height in only two trees. On average, transfusion tracheids were larger and had lumen areas ~3.5 times greater than xylem tracheids in the leaf vein. The cross-sectional area of leaves was related to height in only one tree. The cellular area of leaves was the only anatomical trait not correlated with height in any of the trees (Table 5). Many traits of leaves were more tightly correlated with each other than with height (results not shown), but all 15 traits correlated more strongly with height than with any measure of light availability. This correlation was true for each tree as well as all five trees collectively.
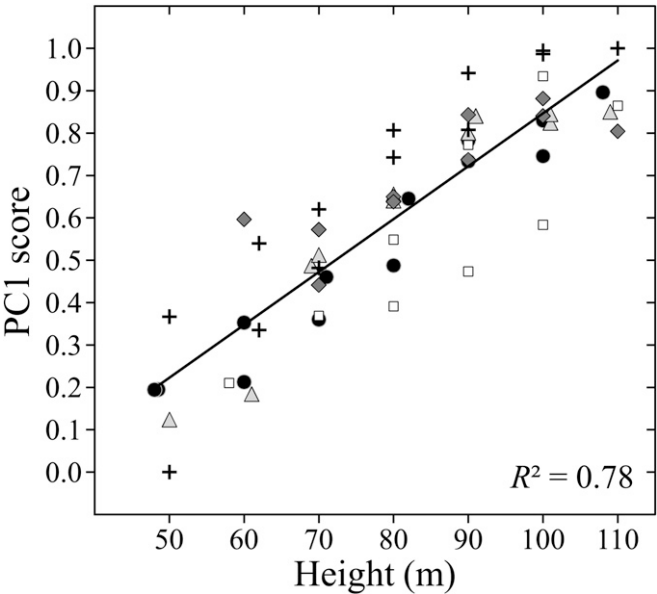
To remove the effect of height, we compared leaves from the inner crown to those from the outer crown. Light availability increased exponentially with height in outer crowns and linearly (or not at all) with height in inner crowns (Fig. 4). The outer crown was much brighter than the inner crown when heights were paired (for percentage sky: $t = 4.66$, df = 48, $P = 0.01$), but none of the 15 anatomical traits of leaves differed significantly between inner and outer crown positions. This lack of anatomical response to crown position was true for crowns as a whole, as well as for upper (above 70 m) and lower (70 m and below) crowns separately.

DISCUSSION

Leaf shape, size of vascular tissues, and degree of mesoporosity all change along the height gradient in tall redwoods. This morphological and anatomical variation reflects hydrostatic constraints on leaf expansion as well as an induced investment in functional traits improving water-stress tolerance. Understanding how these foliar responses to Ψ could impact height growth may help resolve the debate on mechanisms limiting maximum tree height in the tallest species.

The hydrostatic gradient explains over 75% of the variation in key anatomical traits of redwood leaves, while light availability fails to correlate more closely than height to any anatomical variable including leaf width, length, and thickness. Nor are there any differences between leaves of dark inner crowns and bright outer crowns when height is removed as a factor. Eight of the 15 anatomical traits we examined were predicted to respond differently to increasing water stress than to light availability (Table 1). Four of these traits did not vary among trees and responded to the Ψ gradient as predicted, and a fifth trait (cellular area) showed the expected lack of response to Ψ in all five trees. Light availability predicted the direction of variation of only one trait (leaf thickness), the single trait for

Table 4. Anatomical traits of leaves not differing significantly among trees of *Sequoia sempervirens* and their linear relationships to height aboveground (all five trees pooled). All traits measured from midleaf cross sections, except length. All relationships are statistically significant ($P < 0.0001$). The percentage change is increase (+) or decrease (–) in trait between lowermost and uppermost samples averaged for five trees.

Leaf trait	Height		
	R^2	% Change ± SE	Direction
Circularity	0.74	171 ± 47	+
Width	0.69	115 ± 27	–
Length	0.65	303 ± 40	–
Perimeter	0.63	79 ± 21	–
Thickness	0.61	78 ± 12	+
Mean circularity of transfusion tracheids	0.59	24 ± 2	–
Mesoporosity	0.54	134 ± 16	–
Area of xylem	0.44	196 ± 72	–
Area of transfusion tissue	0.42	292 ± 65	+

Fig. 2. Midleaf mesophyll porosity (mesoporosity) decreases with height in *Sequoia sempervirens* ($N = 5$ trees indicated by different symbols).

which we could not make a firm initial guess regarding the potential impact of water stress. These results indicate a striking lack of anatomical responsiveness to light in tall redwood crowns and support the hypothesis that the hydrostatic gradient controls the anatomy of redwood leaves. Absence of an anatomical response to light in the lower crown appears to contradict recent findings of light-determined morphological and physiological variation below 70 m in *S. sempervirens* crowns, including some of the same individual trees in this study (Ishii et al., 2008; Mullin et al., 2009). This discrepancy may simply imply that in the lower crown of very tall trees hydrostatic limitation is already beginning to drive anatomical structure even though light is an important factor controlling shoot morphology.

Our findings add to a growing body of evidence that invalidates the general application of the terms "sun" and "shade" leaves within the crowns of tall trees (Koch et al., 2004; Ishii et al., 2008; Meinzer et al., 2008; Mullin et al., 2009). Additionally, the similarity between inner- and outer-crown leaves suggests that horizontal path length, and by extension hydrodynamic tension, has little effect on internal development of redwood leaves at the branch level. Even in the most water-rich portions of tall redwood crowns, hydrostatic tension appears to have a greater influence on leaf anatomy than does the need to maximize light interception through expansion. Acclimation to irradiance has well-known effects on leaf morphology in short trees (Ellsworth and Reich, 1993; Niinemets and Kull, 1995; Niinemets et al., 1998; Han et al., 2003) in whose crowns hydrostatic tension is likely insufficient to override the impact of light on leaf development. However, because the effects of Ψ and light can covary, the role of water availability in determining leaf structure deserves a closer look in trees of all sizes.

