Does turgor limit growth in tall trees?

D. R. WOODRUFF[1], B. J. BOND[1] & F. C. MEINZER[2]

[1]*Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA and* [2]*USDA Forest Service, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, OR 97331, USA*

ABSTRACT

The gravitational component of water potential contributes a standing 0.01 MPa m^{-1} to the xylem tension gradient in plants. In tall trees, this contribution can significantly reduce the water potential near the tree tops. The turgor of cells in buds and leaves is expected to decrease in direct proportion with leaf water potential along a height gradient unless osmotic adjustment occurs. The pressure–volume technique was used to characterize height-dependent variation in leaf tissue water relations and shoot growth characteristics in young and old Douglas-fir trees to determine the extent to which growth limitation with increasing height may be linked to the influence of the gravitational water potential gradient on leaf turgor. Values of leaf water potential (Ψ_l), bulk osmotic potential at full and zero turgor, and other key tissue water relations characteristics were estimated on foliage obtained at 13.5 m near the tops of young (approximately 25-year-old) trees and at 34.7, 44.2 and 55.6 m in the crowns of old-growth (approximately 450-year-old) trees during portions of three consecutive growing seasons. The sampling periods coincided with bud swelling, expansion and maturation of new foliage. Vertical gradients of Ψ_l and pressure–volume analyses indicated that turgor decreased with increasing height, particularly during the late spring when vegetative buds began to swell. Vertical trends in branch elongation, leaf dimensions and leaf mass per area were consistent with increasing turgor limitation on shoot growth with increasing height. During the late spring (May), no osmotic adjustment to compensate for the gravitational gradient of Ψ_l was observed. By July, osmotic adjustment had occurred, but it was not sufficient to fully compensate for the vertical gradient of Ψ_l. In tall trees, the gravitational component of Ψ_l is superimposed on phenologically driven changes in leaf water relations characteristics, imposing potential constraints on turgor that may be indistinguishable from those associated with soil water deficits.

Key-words: *Pseudotsuga menziesii*; Douglas-fir; gravitational component of water potential; height growth; osmotic adjustment; pressure–volume curve; turgor maintenance.

Correspondence: David R. Woodruff. Fax: +1 541 737 1393; e-mail: david.woodruff@oregonstate.edu

INTRODUCTION

A great deal of attention has recently been focused on the mechanisms responsible for reduced growth in trees as they age and increase in height (Friend 1993; Yoder *et al*. 1994; Ryan & Yoder 1997; Magnani, Mencuccini & Grace 2000; McDowell *et al*. 2002). Much of this research has addressed the hydraulic limitation hypothesis, which proposes that reduced growth in ageing and tall trees may be linked to reductions in leaf-specific hydraulic conductance, which in turn lead to reductions in stomatal conductance and therefore photosynthesis (Ryan & Yoder 1997). In addition to strictly hydraulic constraints that may require stomatal limitation of transpiration to regulate leaf water potential (Ψ_l) within a relatively narrow range, the gravitational component of Ψ influences Ψ_l whether or not transpiration is occurring.

In the absence of transpiration, the gravitational component of water potential should result in a standing xylem tension gradient of 0.01 MPa per metre increase in height (Scholander *et al*. 1965; Hellkvist, Richards & Jarvis 1974; Zimmermann 1983; Bauerle *et al*. 1999). Thus, at the top of a non-transpiring 60-m-tall tree, leaf water potential should be at least 0.6 MPa more negative than foliage at ground level. When transpiration occurs, frictional resistance will lower leaf water potential even further (Hellkvist *et al*. 1974; Connor, Legge & Turner 1977; Bauerle *et al*. 1999). The turgor of cells in leaves and buds is expected to decrease in direct proportion with the leaf water potential if the osmotic potential of these cells remains constant. A variety of growth-related processes, including cell formation, expansion and metabolism are dependent on turgor pressure and cell volume (Boyer 1968; Hsiao *et al*. 1976; Gould & Measures 1977; Ray 1987). However, osmotic adjustment, the active accumulation of symplastic solutes, could maintain turgor and cell volume and may serve to sustain growth and photosynthetic gas exchange with increasing tree height. Nevertheless, compensatory osmotic adjustment may only partially offset reductions in growth with increasing height if it results in a substantial amount of resources being diverted from leaf expansion towards production and accumulation of osmolytes for turgor and volume maintenance.

Osmotic adjustment has been well documented as an adaptation to drought and salinity stress (Hsiao *et al*. 1976; Osonubi & Davies 1978; Turner & Jones 1980; Morgan 1984; McNulty 1985; Heuer & Plaut 1989; Pereira & Pallardy 1989; Ranney, Bassuk & Whitlow 1991; Rieger 1995).

However, we know of no studies that have attempted to evaluate the effects of the gravitational component of water potential on the osmotic, morphological and growth characteristics of foliage along a height gradient in tall trees. The objective of this study therefore was to characterize height-dependent variation in leaf tissue water relations and morphological characteristics in young and old Douglas-fir trees to determine the extent to which shoot growth limitation with increasing height may be linked to the influence of the gravitational water potential gradient on leaf turgor. The pressure–volume technique was employed at various phenological stages during three consecutive growing seasons to assess water relations characteristics of foliage sampled along a height gradient from approximately 14 to 57 m.

