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Biophysical constraints on leaf expansion in a tall conifer

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Summary The physiological mechanisms responsible for reduced extension growth as trees increase in height remain elusive. We evaluated biophysical constraints on leaf expansion in old-growth Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) trees. Needle elongation rates, plastic and elastic extensibility, bulk leaf water (W_t) and osmotic (F_n) potential, bulk tissue yield threshold and final needle length were characterized along a height gradient in crowns of > 50-m-tall trees during the period between bud break and full expansion (May to June). Although needle length decreased with increasing height, there was no height-related trend in leaf plastic extensibility, which was highest immediately after bud break (2.9%) and declined rapidly to a stable minimum value (0.3%) over a 3-week period during which leaf expansion was completed. There was a significant positive linear relationship between needle elongation rates and plastic extensibility. Yield thresholds were consistently lower at the upper and middle crown sampling heights. The mean yield threshold across all sampling heights was 0.12 ± 0.03 MPa on June 8, rising to 0.34 ± 0.03 MPa on June 15 and 0.45 ± 0.05 MPa on June 24. Bulk leaf Ψ_n decreased linearly with increasing height at a rate of 0.004 MPa m^{-1} during the period of most rapid needle elongation, but the vertical osmotic gradient was not sufficient to fully compensate for the 0.015 MPa m^{-1} vertical gradient in Ψ_L, implying that bulk leaf turgor declined at a rate of about 0.011 MPa m^{-1} increase in height. Although height-dependent reductions in turgor appeared to constrain leaf expansion, it is possible that the impact of reduced turgor was mitigated by delayed phenological development with increasing height, which resulted in an increase with height in the temperature during leaf expansion.

Keywords: Douglas-fir, osmotic adjustment, phenology, Pseudotsuga menziesii, tissue extensibility, tree height, turgor.

Introduction

The growth of trees changes dramatically as they mature. Annual height growth of a 50-year-old Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco), for example, averages about one-third less than that of the same tree at age 10 growing in an identical environment, and reductions in annual height increment continue at a rate of about 2 cm m^{-1} of height (Ishii et al. 2000, Bond et al. 2007). Leaves typically become thicker and smaller as trees grow older and larger (Ryan et at. 2006), and new shoots become shorter and thicker. These changes have been evaluated from many perspectives, including developmentally controlled changes in gene expression (Day et at. 2001), tradeoffs between competition for light and mechanical stability (King 1990), interactions between physiological and structural attributes (Ford 1992), allometric constraints on tree form and function (Cannell and Dewar 1994, Niklas 1995, Enquist 2002) and physiological changes associated with increasing tree size (Ryan and Yoder 1997. Magnani et al. 2002, Koch et at. 2004, Mencuccini et al. 2005, Ryan et al. 2006), but the underlying physiological mechanisms responsible for these developmental changes remain elusive.

Nevertheless, recent research has considerably narrowed the field of possible mechanisms for the change in growth characteristics with increasing age. Recent grafting experiments show, for example, that the characteristic decline in height growth in aging trees is a function of tree size rather than meristem age (Mencuccini et at. 2005, Bond et at. 2007), although it is possible that ontogenetic changes in shoot meristems may impact leaf development (Bond et al. 2007). Many studies have demonstrated that trees may become "hydraulically challenged" as they grow larger, and reduced hydraulic conductance with increased height often results in lower stomatal conductance and carbon assimilation (Ryan et at. 2006). However, measured reductions in photosynthesis at the leaf level-whether from hydraulic limitations or other causes-are insufficient alone to explain the observed rates of growth decline as trees grow older and larger (Ryan et al. 2006, Bond et al. 2007, Greenwood et al. 2008). There is increasing evidence that the growth of tall trees is not limited by carbon supply (Minter et al. 2005) and that growth of new shoots is limited by other factors, which, in turn, reduce shoot "sink strength" for carbon (Ryan et at. 2006).