Reduced leaf expansion—An investigation of the foliage of tall redwoods reveals treetop leaves that are on average three times shorter than and only half as wide as leaves in the lower crown (Fig. 5). This reduction in leaf expansion has been well documented in redwood (Jennings, 2002; Koch et al., 2004; Burgess and Dawson, 2007; Ishii et al., 2008; Mullin et al., 2009). Degree of leaf expansion and, thus, final leaf size within individual trees are primarily determined by site-specific environmental conditions influencing water
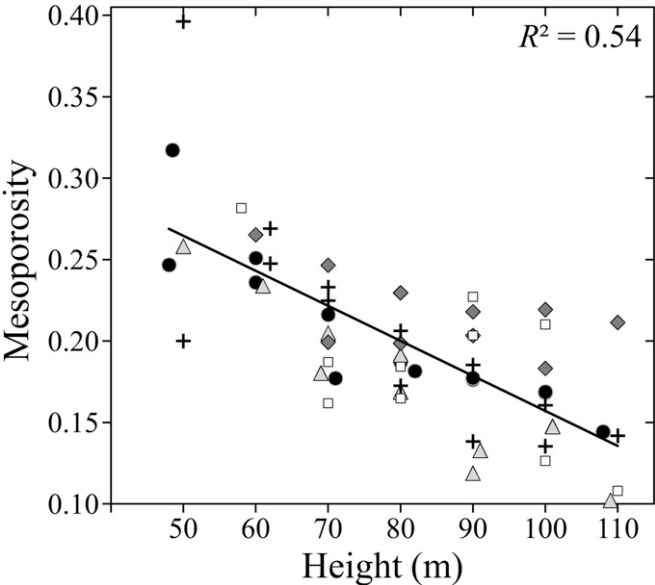
availability and transpiration rates (Koch et al., 2004; Woodruff et al., 2004, 2009; Zwieniecki et al., 2004b, 2006). In tall trees, especially those within intact forests, the dominant factor controlling leaf-level water stress is the hydrostatic component of water potential (Koch et al., 2004). During development, a leaf elongates through tissue production coupled with sufficient turgor to breach the yield threshold of cell walls and allow cellular expansion (Cosgrove, 1993, 2000). Length in single-veined leaves is a function of the xylem-pressure threshold for stomatal closure at the tip of the leaf (Zwieniecki et al., 2006). If the longitudinal expansion of a redwood leaf is
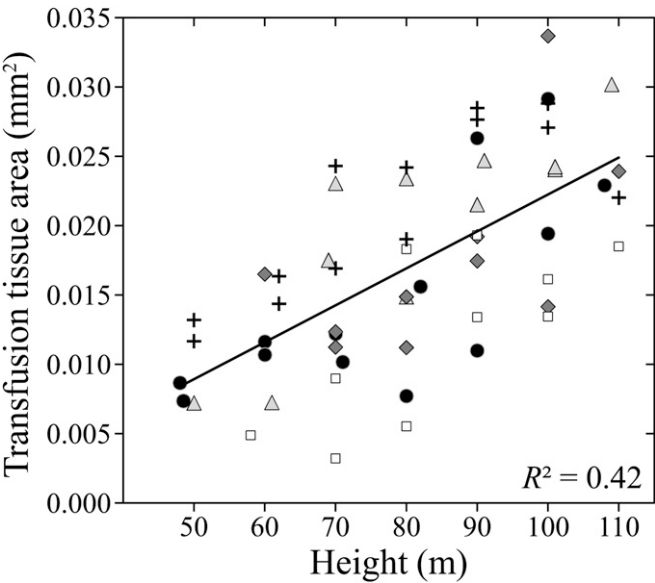