MATERIALS AND METHODS

Field sites

The two study sites were located within 5 km of each other in the Wind River Basin of south-western Washington, USA. The site with the tallest trees was a 450-year-old Douglas-fir/western hemlock (*Pseudotsuga menziesii* (Mirb.) Franco/*Tsuga heterophylla* (Raf.) Sarg.) forest located at the Wind River Canopy Crane Research Facility (WRCCRF) within the T.T. Munger Research Natural Area in the Gifford Pinchot National Forest (Shaw *et al.* 2004). The stand has a mean density of about 437 trees ha^{-1} with a mean height of 62 m. The WRCCRF contains a 75-m-tall construction tower crane with an 85 m jib that provided access to the crowns of the study trees via a suspended gondola. The second site, located in the Wind River Experimental Forest, was clear-cut in 1976 and planted with Douglas-fir seedlings in 1978 (McDowell *et al.* 2002; Phillips *et al.* 2002). During the study period, the mean density was about 1529 trees ha^{-1} with a mean height of 16 m. A 19.5-m-tall scaffold tower provided access to the crowns of the study trees.

The altitudes of the younger and older stands are 561 and 371 m, respectively. The Pacific maritime climate of the region is characterized by wet winters and dry summers. Mean annual precipitation in the region is about 2.5 m, over 70% of which falls as snow, with a dry season from June to September. Very low precipitation between June and September (approximately 119 mm) typically leads to drought conditions. The mean annual temperature is 8.7 °C with means of 0 °C in January and 17.5 °C in July. The soils are well-drained and of volcanic origin.

Sampling and measurements

Branch sampling locations were established near the tops of five 14- to 17-m-tall Douglas-fir trees in the younger stand and at three heights within the crowns of five 54- to 59-m-tall Douglas-fir trees in the old-growth stand. Mean height for the sampling locations was 13.5 m at the tops of the young trees, and 34.7, 44.2 and 55.6 m for the lower,

middle and upper third of the old trees, respectively. Samples were collected during the spring (1 May), early summer (5 July), and late summer (21 August) of 2001, during the spring (2 May) and early summer (27 June) of 2002, and during the early summer (24 June) of 2003.

Bulk leaf water relations characteristics were determined from pressure–volume analysis (Scholander *et al.* 1965; Tyree & Hammel 1972). Twigs approximately 10–15 cm long were excised early in the morning prior to significant transpirational water loss, sealed in plastic bags with moist paper to prevent desiccation, and then stored in a refrigerator until pressure–volume curve determinations began, within 2–6 h of excision. The twigs were not rehydrated artificially to avoid rehydration-induced artifacts reported for a number of woody species (Bowman & Roberts 1985; Meinzer *et al.* 1986; Evans, Black & Link 1990). Pressure–volume curves were initiated by first determining the fresh weight of the twig, and then measuring its water potential (Ψ_l) with a pressure chamber (PMS Instrument Company, Corvallis, OR, USA). Although early morning sampling of foliage was not always conducted prior to dawn due to logistical constraints associated with the use of the canopy crane, the initial value of Ψ_l was considered to be equivalent to pre-dawn Ψ_l and is referred to hereafter as 'pre-dawn' for purposes of convention. Alternate determinations of fresh weight and Ψ_l were repeated during slow dehydration on the laboratory bench until values of Ψ_l exceeded the measuring range of the pressure chamber (–4.0 MPa). The inverse of water potential was plotted against relative water content to create a pressure–volume curve. A characteristic pressure–volume curve consists of an initial curvilinear and sharply declining series of points, followed by a more linear series with a less severe decline. The point of transition between these two portions of the graph represent turgor loss point for the sample (Tyree & Hammel 1972). The key tissue water relations parameters determined from pressure–volume analysis were osmotic potential at full and zero turgor, symplastic water fraction, and the moles of osmotically active solutes in the sample (N_s) calculated according to Tyree *et al.* (1978):

$$N_s = \psi_\Pi^{100} V_{100}/(RT) \tag{1}$$

where ψ_Π^{100} is the osmotic potential at full hydration, V_{100} is the osmotic (symplastic) water volume at full hydration, R is the universal gas constant and T is the Kelvin temperature at which the pressure–volume curve was determined. The saturated weight of each sample, necessary for calculating tissue relative water content, was estimated by fitting a linear regression to a plot of sample fresh weight against Ψ_l for points above the turgor loss point and extrapolating to zero Ψ_l. On some sampling dates, twigs were excised from all sampling heights at midday for determination of midday Ψ_l. Values of turgor at each sampling height were estimated from the difference between values of Ψ_l *in situ* and the value for osmotic potential derived from the pressure–volume curve.
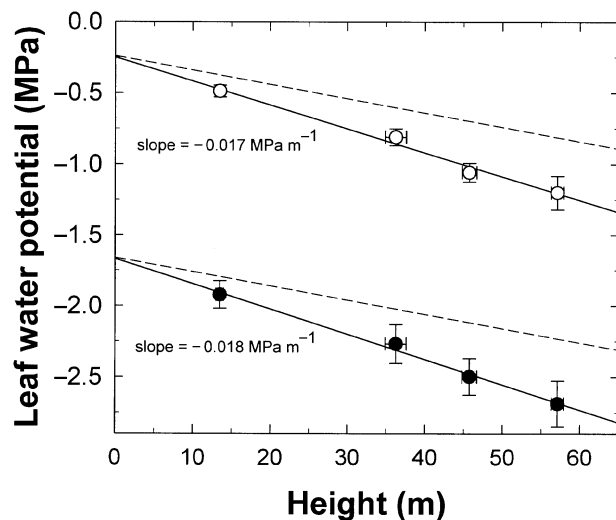
During June 2003, pressure–volume analyses were performed on twig samples bearing only current year, expand-