Reduced turgor during cell expansion has been implicated as a potential mechanism contributing to reduced extension growth and increased thickening of stems and leaves in large trees (Thomas and Winner 2002, Niinemets 2002, Marshall and Monserud 2003). Woodruff et at. (2004) proposed that the vertical gradient in the gravitational component of water po-

tential results in a similar gradient in cell turgor, unless there is a compensatory change in cell osmotic potential with tree height. Experimental tests revealed no variation in cell osmotic potential of foliar mesophyll cells with increasing tree height during bud break and stem elongation in Douglas-fir (Woodruff et al. 2004). Thus, to the extent that vertical gradients in turgor of mesophyll cells reflect conditions in apical initial cells, and that turgor controls the expansion and division of new cells, height-related differences in stem elongation may be a direct consequence of the vertical water potential gradient in trees. This hypothesis predicts an inverse relationship between tree height and elongation of new stems, as observed. The hypothesis also predicts changes in growth that are independent of carbon supply, although reduced growth may result in reduced sink strength in growing shoots, which could then feed back to changes in carbon assimilation or allocation.

One important test of this hypothesis is to examine the relationships between turgor and growth. The current study begins that examination with a focus on the biophysics of cell expansion. Biophysical controls on cell expansion have traditionally been analyzed within the framework of a set of growth equations formulated by Lockhart (1965). The simplest equation relates the rate of irreversible increase in cell volume to turgor and wall yielding properties:

$$\frac{dV}{Vdt} = m(\Psi_v - Y) \quad (1)$$

where dV/Vdt is growth rate normalized by the volume of the cell, m is volumetric plastic extensibility of the cell wall, is turgor pressure and Y is the minimum turgor or "yield threshold" necessary for expansion. Sustaining a steady-state growth rate requires continuous transport of water into the cell so:

$$Vdt \quad m_{+L} \quad (WIC - Y) \quad (2)$$

where L is cell hydraulic conductance and is the difference in osmotic potential between the inside and outside of the cell, which is equivalent to turgor if reflection coefficients for solutes are =1. In isolated cells and simple tissues, L may be large relative *to m* and its effect on rates of cell expansion may be negligible (Frensch and Hsiao 1995, Hsiao and Xu 2000). It has been suggested, however, that L can impose a major limitation on rates of expansion in complex tissues such as leaves and stems (Nonami and Boyer 1989, 1990, Schultz and Matthews 1993). In these cases, water entry apparently does not keep pace with continuous cell wall relaxation, resulting in reduced turgor in the growing zone and, therefore, a substantial water potential disequilibrium between adjacent regions of growing and non-growing tissue, the so-called growth-induced water potential that is necessary to sustain water uptake by expanding cells (Boyer et al. 1985). However, in other investigations of the origin of growth-induced water potentials it has been concluded that they are attributable largely to the presence of apoplastic solutes (Cosgrove and Cleland 1983, Meinzer and Moore 1988). In either case, even though wall relaxation creates the initial driving force for water uptake by lowering the turgor of expanding tissue, a continuous supply of solutes to the growing zone is necessary to maintain turgor above the yield threshold.

Because Lockhart's equations were developed to describe growth of individual cells, several ambiguities and uncertainties arise when applying them to the growth of complex tissues such as leaves and stems. These constraints are both geometrical and methodological in origin. For example, although m was originally defined as a three-dimensional property, "extensibility" is frequently estimated by applying a unidirectional force to a tissue. This is justifiable because anisotropic organization of cellulose microfibrils forces cell expansion along a pre-determined axis (Darley et al. 2001); in other words, m is unequally distributed across the cell surface. But the mathematical relationships among volumetric or linear expansion, wall pressure and wall extensibility are more complex than suggested by the simple expressions in Equations 2 and 3. Furthermore, use of thermocouple psychrometry to measure `Y and turgor of excised growing tissue will result in underestimates of 1' and turgor, because both decline as walls continue to relax during the time required for vapor equilibration in the psychrometer chamber. These and other factors can make it difficult to distinguish between changes in m and Yin complex tissues. Despite these and other constraints, some success has been achieved in developing techniques that permit partitioning of the relative influences of turgor, extensibility and yield threshold on growth of whole tissues (Cosgrove et al. 1984, Hsiao and Xu 2000). Examples include use of extensiometers to estimate relative extensibility of living and killed tissue (Cleland 1967, Van Volkenburgh et al. 1983, Metcalfe et al. 1991, Lu and Neumann 1998), use of the pressure block technique to estimate Y(Cosgrove 1987), use of the pressure jump method to estimate m and Y (Okamoto et al. 1989), and use of non-permeating osmotica to rapidly change the of the solution surrounding growing tissues (Hsiao and Jing 1987, Frensch and Hsiao 1994).