Fig. 3. Cross-sectional area of leaf transfusion tissue increases with height in *Sequoia sempervirens* ($N = 5$ trees indicated by different symbols).
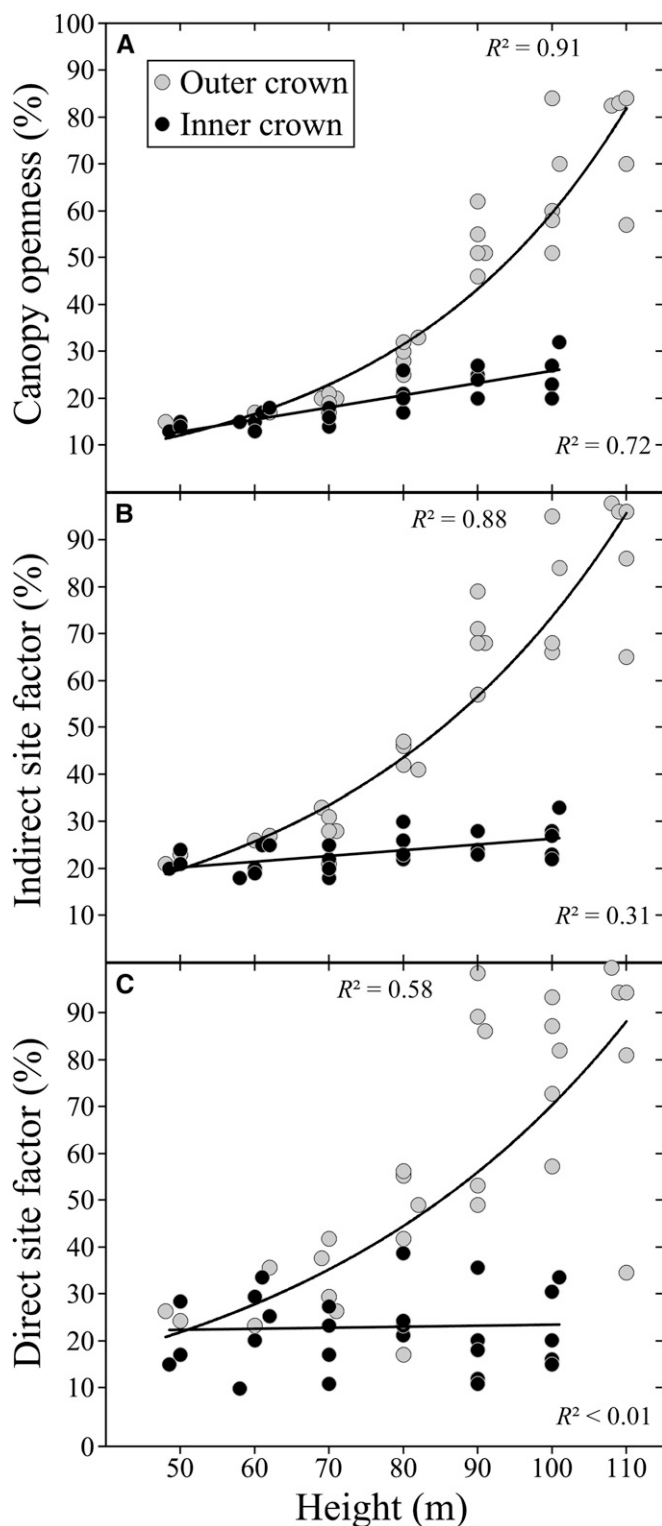
Fig. 4.   Light availability, measured as (A) canopy openness, (B) indirect site factor and (C) direct site factor, increases with height in *Sequoia sempervirens* (*N* = 5 trees). Gray symbols indicate outer crown samples. Black symbols indicate inner crown samples.

xylem cross-sectional area diminishes with height, short leaves in the upper crown come as no surprise. The potential width of single-veined leaves is related to the radial hydraulic resistances of both the vein and mesophyll (Zwieniecki et al., 2004a). In combination with the need for turgor sufficient to drive cellular expansion, this relation explains why lateral expansion is also likely to be controlled by Ψ in tall trees, overriding acclimation to exploit light.

Reduced expansion has implications for the physiological performance of leaves (Parkhurst, 1994; Niinemets, 1999; Koch et al., 2004; Vanderklein et al., 2007). A simultaneous decrease in both length and width of leaves results in a sharp decline in leaf surface area with height, which partially explains previously reported increases in the ratio of leaf mass to area (LMA) and shoot mass to area (SMA) with height in redwood (Jennings, 2002; Koch et al., 2004; Burgess and Dawson, 2007; Ishii et al., 2008; Mullin et al., 2009). High LMA is associated with a reduction in mass-based photosynthetic capacity (Niinemets, 1999; Wright et al., 2004; Ishii et al., 2008). Averaged across species, a 10-fold increase in LMA generates a 21-fold decrease in photosynthetic capacity (Wright et al., 2004). This decrease is caused not only by lower light interception due to less surface area for a given mass, but also by reduction of mesoporosity (Parkhurst, 1994; Niinemets and Kull, 1998; Hanba et al., 1999), which contributes to the increase in LMA and SMA.

Diminished intercellular air space in denser upper crown leaves slows internal conductance of CO_2 and thus impedes carbon assimilation (Flexas et al., 2008). Accordingly, the previously unquantified reduction in mesoporosity with height in redwood corroborates recent measurements of impaired internal CO_2 conductance (Mullin et al., 2009) and lower maximum photosynthetic rate in treetops of this species (Ishii et al., 2008, Ambrose et al., 2009; Mullin et al., 2009). A similar pattern of diminished mesoporosity likely explains the height-driven reduction in mesophyll CO_2 conductance in another tall conifer species, *Pseudotsuga menziesii* (Douglas-fir; Woodruff et al., 2009). Moreover, a decrease in volume of substomatal chambers means that less CO_2 is stored within the leaf when stomata close, further constraining photosynthesis.

Improved water-stress tolerance—Another set of height-induced anatomical changes, we observed represent functional traits that improve water-stress tolerance. In tall redwoods, there is a dramatic increase in both transfusion tissue cross-sectional area and leaf thickness from the lowermost branches to treetops. Although they are probably not direct results of lower turgor pressure, these traits can be seen as investments in foliar survival and photosynthetic maximization in the face of hydrostatic limitations and risks.

Transfusion tissue—Characteristic of gymnosperms, transfusion tissue is involved in bidirectional radial transport between the leaf vein and mesophyll and has been associated with water storage, xylem protection, low radial resistance, solute retrieval, and increased surface area for contact between the vein and mesophyll (Thoday, 1931; Esau, 1977; Canny, 1993; Zwieniecki et al., 2004a; Brodribb and Holbrook, 2005). Like all tracheids, those in transfusion tissue are nonphotosynthetic and so represent a cost in terms of lost opportunity for photosynthesis. The expense of transfusion tissue and the increase in transfusion tissue with height together imply that this tissue may provide functional advantages of increasing importance with height and

regulated by its ability to maintain turgor in the most distal portion of the vein, then Ψ should determine optimal leaf length for a given hydraulic conductivity. Considering that

TABLE 5.   Anatomical traits of leaves and their linear relationships to height in five trees of *Sequoia sempervirens*. Sample heights indicated for each tree. All traits measured from midleaf cross sections, except length. Values are coefficients of determination (R^2). In all cases, direction of change was identical when data from all five trees were pooled. Only traits listed in italics were significantly different among trees (see Table 2). Data arranged to reflect patterns of significance within trees. All R^2 values marked with asterisk correlated significantly with height ($P < 0.05$).