ing foliage to determine whether height-dependent trends in tissue water relations parameters were similar to those previously observed in old-growth trees using twig samples bearing multiple years of foliage. In addition, bulk foliage osmotic potential was determined with a vapour pressure osmometer (Vapro 5520; Wescor, Inc., Logan, UT, USA) to compare values obtained with this direct method to those derived from pressure–volume analysis. Sample twigs were initially frozen at −80 °C to facilitate subsequent sap extraction. Current year foliage was removed from frozen sample twigs, placed in a sleeve of Tygon tubing, which was squeezed with a bench vice to express foliage sap. A 10-μL sample of sap was immediately obtained for determination of osmotic potential.
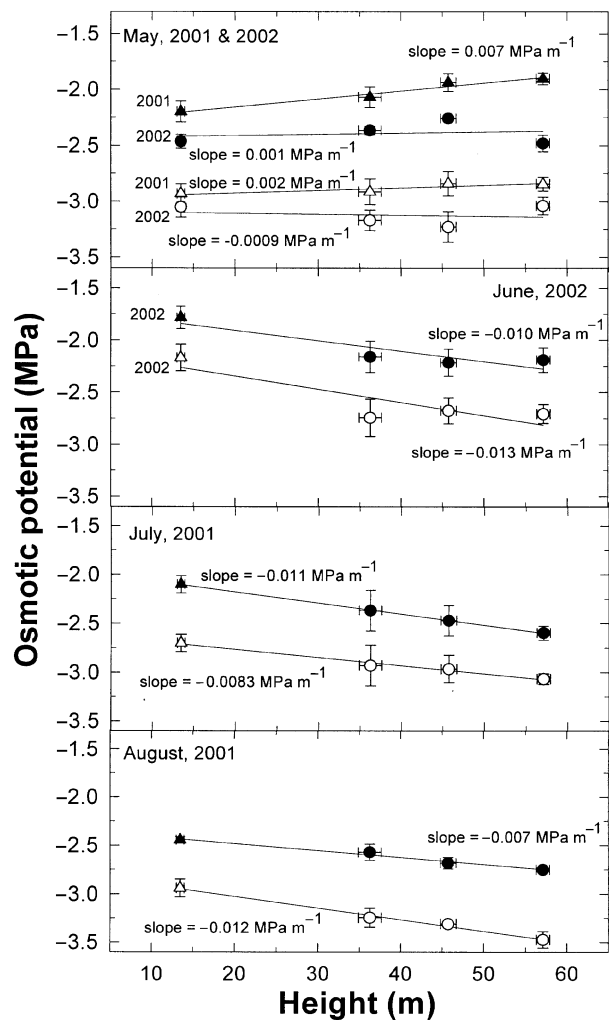
Leaf and branch growth and morphological characteristics were determined on foliage samples collected at the same locations as those used for the pressure–volume curves and osmometer samples. Needle linear dimensions and branch annual extension increments were determined with digital calipers. Leaf mass per area was obtained by determining needle areas with a scanner and IMAGEJ version 1.27 image analysis software (http://rsb.info.nih.gov/ij/), then weighing the needles after oven-drying.

RESULTS

Both pre-dawn and midday Ψ_l decreased in a linear fashion with increasing height (Fig. 1). The vertical water potential gradient was substantially steeper than that expected from the gravitational component alone (Fig. 1, dashed line). Even though values of Ψ_l for the lowest sampling height (13.5 m) were obtained from younger trees in a nearby

Figure 2. Leaf osmotic potential at full (filled symbols) and zero (open symbols) turgor as a function of height on young (triangles) and old (circles) trees during the 2001 and 2002 growing seasons. Bars represent standard errors for measurements on one sample from each of five trees (*n* = 5).

stand, single regression lines adequately described the relationship between Ψ_l and height.

Height-dependent trends in the characteristics of leaf water relations varied seasonally and from year to year. In May of 2001, osmotic potential at full turgor increased with height (*P* = 0.02; Fig. 2), whereas in May of 2002 no vertical trend in osmotic potential was detected (Fig. 2). In both July and August 2001, osmotic potential decreased linearly with increasing height (*P* ≤ 0.01) at a mean rate consistent with the gravitational component of water potential (Fig. 2). A similar decrease in osmotic potential with increasing height was observed in June of 2002 (Fig. 2). As with the vertical trends in Ψ_l, single regression lines adequately described the dependence of osmotic potential on height when data for young and old trees were combined. Seasonal changes in vertical profiles of symplast solute content on a tissue dry weight basis indicated that seasonal changes in vertical profiles of osmotic potential were asso-
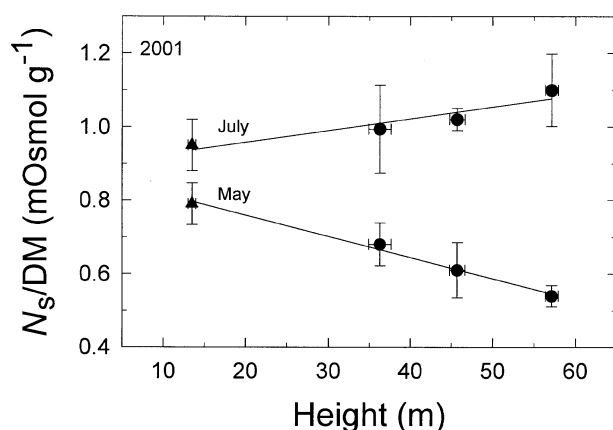