In this study, we used an extensiometer to measure relative cell wall extensibility and a pressure chamber technique (Sands et al. 1992) to estimate yield threshold in newly expanding foliage of Douglas-fir collected from different heights above the ground and at frequent intervals during the period between bud break and full expansion. We studied the same trees as studied by Woodruff et al. (2004) in order to build on the knowledge gained in that study of vertical gradients in turgor during the period of stem elongation and leaf expansion. Our objective was to evaluate the relative roles of extensibility, yield threshold and osmotic adjustment in controlling new tissue growth.

Materials and methods

Study site and plant material

The study took place between May and July 2005 at the Wind River Canopy Crane Research Facility (WRCCRF), Wind River Experimental Forest, in southwest Washington State

(45°49'14" N, 121°57'7" W, 371 m a.s.l.), where the annual precipitation is 2223 mm, of which < 10% falls during June through September, and annual temperature is 8.7 °C (Shaw et al. 2004). The study trees were located in a 4-ha plot of old-growth (~450-year-old) Douglas-fir, western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and western red cedar (*Thuja plicata* Donn) forest under the canopy crane. In 2005, stand density was 427 trees ha^{-1} and basal area was 82.9 m^2 ha^{-1}. The Douglas-fir trees (35 ha^{-1}) in the stand had a mean height of 52 m and a mean diameter at breast height (DB H) of 1.11 m, and were from the original population that colonized the site after a stand-clearing fire.

Three Douglas-fir trees ranging in height from 56.8 to 59.0 m were selected for intensive study (Trees 2173, 3013, 3139). The WRCCRF contains a 75-m-tall crane with an 85-m jib that provided access to the crowns of the study trees via a suspended gondola. Sampling locations were established at three heights within the crown of each tree for a total of nine sampling locations. Sampling heights ranged from 32.2 to 57.4 m with means of 34.4 ± 1.9, 45.4 ± 1.2 and 56.8 ± 0.4 m for the lower-, middle- and upper-crown locations, respectively. Shoot growth of Douglas-fir is mainly determinate: the majority of leaf primordia of current-year growth being formed during the previous year and overwintered in dormant buds.

Leaf extensibility

Material for measurement of cell wall extensibility was collected on seven occasions at 6- to 13-day intervals between May 18 and July 7, 2005. May 18 was the earliest date on which bud break was sufficiently advanced at all sampling locations on the three study trees to yield needles long enough to permit reliable estimates of extensibility. Branch tips containing the entire current-year growth increment were excised between 0600 and 1200 h local time and immediately sealed in glass vials containing 70% (vlv) methanol. Before measurement of extensibility, individual needles were removed from the preserved shoot tips and rehydrated in distilled water for at least 4 h. Estimates of relative cell wall extensibility were obtained with an extensiometer (In-Spec 2200, Instron Corporation, Canton, MA) as described by Cleland (1967). With the portable extensiometer configured in a horizontal orientation, rehydrated needles were held with clamps placed 10 mm apart and extended twice to a load of 20 g (at 2 mm min^{-1}). Relative extensibility for each load-extension relationship was calculated according to Van Volkenburgh et al. (1983):

$$\% \text{ change in Ill 0 g load} = \frac{l_f - l_i}{21,} \cdot 100 \quad (3)$$

where If and l_i are the final and initial length of the sample, respectively. Plastic (irreversible) extensibility was calculated from the difference between the first (plastic + elastic) and the second (elastic) load-extension relationship. Typical plastic and elastic extension curves for rapidly elongating and non-elongating needles are shown in Figure 1. Values of i_f were calculated from the x-intercepts observed during the relaxation portion of the load-extension cycles. Extensibility was determined for five needles from each sampling location on the first five sampling dates. Measurements were not completed for the two remaining sampling dates because extensibility had already decreased to constant minimum values by the fourth and fifth sampling dates. Because estimates of extensibility depend in part on the cross-sectional area to which the force is applied, the influence of differences in needle cross-sectional area among individuals and sampling locations and dates was evaluated in a subset of 26 needles for which cross-sectional area was measured. When the original estimates of extensibility were plotted against values normalized by cross-sectional area, a strongly linear relationship was obtained ($r^2 = 0.93$, $P < 0.0001$) with a y-intercept not significantly different from zero. Based on these results, cross-sectional areas were not measured for the remaining samples and the values reported here represent the non-normalized extensiometer measurements.