Leaf traits	Tree 1 50–110 m		Tree 2 50–109 m		Tree 3 48–108 m		Tree 4 58–110 m		Tree 5 60–110 m		Direction
Circularity	0.87	*	0.76	*	0.91	*	0.73	*	0.70	*	+
Thickness	0.54	*	0.76	*	0.79	*	0.64	*	0.71	*	+
Length	0.72	*	0.86	*	0.82	*	0.79	*	0.69	*	−
Area of transfusion tissue	0.68	*	0.74	*	0.59	*	0.55	*	0.37		+
Width	0.84	*	0.80	*	0.86	*	0.72	*	0.34		−
Mean circularity of transfusion tracheids	0.78	*	0.48	*	0.89	*	0.56	*	0.28		−
Perimeter	0.82	*	0.67	*	0.84	*	0.76	*	0.24		−
Maximum lumen area of a transfusion tracheid	0.41	*	0.59	*	0.49	*	0.62	*	0.18		+
Tracheids in xylem	0.87	*	0.66	*	0.73	*	0.51	*	0.18		−
Mesoporosity	0.62	*	0.85	*	0.80	*	0.35		0.34		−
Width of xylem	0.93	*	0.83	*	0.56	*	0.36		0.16		−
Area of xylem	0.87	*	0.69	*	0.57	*	0.30		0.16		−
Area of phloem	0.85	*	0.76	*	0.27		0.02		0.03		−
Total area	0.40	*	0.00		0.16		0.03		0.05		−
Cellular area	0.06		0.10		0.01		0.03		0.13		n.s.

enough significance to justify allocating that growth potential to leaf-level hydraulic tissue instead of elsewhere.

While the cross-sectional area and number of tracheids in transfusion tissue are greater in upper crown leaves, the cross-sectional area and tracheid numbers in xylem decrease with increasing height. The height-associated rise in the total leaf cross-sectional area devoted to vascular tissue (Jennings, 2002) is therefore entirely due to increased investment in transfusion tissue. Because of this increase in cross-sectional area, total vascular volume of redwood leaves decreases only slightly with height despite drastic shortening of leaves. Consequently, the ratio of vascular volume (and, therefore, hydraulic capacity) to leaf surface area increases strongly with height, which has great potential for improving the water-stress tolerance of individual leaves as height increases.

Hydraulic capacity is directly related to vascular area; higher capacity leaves are capable of keeping stomata open longer when detached from the water column (Brodribb et al., 2005). The turgor of a leaf is related to its hydraulic capacity, and stomatal closure occurs at or near the turgor loss point (Brodribb and Holbrook, 2003; Woodruff et al., 2004). Assuming equal conductivity, higher capacity leaves should maintain turgor longer under water stress (Brodribb et al., 2003; Brodribb and Holbrook, 2003) and increase the time that stomata are open. Hydraulic capacity is positively correlated with photosynthetic rate primarily during times of water stress (Brodribb et al., 2002), so it may be particularly important in the upper crowns of tall trees. Indeed, tall Douglas-fir trees compensate for hydraulic limitations by greater reliance on water stored in the xylem than short trees (Phillips et al., 2003). At the whole-tree level, the capacity to store water is positively linked to annual net carbon assimilation (McDowell et al., 2005). Thus, hydraulic capacity of leaves should impact photosynthetic yield in a similar manner.

Redwoods possess the ability to uptake fog water directly through their leaves in sufficient quantities as to reverse xylem flow (Burgess and Dawson, 2004). Before trunk-level sensors can detect this flow reversal, not only the whole branch, but also all the leaves must first fill with water (Burgess and Dawson, 2004), implying that the vascular capacity of a leaf is part of what determines its ability to act as a local reservoir for fog

water and maximize the impact of summer fog events on its own Ψ and photosynthetic output. In other conifers, foliar absorption increases with water stress (Breshears et al., 2008), which suggests that upper crowns of tall redwoods may be capable of greater foliar uptake of fog than lower crowns.

The most striking difference between transfusion and xylem tracheids in redwood is cell size; transfusion tracheids have mean lumen areas much larger than those of xylem tracheids in the leaf vein. Such a difference, coupled with the effects of larger vascular area, may underlie the functional significance of observed anatomical variation in redwood vascular tissue with height. As tracheid diameter increases, resistance decreases, resulting in greater hydraulic efficiency in vascular tissue with larger cells (Pittermann et al., 2005, 2006; Sperry et al., 2006; Westoby and Wright, 2006). Because water travels more efficiently through tracheids than mesophyll cells, increased transfusion tissue volume should boost leaf-level hydraulic conductivity, thereby decreasing radial resistance (Brodribb et al., 2007) and increasing maximum rates of photosynthesis (Hubbard et al., 1999; Brodribb et al., 2002, 2007).