Figure 1. Pre-dawn (open symbols) and midday (filled symbols) leaf water potentials measured along a height gradient on young and old trees in July 2001. Bars represent standard errors for measurements on three samples at each location from each of five trees (*n* = 15). The dashed lines represent the theoretical gravitational gradient in the absence of transpiration (−0.01 MPa m^{-1}).

Figure 3. Symplast solute content on a dry matter basis (N_s/DM) as a function of height on young (triangles) and old (circles) trees for May and July 2001. Bars represent standard errors for measurements on one sample from each of five trees ($n = 5$).

ciated with true osmotic adjustment (Fig. 3). In May of 2001, symplast solute content decreased by 30% between 13.5 and 55.6 m, whereas by July solute content was greater at all heights and increased by 15% between 13.5 and 55.6 m.

Vertical trends in turgor varied seasonally in a manner consistent with those of osmotic potential. In May of 2001, when osmotic potential increased slightly with height, pre-dawn turgor decreased with increasing height ($P < 0.02$) at a rate similar to that of the gravitational component of Ψ_l (Fig. 4). At midday, the decline in turgor with height was even steeper than it was before dawn, with estimated turgor values near zero at the 55.6 m sampling height (Fig. 4). Similarly, in May of 2002, when osmotic potential was independent of height, the vertical decline in pre-dawn turgor ($P = 0.03$) was approximately 0.015 MPa m^{-1} (Fig. 4). Although the vertical decline in pre-dawn turgor was substantially smaller later in the season in both 2001 and 2002 (Fig. 4), it still amounted to about 0.25 MPa between 13.5 and 55.6 m in July and August of 2001.

Height-dependent trends in osmotic potential and turgor for twigs bearing only current-year expanding foliage were similar to those of twigs bearing multiple years of foliage. In June 2003, osmotic potential at zero turgor decreased linearly with increasing height ($P < 0.01$) at a rate similar to that of the gravitational component of Ψ_l (Fig. 5a), whereas osmotic potential at full turgor was independent of height. Pre-dawn turgor decreased with increasing height ($P = 0.067$; Fig. 5b). Midday turgor showed the same trend with height, although not significantly. The values of foliage osmotic potential determined by osmometry (Fig. 5a, squares) were slightly, but not significantly more negative than values of osmotic potential at full turgor obtained from pressure–volume curves.

Leaf and branch growth and morphological characteristics also exhibited marked changes along a vertical gradient. In the old-growth trees, leaf mass/area increased sharply with height (Fig. 6). Strong vertical trends of

decreasing needle length, needle width, and branch extension growth with increasing height were also observed (Fig. 7).