Leaf water relations

Bulk leaf osmotic potential (`f') was determined with a vapor

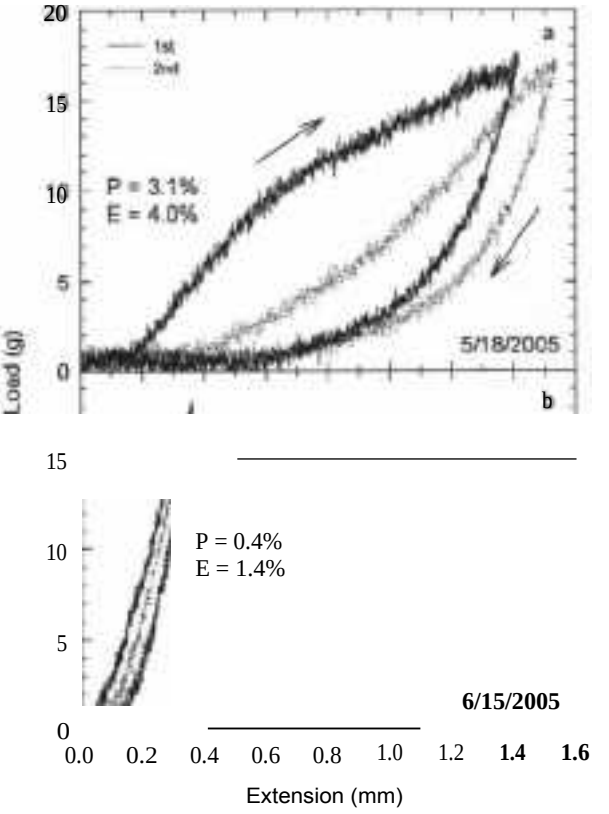

Figure 1. Typical first (combined plastic and elastic) and second (elastic) extension curves for (a) rapidly elongating and (h) fully elongated needles collected from the same tree at a height of 32.2 m on the dates shown. Relative plastic (P) and elastic (E) extensibilities are shown. Arrows in (a) indicate the trajectories of the load-extension curves. Initial needle lengths were > 10 mm, but extensiometer clamps were spaced 10 mm apart.

pressure osmometer (Vapro 5520, Wescor, Logan, UT) on sap expressed from frozen and thawed current-year needles collected at each sampling location. The needles were sealed in plastic vials immediately following removal from the trees and placed in a cooler for transport to the laboratory where they were stored at -20 °C until thawed for osmometric measurements. Data reported here are mean values of $'F_n$ for needles collected between 0610 and 1030 h local time on six occasions between May 18 and July 7. Needle elongation was negligible after June 8. The influence of variable tissue hydration on $'P_R$ of expressed sap was minimized by collecting needles when their natural hydration in situ was near maximal. Wet foliage was blotted dry and sealed in vials. Previous work conducted on foliage from Douglas-fir trees at this site showed that values of bulk leaf 'l' determined by vapor pressure osmometry and by the pressure-volume technique were similar (Woodruff et al. 2004).

The vertical gradient in $'P_L$ at dawn was estimated from measurements taken with a pressure chamber (PMS Instrument Company, Albany, OR) at the nine sampling locations on June 1, 15 and 24. The slope and intercept of a linear regression fitted to the data were -0.015 MPa m $^{-}$' and -0.29 MPa, respectively. The slope was similar to the vertical $'F_L$ gradient of about -0.017 MPa m ' previously reported in two independent studies conducted at the site in different years (Bauerle et al. 1999, Woodruff et al. 2004). Soil water content measured by frequency domain capacitance sensors positioned at six depths between 20 and 100 cm yielded a mean volumetric water content of 0.34 m3 m-3 in the upper 1 m of soil during the study period, which corresponds to a value of soil 'P indistinguishable from zero MPa as measured with soil psychrometers (Warren et al. 2005).