Larger diameter cells are less able to withstand tension than smaller cells. Transfusion tracheids in redwood leaves become less circular with height and occasionally seem to be collapsed in the upper crown (Fig. 6). Accessory transfusion tracheids have been observed to deform under water stress and then to return to their normal shapes when the stress is removed in *Podocarpus* (Brodribb and Holbrook, 2005). In that case, the collapsible vascular tissue was hypothesized to provide a Ψ buffer, temporarily relieving tension in the xylem long enough for the stomata to close before embolism occurs in the vein. Tracheid collapse has also been observed in two Cupressaceae species subjected to water stress, resulting in a 50% loss of leaf hydraulic conductivity, and was implicated in the ability of those leaves to regain the lost conductivity overnight (Brodribb and Cochard, 2009). Likewise, transfusion tracheids in excised *Pinus* leaves collapse or are distorted during desiccation, while xylem tracheids do not change shape (Parker, 1952). Xylem cavitation may have a capacitive effect by releasing water into the transpiration stream during times of peak irradiance. Although little water is stored in the leaf, release of water within

A
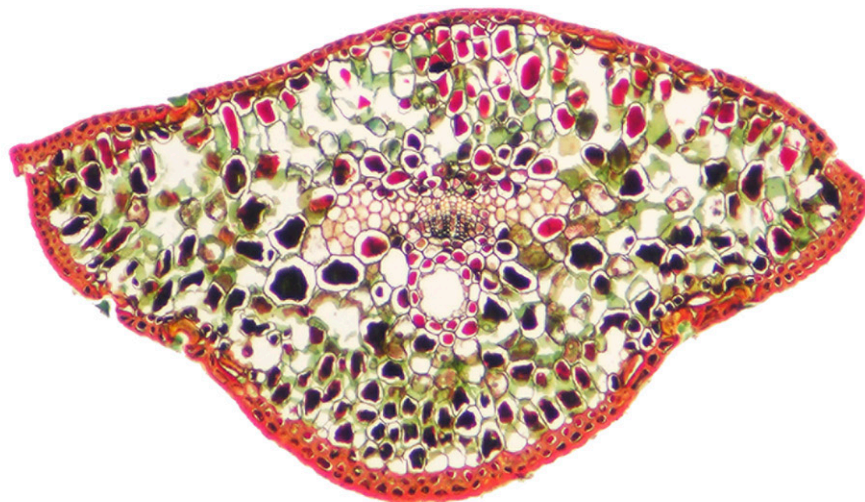
B

Fig. 5. Leaf cross sections of *Sequoia sempervirens* from (A) 110 and (B) 48 m aboveground show clear decreases in expansion and mesoporosity with height. Scale bar = 200 μm.

the leaf has a stronger effect than elsewhere because of proximity to the site of transpiration (Hölttä et al., 2009). Temporary leaf cavitation is a mechanism that may help regulate stem hydraulic conditions in Douglas-fir (Woodruff et al., 2007). Similarly, trees may sacrifice highly vulnerable twigs to improve the water status of adjacent branches during drought events (Zimmerman, 1983; Tyree and Sperry, 1988). Collapse of tracheids should have a water-release effect similar to that of cavitation and with presumably less risk of tissue loss. Futhermore, the maximum cross-sectional area of tracheid lumens in redwood transfusion tissue increased with height, possibly signifying that size is being used to enhance cell collapsibility. These observations support the notion that transfusion tissue serves a protective function for the vein through collapse that likely decreases leaf (and thus branch and treetop) mortality during times of extreme water stress.

Leaf thickness—In addition to investments in transfusion tissue, thickness of redwood leaves also increases substantially with height. Not only does added tissue have construction costs and contribute to rising LMA, but it is also linked to observations of greater respiratory demands and increased hydraulic resistance at the tops of tall trees (Mullin et al., 2009; Woodruff et al., 2009). At the same time, leaf thickness is a functional trait related to improved water stress tolerance. Thick leaves are considered more xeromorphic than thin ones because the sur-

face-area-to-volume ratio is minimized (Wright et al., 2005; England and Attiwill, 2006; Westoby and Wright, 2006). Increasing leaf thickness in redwood correlates with the height-related decrease in leaf width, such that leaf cross-sectional area is nearly height constant. Uniformity in midleaf cross-sectional area implies that increasing thickness may be an area-preserving mechanism compensating for loss of width and, thus, potentially a fingerprint of developmental constraints on cellular proliferation and growth (Tsukaya, 2003, 2006; Fleming, 2006; Horiguchi et al., 2006).

Among-tree developmental variation in leaf size—Remarkably, leaf traits directly linked to both reduced leaf expansion and water stress tolerance do not vary significantly among trees, while traits reflecting organ size do (Table 2). Evidence of hydrostatic constraints overriding tree individuality supports the suggestion that genetic variation may have more control on structural development where impacts of highly negative Ψ are less directly influential (Fabre et al., 2007). A possible height-related transition from primarily genetic to increasingly hydrostatic control on leaf development fits with recent observations of greater among-tree physiological variability in lower-crown and short-tree redwood branches (Ambrose et al., 2009; Mullin et al., 2009).

Only one tree exhibited correlations between leaf size and height. At the cellular level, this tree generally had stronger
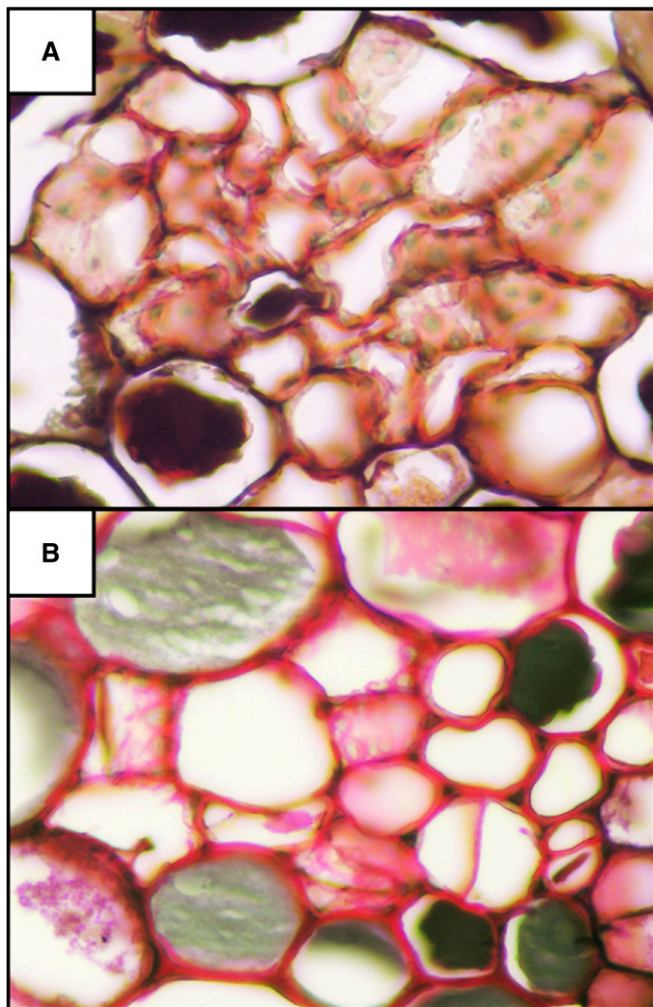
Fig. 6. Higher magnification view of redwood leaf cross sections showing transfusion tracheids from (A) 110 and (B) 48 m aboveground. Note deformation of tracheids in 110 m sample. Scale bar = 25 μm.