DISCUSSION

Vertical gradients of Ψ_l and pressure–volume analyses of Douglas-fir twigs collected at different heights suggested that turgor decreased with increasing height, particularly during the late spring when vegetative buds began to swell. The decline in turgor with increasing height was evident both near dawn and at midday, although the rate of change with respect to height was greater at midday due to a steeper water potential gradient. Vertical trends in leaf physical and morphological characteristics were consistent with the hypothesis that reduced turgor may limit leaf expansion at greater heights. During the late spring, no osmotic adjust-
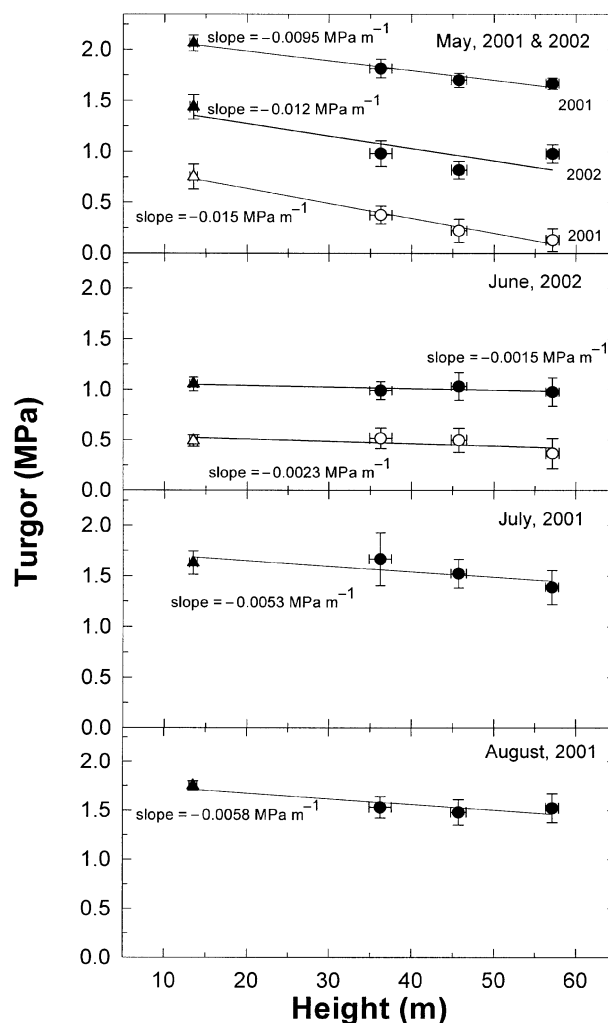
Figure 4. Pre-dawn (filled symbols) and midday (open symbols) turgor as a function of height on young (triangles) and old (circles) trees during the 2001 and 2002 growing seasons. Midday values were not available for sampling sessions when midday water potentials were not able to be collected. Bars represent standard errors for measurements on one sample from each of five trees ($n = 5$).
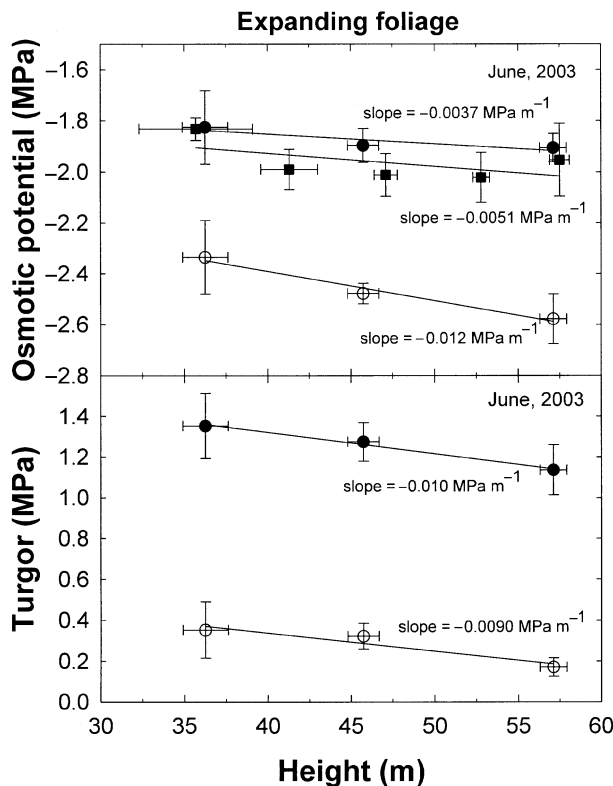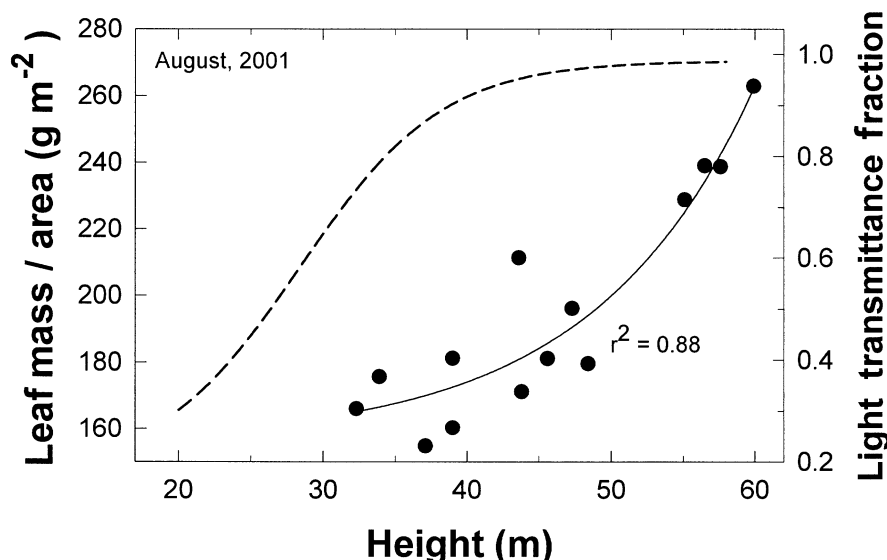
Expanding foliage

Figure 5. Water relations characteristics of expanding foliage as a function of height on old-growth trees during the 2003 growing season. (a) Osmotic potential at full (filled circles) and zero (open circles) turgor estimated from pressure–volume curves and osmotic potential of sap expressed from frozen and thawed needles (squares) determined by vapour pressure osmometry. (b) Pre-dawn (filled circles) and midday (open circles) turgor.

compensate for the vertical gradient of Ψ_l, which was nearly identical to the gradient observed in an earlier study conducted in the old-growth stand (Bauerle *et al*. 1999). It should be noted that although some of our samples used to estimate osmotic potential, and therefore turgor, included both mature and expanding foliage, subsequent analyses on samples containing only current year expanding foliage showed similar results (Fig. 5) thus validating the use of twig samples containing multiple years of foliage. This is not surprising given the fact that in developing needles, which constituted a lesser fraction of the multiyear sample weight early in the season, solute concentration and turgor were likely to have been considerably lower than in older needles. By June and July, expanding foliage made up half or more of the foliage on twig samples.