The yield threshold was estimated on June 8, 15 and 24 by the pressure chamber technique described by Sands et al. (1992). Pairs of small branches comprising at least two adjacent terminal shoots with current-year foliage were excised between 0610 and 0745 h and wrapped in aluminum foil and sealed in plastic bags immediately after excision. Within 1 h of excision, initial values of $'F_L$ were measured with a pressure chamber on one set of samples, and the remaining samples were incubated at about 20 °C for 12 h to allow turgor to relax to the yield threshold. The samples were then unwrapped for a final determination of $"F_L$ after which the current-year needles were removed, sealed in plastic vials and frozen and thawed for determination of $'F_R$ on expressed sap. The yield threshold turgor was estimated as the difference between $'P_R$ and $'P_L$ measured after turgor relaxation. Two to five usable measurements were obtained for each mean sampling height on each of the three sampling dates.

Leaf growth

Lengths of a minimum of five current-year needles from each sampling location were measured with digital calipers on seven occasions between May 18 and July 7, 2005. The needles were obtained from shoot tips preserved in methanol for extensibility analyses. Time courses of needle extension were generated for the lower, middle and upper crown by calculating the overall mean needle lengths of the three study trees at the corresponding sample locations.

Data analysis

Differences in tissue biophysical properties among the three mean sampling heights on a given date were evaluated by one-way analysis of variance (ANOVA). Vertical trends in $'P_R$, $'F_L$ and needle length across the nine sampling heights were assessed by linear regression analysis with each sampling location taken as the sampling unit and the values for individual needles considered as subsamples.

Results

Bulk leaf $'P_R$ decreased linearly with increasing height at a rate of -0.004 MPa m^{-}' ($r^2 = 0.47$, $P = 0.04$) during the period of most rapid needle elongation (Figure 2a). However, the vertical osmotic gradient was insufficient to fully compensate for the 0.015 MPa m^{-}' vertical gradient in $'F_L$ ($r^2 = 0.58$, $P < 0.02$), implying that bulk leaf turgor declined at a rate of about 0.011 MPa m^{-}' increase in height. The final length of fully expanded needles also decreased linearly with increasing height ($r^2 = 0.89$, $P < 0.001$; Figure 2b).

Load-extension relationships were distinct for rapidly elongating versus fully elongated needles (Figure 1). On the earliest sampling date (May 18), the irreversible component of extension was readily visible as a displacement of the two consecutive load-extension curves (Figure la). By the last sampling date (June 15), the needles were considerably less exten-

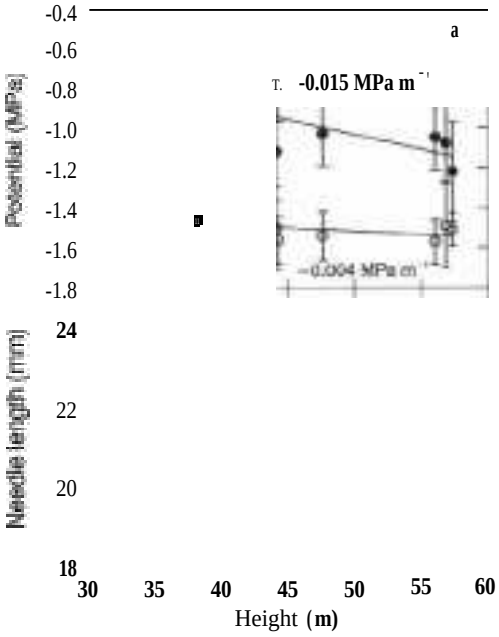

Figure 2. Height-dependent variation in (a) bulk leaf osmotic potential (T_R) and water potential ($'P_L$) of current-year needles in May and June, and (b) length of fully expanded needles in June and July.

sible, and the two load-extension curves appeared to be nearly superimposed, indicating that the plastic component of extensibility was near zero (Figure lb). Plastic extensibility declined by an order of magnitude, from about 3% to 0.3%, during the 4 weeks from May 18 to June 15 (Table I). There were no significant differences in plastic extensibility among the three mean sampling heights on any date, indicating no consistent height-related trends in extensibility. Nevertheless, the time dependence of plasticity suggested that phenological development of needles sampled at a mean height of 34.4 m may have been more advanced than that of needles at 45.4 and 56.8 m, because at 34.4 m, plasticity had declined to stable minimum values by June 1, but continued to decline steadily through June 15 at 45.4 and 56.8 m (Table 1).