responses and tighter correlations with height than other trees in this study (Table 4, tree 1). A stronger response to water stress is intriguing in light of dendrochronological evidence, which shows that this particular tree is 30–50% older than the other trees (Sillett et al., 2010). Previous research on other species has not shown age-related effects on either growth rates or carbon assimilation when cuttings from old trees are grown alongside those from young trees (Mencuccini et al., 2005; Bond et al., 2007). However, this age-related work focused on "old" trees much younger than those studied here. The possibility that tree age may enhance anatomical susceptibility to gravitational forces has fascinating implications for age-related constraints on height growth and should be explored further.

Conclusions—Height-related declines in Ψ drive leaf anatomical gradients, potentially impacting physiological performance of leaves near the tops of tall trees, while light apparently has little influence on leaf anatomy. With increasing height, rates of mitochondrial respiration increase (Mullin et al., 2009), net photosynthesis decreases (Ishii et al., 2008), and stomatal con-

ductance likely more strongly limits CO_2 assimilation (Koch et al., 2004; see a critical discussion of this work in Netting, 2009 and Koch and Sillett, 2009). These physiological processes limiting carbon uptake appear to be heavily influenced by gravitational constraints on leaf expansion leading to increased LMA and reduced mesoporosity with increasing height. Highly negative Ψ may also drive tissue investments in water stress tolerance (i.e., transfusion tissue and leaf thickness) that promote organ survival and maintain photosynthetic activity in the upper crown. Indeed, these may be some of the very functional traits that allow redwoods to reach such great heights. A synergistic effect of all these height-associated anatomical changes is likely to be reduced carbon gain per unit leaf mass in the upper crown, which could contribute to diminishing rates of height growth with increasing height in redwoods (Sillett et al., 2010). Regardless of developmental variation among trees, hydraulic constraints on leaf anatomy appear to underlie the complex set of factors determining fundamental limits to tree height.

LITERATURE CITED

AMBROSE, A. R., S. C. SILLETT, AND T. E. DAWSON. 2009. Effects of tree height on branch hydraulics, leaf structure, and gas exchange in California redwoods. *Plant, Cell & Environment* 32: 743–757.

BOND, B. J., N. M. CZARNOMSKI, C. COOPER, M. E. DAY, AND M. S. GREENWOOD. 2007. Developmental decline in height growth in Douglas-fir. *Tree Physiology* 27: 441–453.

BOND, B. J., B. T. FARNSWORTH, R. A. COULOMBE, AND W. E. WINNER. 1999. Foliage physiology and biochemistry in response to light gradients in conifers with varying shade tolerance. *Oecologia* 120: 183–192.

BOYER, J. S., AND W. K. SILK. 2004. Hydraulics of plant growth. *Functional Plant Biology* 31: 761–773.

BRESHEARS, D. D., N. G. MCDOWELL, K. L. GODDARD, K. E. DAYEM, S. N. MARTENS, C. W. MEYER, AND K. M. BROWN. 2008. Foliar absorption of intercepted rainfall improves woody plant water status most during drought. *Ecology* 89: 41–74.

BRODRIBB, T. J., AND H. COCHARD. 2009. Hydraulic failure defines the recovery and point of death in water-stressed conifers. *Plant Physiology* 149: 575–584.

BRODRIBB, T. J., T. S. FIELD, AND G. J. JORDAN. 2007. Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology* 144: 1890–1898.

BRODRIBB, T. J., AND N. M. HOLBROOK. 2003. Stomatal closure during leaf dehydration, correlation with other physiological traits. *Plant Physiology* 132: 2166–2173.

BRODRIBB, T. J., AND N. M. HOLBROOK. 2005. Water stress deforms tracheid peripheral to the leaf vein of a tropical conifer. *Plant Physiology* 137: 1139–1146.

BRODRIBB, T. J., N. M. HOLBROOK, E. J. EDWARDS, AND M. V. GUTIERREZ. 2003. Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. *Plant, Cell & Environment* 26: 443–450.

BRODRIBB, T. J., N. M. HOLBROOK, AND M. GUTIÉRREZ. 2002. Hydraulic and photosynthetic coordination in seasonally dry tropical forest trees. *Plant, Cell & Environment* 25: 1435–1444.

BRODRIBB, T. J., N. M. HOLBROOK, M. A. ZWIENIECKI, AND B. PALMA. 2005. Leaf hydraulic capacity in ferns, conifers and angiosperms: Impacts on photosynthetic maxima. *New Phytologist* 165: 839–846.