Seasonal trends in leaf water relations characteristics, particularly osmotic potential, have been reported in previous studies (Tyree *et al*. 1978; Davies & Lakso 1979; Ritchie & Shula 1984; Kubiske & Abrams 1991) and seem to be related to stages of phenological development. In a previous study of Douglas-fir seedlings, leaf osmotic potential began to increase during late winter and early spring, reaching maximum (least negative) values between April and May, then rapidly decreasing to a new stable minimum value by mid-June (Ritchie & Shula 1984). The timing of water stress in relation to phenological stage is important in terms of internal competition for water (Hale & Orcutt 1987). In tall trees, the gravitational component of Ψ_l is superimposed on phenologically driven changes in leaf water relations characteristics, imposing potential constraints on turgor that may be indistinguishable from those associated with soil water deficits. Even near dawn, the vertical Ψ_l gradient was markedly steeper than the expected gravitational gradient in the absence of transpiration (Fig. 1), suggesting that nocturnal stomatal opening and transpiration may have prevented Ψ_l from fully equilibrating with that of the soil during the night (Reed & Waring 1974; Meinzer 1982; Donovan, Richards & Linton 2003).

ment to compensate for the gravitational gradient of Ψ_l was observed. In fact, symplast solute content actually decreased with increasing height in May. By July, osmotic adjustment had occurred, but it was not sufficient to fully

Figure 6. Leaf mass per area and light transmittance fraction in relation to height for five old-growth Douglas-fir trees accessible from the canopy crane. Light transmittance data (dashed line) are from Parker *et al*. (2002).
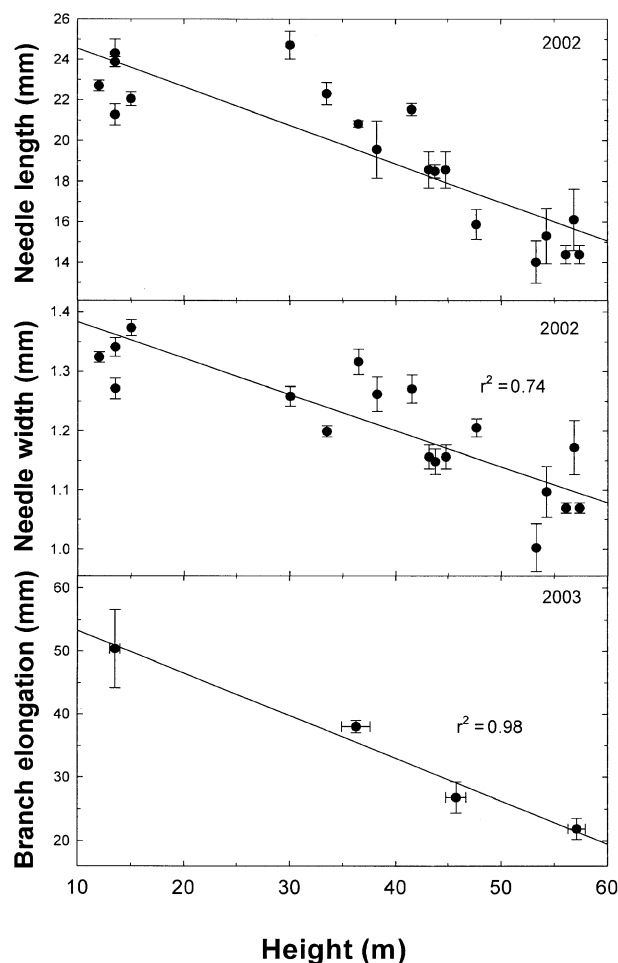
Figure 7. Needle length and width in relation to height for the 2002 cohort of foliage and branch extension growth in relation to height for the 2003 cohort of foliage on the five young and the five old-growth Douglas-fir trees accessible from the canopy crane. Bars represent standard errors for measurements on 10 needles from each sampling location in needle dimension plots and standard errors for measurements on five twigs for branch extension plot.

When buds swell in Douglas-fir, foliar sugars are metabolized more rapidly than they are produced and therefore tend to be limited (Krueger & Trappe 1967; Billow, Matson & Yoder 1994). The rapid decline in osmotic solute concentration of previous years' foliage of Douglas-fir and other evergreen conifers observed during early spring may be a manifestation of this phenomenon and suggests that sugars and other soluble carbohydrates may comprise a significant fraction of cellular osmolytes. The decline in symplast solute content of mature foliage with increasing height observed during May 2001 (Fig. 3), a period when buds are normally swelling, was also consistent with low availability of solutes relative to the demands of developing foliage. By July, when needles are normally fully expanded, symplast solute content was greater at all heights and increased with height, resulting in a doubling of solute content at 55.6 m between May and July. Billow *et al.* (1994) found that sugar concentrations of unfertilized Douglas-fir foliage nearly doubled between May and October