Based on the results in Table 1, a single sigmoid function was fitted to the data from all three mean sampling heights to describe the time course of plastic extensibility over the four-week measurement period (Figure 3a). Extensibility decreased by about 17% during the 6 days between the first two sampling dates, then dropped precipitously (80%) during the subsequent 8 days, remaining nearly constant at low values for the next 2 weeks. Height-dependent trends in needle growth were evident at the earliest sampling date when needles at 34.4 m were already 2.8 mm longer than needles at 56.8 m (Table 2). However, the general trajectories of the time courses of needle elongation at the three mean heights were similar. The mean time course of needle elongation for the three sampling heights (Figure 3b) showed a general association between elongation rate and plastic extensibility, with needles having nearly reached their maximum length by the time extensibility had fallen to its minimum value. Bulk leaf T_{it} declined by about 0.6 MPa over the 50-day interval between the first and last sampling dates (Figure 3c). The rate of change in $'1'_n$ was highest before cessation of needle elongation. Needle elongation rates and needle plastic extensibility were strongly correlated ($r^2 = 0.91$, P = 0.01) between May 18 and June 15 (Figure 4). Needle elongation rates were calculated as the instantaneous slope of the curve in Figure 3b on the dates when needle length and extensibility were measured.

Yield thresholds were consistently lower at the upper- and middle-crown sampling heights (P = 0.03) and increased sharply between June 8 and 24 (Figure 5). The mean yield threshold across all sampling heights was 0.12 ± 0.03 MPa on June 8, rising to 0.34 ± 0.03 MPa on June 15 and 0.45 ± 0.05 MPa on June 24.

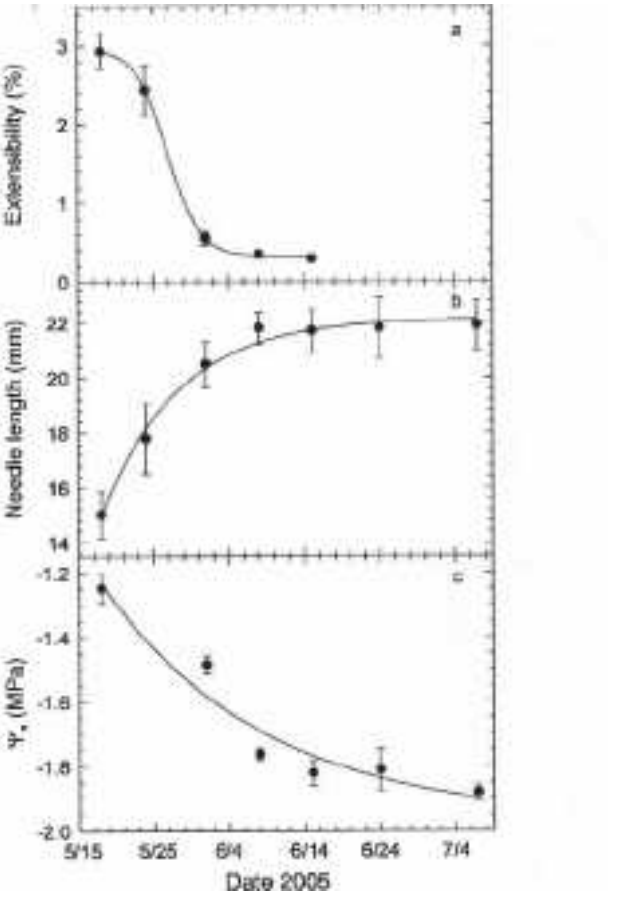

Figure 3. Time courses of mean (± SE) (a) needle plastic extensibility, (b) needle length and (c) bulk leaf osmotic potential ('Pn). Means were obtained from values for the three mean sampling heights shown in Tables 1 and 2.