BURGESS, S. S. O., AND T. E. DAWSON. 2004. The contribution of fog to the water relations of *Sequoia sempervirens*: Foliar uptake and prevention of dehydration. *Plant, Cell & Environment* 27: 1023–1034.

BURGESS, S. S. O., AND T. E. DAWSON. 2007. Predicting the limits to tree height using statistical regressions of leaf traits. *New Phytologist* 174: 626–636.

CANNY, M. J. 1993. Transfusion tissues of pine needles as a site of retrieval of solutes from the transpiration stream. *New Phytologist* 123: 227–232.

COSGROVE, D. J. 1993. How do plant cell walls extend? *Plant Physiology* 102: 1–6.

COSGROVE, D. J. 2000. Loosening of plant cell walls by expansins. *Nature* 407: 321–326.

ELLSWORTH, D. S., AND P. B. REICH. 1993. Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia* 96: 169–178.

ENGLAND, J. R., AND P. M. ATTIWILL. 2006. Changes in leaf morphology and anatomy with tree age and height in the broadleaved evergreen species, *Eucalyptus regnans*. *Trees* 20: 79–90.

ESAU, K. 1977. Anatomy of seed plants, 2nd ed. Wiley, New York, New York, USA.

FABRE, N., I. M. REITER, N. BECUWE-LINKA, B. GENTY, AND D. RUMEAU. 2007. Characterization and expression analysis of genes encoding α and β carbonic anhydrases in *Arabidopsis*. *Plant, Cell & Environment* 30: 617–629.

FLEMING, A. J. 2006. The integration of cell proliferation and growth in leaf morphogenesis. *Journal of Plant Research* 119: 31–36.

FLEXAS, J., M. RIBAS-CARBÓ, A. DIAZ-ESPEJO, J. GALMÉS, AND H. MEDRANO. 2008. Mesophyll conductance to CO_2: Current knowledge and future prospects. *Plant, Cell & Environment* 31: 602–621.

HAN, Q. M., T. KAWASAKI, S. KATAHATA, Y. MUKAI, AND Y. CHIBA. 2003. Horizontal and vertical variations in photosynthetic capacity in a *Pinus densiflora* crown in relation to leaf nitrogen allocation and acclimation to irradiance. *Tree Physiology* 23: 851–857.

HANBA, Y. T., S. I. MIYAZAWA, AND I. TERASHIMA. 1999. The influence of leaf thickness on the CO_2 transfer conductance and leaf stable carbon isotope ratio for some evergreen tree species in Japanese warm-temperate forests. *Functional Ecology* 13: 632–639.

HINTZE, J. 2002. Number cruncher statistical system, version 2001. NCSS Software, Kaysville, Utah, USA.

HÖLTTA, T., H. COCHARD, E. NIKINMAA, AND M. MENCUCCINI. 2009. Capacitative effect of cavitation in xylem conduits: Results from a dynamic model. *Plant, Cell & Environment* 32: 10–21.

HORIGUCHI, G., A. FERJANI, U. FUJIKURA, AND H. TSKAYA. 2006. Coordination of cell proliferation and cell expansion in the control of leaf size in *Arabidiopsis thaliana*. *Journal of Plant Research* 119: 37–42.

HUBBARD, R. M., B. J. BOND, AND M. G. RYAN. 1999. Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. *Tree Physiology* 19: 165–172.

ISHII, H. T., G. M. JENNINGS, S. C. SILLETT, AND G. W. KOCH. 2008. Hydrostatic constraints on morphological exploitation of light in tall *Sequoia sempervirens* trees. *Oecologia* 156: 751–763.

JENNINGS, G. M. 2002. Vertical hydraulic gradients and the cause of foliar variation in tall redwood trees. Masters thesis, Humboldt State University, Arcata, California, USA.

KOCH, G. W., AND S. C. SILLETT. 2009. A response to: Limitations within "The limits to tree height". *American Journal of Botany* 96: 545–547.

KOCH, G. W., S. C. SILLETT, G. M. JENNINGS, AND S. D. DAVIS. 2004. The limits to tree height. *Nature* 428: 851–854.

MCCUNE, B., AND M. J. MEFFORD. 2006. PC-ORD: Multivariate analysis of ecological data, version 5.10. MjM Software, Gleneden Beach, Oregon, USA.

MCDOWELL, N. G., J. LICATA, AND B. J. BOND. 2005. Environmental sensitivity of gas exchange in different-sized trees. *Oecologia* 145: 9–20.

MEINZER, F. C., B. J. BOND, AND J. A. KARANIAN. 2008. Biophysical constraints on leaf expansion in a tall conifer. *Tree Physiology* 28: 197–206.

MENCUCCINI, M., J. MARTÍNEZ-VILALTA, D. VANDERKLEIN, H. A. HAMID, E. KORAKAKI, S. LEE, AND B. MICHIELS. 2005. Size-mediated ageing reduced vigour in trees. *Ecology Letters* 8: 1183–1190.

MULLIN, L. P., S. C. SILLETT, G. W. KOCH, K. TU, AND M. E. ANTOINE. 2009. Physiological consequences of height-related morphological variation in *Sequoia sempervirens* foliage. *Tree Physiology* 29: 999–1010.

NETTING, A. G. 2009. Limitations within "The limits to tree height". *American Journal of Botany* 96: 542–544.

NIINEMETS, U. 1999. Research review: Components of leaf dry mass per area—thickness and density—alter leaf photosynthetic capacity in reverse directions in woody plants. *New Phytologist* 144: 35–47.

NIINEMETS, U., AND O. KULL. 1995. Effects of light availability and tree size on the architecture of assimilative surface in the canopy of *Picea abies*: Variation in needle morphology. *Tree Physiology* 15: 307–315.