Height-dependent variation in needle length, width, leaf mass per area and shoot extension was consistent with diminished capacity for cell expansion with increasing height (Figs 6 & 7). Trends in leaf morphological and physical characteristics with canopy position have been well documented, but are typically attributed to variation in the light environment (Ellsworth & Reich 1993; Sprugel, Brooks & Hinckley 1996; Niinemets & Tenhunen 1997). However, a recent study investigating the impact of shading and leaf water status upon leaf morphology along a height gradient within tall conifers reported a lack of any strong shading effect with decreasing height; consistent with the hypothesis that the gravitational component of leaf water potential causes specific leaf area to decrease with increasing height (Marshall & Monserud 2003). Similarly, the sharp decline in leaf mass per area with decreasing height observed in old-growth trees in the present study did not appear to be associated with height-dependent variation in the light environment because the light transmittance fraction measured in an earlier study in the same stand (Parker, Davis & Chapotin 2002) did not change appreciably in the height interval over which our foliage samples were collected (Fig. 6). Moreover, the length and width size of needles from young trees at 13.5 m, near the fully illuminated tops, was substantially greater than that of needles at 55.6 m height in the old-growth stand (Fig. 7). In a study of changes in needle anatomy with tree age, Apple *et al.* (2002) found that needles from the tops of Douglas-fir saplings had a significantly greater percentage of photosynthetic mesophyll tissue than needles from the tops of old-growth trees. Higher astrosclereid abundance and larger vascular cylinders in needles from old-growth trees were identified as probable causes of reduced photosynthetic cell area in needles from tall trees. However, mesophyll cell size, which was not reported in their study, may also have contributed to the patterns observed.

If environmental factors such as relative humidity, wind speed and temperature vary with canopy height, they might have effects on leaf water balance that are additive to the effects of the hydrostatic gradient, and the impact on leaf water balance might also affect the physicochemical properties of leaves. However, gradients in leaf mass per area have been noted even in the absence of any vertical environmental variation. Marshall & Monserud (2003) measured vertical trends in leaf mass per area of similar magnitude to those reported here in stands with densities sufficiently low as to result in little variability with height in such environmental parameters. Taken together, the preceding observations suggest that the gravitational component of water potential plays a dominant role in governing morphological changes in Douglas-fir needles along a height gradient.

CONCLUSIONS

Physiological constraints on turgor imposed by the gravitational component of plant water potential imply a series of negative feedbacks that limit leaf expansion and branch

extension growth in tall Douglas-fir trees. Increasingly small annual increments of photosynthetically active leaf area not only limit the overall potential for photosynthate production, but also reduce the allocation of photosynthate to growth of other organs because of the greater investment of photosynthate required to produce a given amount of leaf area as height increases. The osmotic adjustment required to maintain turgor as height increases also implies an enhanced expenditure of energy to produce and maintain a given amount of photosynthetic surface area. Energy is expended and photosynthate diverted in the production of organic solutes, and in the uptake, accumulation and sequestering of inorganic solutes. Future research to identify and quantify the principal solutes involved in this osmotic adjustment would contribute to a more complete understanding of its energy requirements.

ACKNOWLEDGMENTS

REFERENCES

Apple M., Tiekotter K., Snow M., Young J., Soelder A., Phillips D., Tingey D. & Bond B. (2002) Needle anatomy changes with increasing tree age in Douglas-fir. *Tree Physiology* **22,** 129–136.

Bauerle W.L., Hinckley T.M., Cermak J., Kucera J. & Bible K. (1999) The canopy water relations of old-growth Douglas-fir trees. *Trees* **13,** 211–217.

Billow C., Matson P. & Yoder B. (1994) Seasonal biochemical changes in coniferous forest canopies and their response to fertilization. *Tree Physiology* **14,** 563–574.

Bowman W.D. & Roberts S.W. (1985) Seasonal changes in tissue elasticity in chaparral shrubs. *Physiologia Plantarum* **65,** 233–236.

Boyer J.S. (1968) Relationship of water potential to growth of leaves. *Plant Physiology* **43,** 1056–1062.

Connor D.J., Legge N.C. & Turner N.C. (1977) Water relations of mountain ash (*Eucalyptus regnans* F. Muell.) forests. *Australian Journal of Plant Physiology* **4,** 753–762.

Davies F.S. & Lakso A.N. (1979) Diurnal and seasonal changes in leaf water potential components and elastic properties in response to water stress in apple trees. *Physiologia Plantarum* **46,** 109–114.

Donovan L.A., Richards J.H. & Linton M.J. (2003) Magnitude and mechanisms of disequilibrium between predawn plant and soil water potentials in desert shrubs. *Ecology* **84,** 463–470.

Ellsworth D.S. & Reich P.B. (1993) Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia* **96,** 169–178.

Evans R.D., Black R.A. & Link S.O. (1990) Rehydration-induced changes in pressure-volume relationships of *Artemisia tridentata* Nutt. ssp. *tridentata*. *Plant, Cell and Environment.* **13,** 455–461.

Friend A.D. (1993) The prediction and physiological significance of tree height. In *Vegetation Dynamics and Global Change* (eds A.M. Solomon & H.H. Shugart), pp. 101–115. Chapman & Hall, New York, USA.

Gould G.W. & Measures J.C. (1977) Water relations in single cells. *Philosophical Transactions of the Royal Society of London, Serial B, Biology Sciences* **278,** 151–166.

Hale M.G. & Orcutt D.M. (1987) *The Physiology of Plants Under Stress*. John Wiley and Sons, New York, USA.

Hellkvist J., Richards G.P. & Jarvis P.G. (1974) Vertical gradients of water potential and tissue water relations in Sitka spruce trees measured with the pressure chamber. *Journal of Applied Ecology* **11,** 637–667.

Heuer B. & Plaut Z. (1989) Photosynthesis and osmotic adjustment of two sugarbeet cultivars grown under saline conditions. *Journal of Experimental Botany* **40,** 437–440.

Hsiao T.C., Acevedo E., Fereres E. & Henderson D.W. (1976) Stress metabolism; water stress, growth and osmotic adjustment. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences* **273,** 479–500.