NIINEMETS, U., AND O. KULL. 1998. Stoichiometry of foliar carbon constituents varies along light gradients in temperate woody canopies: Implications for foliage morphological plasticity. *Tree Physiology* 18: 467–479.

NIINEMETS, U., O. KULL, AND J. D. TENHUNEN. 1998. An analysis of light effects on foliar morphology, physiology and light interception in temperate deciduous woody species of contrasting shade tolerance. *Tree Physiology* 18: 681–698.

NOBEL, P. S., L. J. ZARAGOZA, AND W. K. SMITH. 1975. Relation between mesophyll surface area, photosynthetic rate, and illumination level during development for leaves of *Plectranthus parviforus* Henckel. *Plant Physiology* 55: 1067–1070.

PARKER, J. 1952. Desiccation in conifer leaves: Anatomical changes and determination of the lethal level. *Botanical Gazette* 114: 189–198.

PARKHURST, D. F. 1994. Diffusion of CO_2 and other gases inside leaves. *New Phytologist* 126: 449–479.

PERES-NETO, P. R., D. A. JACKSON, AND K. M. SOMERS. 2005. How many principal components? Stopping rules for determining the number of non-trivial axes revisited. *Computational Statistics & Data Analysis* 49: 974–997.

PHILLIPS, N. G., M. G. RYAN, B. J. BOND, N. G. MCDOWELL, T. M. HINCKLEY, AND J. ČERMÁK. 2003. Reliance on stored water increases with tree size in three species in the Pacific Northwest. *Tree Physiology* 23: 237–245.

PITTERMANN, J., J. S. SPERRY, U. G. HACKE, J. K. WHEELER, AND E. H. SIKKEMA. 2005. Torus margo pits help conifers compete with angiosperms. *Science* 310: 1924.

PITTERMANN, J., J. S. SPERRY, U. G. HACKE, J. K. WHEELER, AND E. H. SIKKEMA. 2006. Inter-tracheid pitting and the hydraulic efficiency of conifer wood: The role of tracheid allometry and cavitation protection. *American Journal of Botany* 93: 1265–1273.

RYAN, M. G., AND B. J. YODER. 1997. Hydraulic limits to tree height and growth. *Bioscience* 47: 235–242.

SILLETT, S. C., R. VAN PELT, G. W. KOCH, A. R. AMBROSE, A. L. CARROLL, M. E. ANTOINE, AND B. M. MIFSUD. 2010. Increasing wood production through old age in tall trees. *Forest Ecology and Management* 259: 976–994.

SPERRY, J. S., U. G. HACKE, AND J. PITTERMANN. 2006. Size and function in conifer tracheid and angiosperm vessels. *American Journal of Botany* 93: 1490–1500.

THODAY, D. 1931. The significance of reduction of size in leaves. *Journal of Ecology* 19: 297–303.

TSUKAYA, H. 2003. Organ shape and size: A lesson from studies of leaf morphogenesis. *Current Opinion in Plant Biology* 6: 57–62.

TSUKAYA, H. 2006. Mechanism of leaf-shape determination. *Annual Review of Plant Biology* 57: 477–496.

TYREE, M. T., AND J. S. SPERRY. 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? *Plant Physiology* 88: 574–580.

VANDERKLEIN, D., J. MARTINEZ-VILALTA, S. LEE, AND M. MENCUCCINI. 2007. Plant size, not age, regulates growth and gas exchange in grafted Scots pine trees. *Tree Physiology* 27: 71–79.

WESTOBY, M., AND I. J. WRIGHT. 2006. Land-plant ecology on the basis of functional traits. *Trends in Ecology & Evolution* 21: 261–268.

WOODRUFF, D. R., B. J. BOND, AND F. C. MEINZER. 2004. Does turgor limit growth in tall trees? *Plant, Cell & Environment* 27: 229–236.

WOODRUFF, D. R., K. A. MCCOLLOH, J. M. WARREN, F. C. MEINZER, AND B. LACHENBRUNCH. 2007. Impacts of tree height on leaf hydraulic architecture and stomatal control in Douglas-fir. *Plant, Cell & Environment* 30: 559–569.

WOODRUFF, D. R., F. C. MEINZER, B. LACHENBRUNCH, AND D. M. JOHNSON. 2009. Coordination of leaf structure and gas exchange along a height gradient in a tall conifer. *Tree Physiology* 29: 261–272.

WRIGHT, I. J., P. B. REICH, J. H. C. CORNELISSEN, D. S. FALSTER, P. K. GROOM, K. HIKOSAKA, W. LEE, ET AL. 2005. Modulation of leaf economic traits and trait relationships by climate. *Global Ecology and Biogeography* 14: 411–421.

WRIGHT, I. J., P. B. REICH, M. WESTOBY, D. D. ACKERLY, Z. BARUCH, F. BONGERS, J. CAVENDER BARES, ET AL. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821–827.

ZIMMERMANN, M. H. 1983. Xylem structure and the ascent of sap. Springer, Berlin, Germany.

ZWIENIECKI, M. A., C. K. BOYCE, AND N. M. HOLBROOK. 2004a. Functional design space of single-veined leaves: Role of tissue hydraulic properties in constraining leaf size and shape. *Annals of Botany* 94: 507–513.

ZWIENIECKI, M. A., C. K. BOYCE, AND N. M. HOLBROOK. 2004b. Hydraulic limitations imposed by crown placement determine final size and shape of *Quercus rubra* leaves. *Plant, Cell & Environment* 27: 357–365.

ZWIENIECKI, M. A., H. STONE, A. LEIGH, C. K. BOYCE, AND N. M. HOLBROOK. 2006. Hydraulic design of pine needles: One-dimensional optimization for single-veined leaves. *Plant, Cell & Environment* 29: 803–809.