Krueger K.W. & Trappe J.M. (1967) Food reserves and seasonal growth in Douglas-fir seedlings. *Forest Science* **2,** 192–202.

Kubiske M.E. & Abrams M.D. (1991) Rehydration effects on pressure-volume relationships in four temperate woody species: variability with site, time of season and drought conditions. *Oecologia* **85,** 537–542.

Magnani F., Mencuccini M. & Grace J. (2000) Age-related decline in stand productivity: the role of structural acclimation under hydraulic constraints. *Plant, Cell and Environment* **23,** 251–263.

Marshall J.D. & Monserud R.A. (2003) Foliage height influences specific leaf area of three conifer species. *Canadian Journal of Forest Research* **33,** 164–170.

McDowell N.G., Phillips N., Lunch C., Bond B.J. & Ryan M.G. (2002) An investigation of hydraulic limitation and compensation in large, old Douglas-fir trees. *Tree Physiology* **22,** 763–774.

McNulty I.B. (1985) Rapid osmotic adjustment by a succulent halophyte to saline shock. *Plant Physiology* **78,** 100–103.

Meinzer F.C. (1982) The effect of light on stomatal control of gas exchange in Douglas-fir (*Pseudotsuga menziesii*) saplings. *Oecologia* **54,** 270–274.

Meinzer F.C., Rundel P.W., Sharifi M.R. & Nilsen E.T. (1986) Turgor and osmotic relations of the desert shrub *Larrea tridentata*. *Plant, Cell and Environment* **9,** 467–475.

Morgan J.M. (1984) Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiology* **35,** 299–319.

Niinemets Û. & Tenhunen J.D. (1997) A model separating leaf structure and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer sacharum*. *Plant, Cell and Environment* **20,** 845–866.

Osonubi O. & Davies W.J. (1978) Solute accumulation in leaves and roots of woody plants subjected to water stress. *Oecologia* **32,** 323–332.

Parker G.G., Davis M.M. & Chapotin S.M. (2002) Canopy light transmittance in Douglas-fir-western hemlock stands. *Tree Physiology* **22,** 147–157.

Pereira J.S. & Pallardy S. (1989) Water stress limitations to tree productivity. In *Biomass Production by Fast-Growing Trees* (eds J.S. Pereira & J.J. Landsberg), pp. 37–56. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Phillips N., Bond B.J., McDowell N.G. & Ryan M.G. (2002) Canopy and hydraulic conductance in young, mature and old Douglas-fir trees. *Tree Physiology* **22,** 205–211.

Ranney T.G., Bassuk N.L. & Whitlow T.H. (1991) Osmotic adjustment and solute constituents in leaves and roots of water-stressed cherry (*Prunus*) trees. *Journal of the American Society for Horticultural Science* **4,** 684–688.

Ray P.M. (1987) Principles of plant cell expansion. In *Physiology of Cell Expansion During Plant Growth* (eds D.J. Cosgrove &

D.P. Knievel), pp. 1–17. American Society of Plant Physiologists, Rockville, MD, USA.

Reed K.L. & Waring R.H. (1974) Coupling of environment to plant response: a simulation model of transpiration. *Ecology* **55,** 62–72.

Rieger M. (1995) Offsetting effects of reduced root hydraulic conductivity and osmotic adjustment following drought. *Tree Physiology* **15,** 379–385.

Ritchie G.A. & Shula R.G. (1984) Seasonal changes of tissue-water Relations in shoots and root systems of Douglas-fir seedlings. *Forest Science* **30,** 538–548.

Ryan M.G. & Yoder B.J. (1997) Hydraulic limits to tree height and tree growth. *Bioscience* **47,** 235–242.

Scholander P.F., Hammel H.T., Bradstreet E.D. & Hemmington E.A. (1965) Sap pressure in vascular plants. *Science* **148,** 339–346.

Shaw D.C., Franklin J.F., Bible K., Klopatek J., Freeman E., Greene S. & Parker G.G. (2004) Ecological setting of the Wind River old-growth forest. *Ecosystems* in press.

Sprugel D.G., Brooks J.R. & Hinckley T.M. (1996) Effects of light on shoot geometry and needle morphology in *Abies amabilis. Tree Physiology* **16,** 91–98.

Turner N.C. & Jones M.M. (1980) Turgor maintenance by osmotic adjustment: A review and evaluation. In *Adaptation of Plants to Water and High Temperature Stress* (eds N.C. Turner & P.J. Kramer), pp. 87–103. Wiley-Interscience, New York, USA.

Tyree M.T., Cheung Y.N.S., MacGregor M.E. & Talbot A.J.B. (1978) The characteristics of seasonal and ontogenic changes in the tissue-water relations of *Acer*, *Populus*, *Tsuga*, and *Picea. Canadian Journal of Botany* **56,** 635–647.

Tyree M.T. & Hammel H.T. (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *Journal of Experimental Botany* **23,** 267–282.

Yoder B., Ryan M.G., Waring R.H., Schoettle A.W. & Kaufmann M.R. (1994) Evidence of reduced photosynthetic rates in old trees. *Forest Science* **40,** 513–527.

Zimmermann M.H. (1983) *Xylem Structure and the Ascent of Sap*. Springer-Verlag, Berlin, Germany.