Discussion

Plastic extensibility of leaves did not differ with height during the study, but decreased rapidly after bud break in a similar fashion at all heights, limiting the duration of rapid leaf expansion to about 3 weeks. It was unlikely that the yield threshold for tissue expansion played an important role in height-dependent variation in leaf growth, because it remained low (-0.1 MPa) at all heights at the end of rapid leaf elongation and was likely to have been even lower when leaves were elon-

Table 1. Elastic and plastic extensibility (% per 10 g load) of Douglas-fir needles in relation to sampling date (in 2005) and height. Values are means of three sampling locations ± SE.

Date	Day of year	Elasticity			Plasticity		
		56.8 m	45.4 m	34.4 m	56.8 m	45.4 m	34.4 m
May 18	138	4.08 ± 0.28	3.79 ± 0.27	4.10 ± 0.17	3.37 ± 0.35	2.70 ± 0.90	2.72±0.47
May 24	144	3.32±0.10	4.00±0.16	3.73±0.19	1.86 ± 0.20	2.95 ± 0.73	2.52±0.67
June 1	152	2.16 ± 0.05	2.29 ± 0.13	1.91 ± 0.10	0.59 ± 0.06	0.67 ± 0.25	0.40 ± 0.09
June 8	159	1.61 ± 0.06	1.45 ± 0.05	1.69 ± 0.07	0.34 ± 0.09	0.35 ± 0.05	0.37 ± 0.06
June 15	166	1.23 ± 0.06	1.27 ± 0.06	1.32 ± 0.07	0.27 ± 0.06	0.25 ± 0.01	0.35±0.05

Table 2. Length and osmotic potential of Douglas-fir needles in relation to sampling date (in 2005) and height. Values are means of three sampling locations ± SE.

Date	Day of year	Length (mm)			Osmotic potential (MPa)		
		56.8 m	45.4 m	34.4 m	56.8 *m*	45.4 *m*	34.4 *m*
May 18	138	13.3 ± 1.1	15.5 -0.7	16.1±0.9	-1.30±0.07	-1.29±0.09	-1.16±0.05
May24	144	15.4±1.0	18.0 ± 1.1	19.9 - 1.6	-	-	-
June 1	152	18.9 ± 0.3	20.7 ± 0.6	21.8±0.6	-1.52±0.06	-1.51±0.04	-1.43±0.02
June 8	159	20.8 ± 1.1	21.8 t 1.1	22.8 t 1.6	-1.75 t 0.08	-1.80 ± 0.02	-1.74 ± 0.02
June 15	166	20.2 ± 2.4	22.0 ± 4.1	22.9 ± 0.6	-1.88-0.04	-1.84 ± 0.01	-1.74 ± 0.02
June 24	175	19.7 ± 0.2	22.61 1.0	23.2-0.5	-1.87±0.03	-1.88 ± 0.08	-1.68 ± 0.04
July 7	188	20.5 ± 0.5	21.6 ± 0.6	23.6 ± 1.7	-1.93 ± 0.00	-1.8.5 ± 0.02	-1.87 ± 0.04

gating rapidly (Metcalfe et al. 1991). The steeper vertical gradient in $'P_L$ than in $'F_n$ (Figure 2a) was consistent with earlier findings of Woodruff et al. (2004) working at the same site and is suggestive of a vertical gradient of declining turgor with increasing height. However, the impact of reduced turgor on leaf growth may have been partially mitigated by delayed phonological development in the upper canopy, which would displace leaf expansion toward periods of potentially warmer and therefore more favorable temperatures slightly later in the spring.

Biophysical control of leaf growth

Although Lockhart's equations (Lockhart 1965) were originally formulated to model the irreversible expansion of single cells, they have proved useful for identifying relative biophysical constraints on expansive growth of complex plant tissues and organs. Plastic extensibility, turgor and yield turgor are represented as constants for short-term analyses of growth rates; however, these three biophysical properties can undergo rapid adjustments in concert with changing environmental conditions. Inferences concerning their relative importance in controlling growth rates depend largely on the plant organ studied, the environmental stress experienced, and the conditions under which stress is imposed. In addition, reduced tissue hydraulic conductivity may appreciably constrain rates of tissue expansion by steepening the 'F gradient required to sustain water uptake by expanding cells. Although we did not measure the hydraulic conductivity of expanding tissues, previous studies of vertical trends in tracheid structure (Domec et at. 2006) and leaf hydraulic architecture (Woodruff et al. 2007) in Douglas-fir suggest that localized hydraulic constraints on water uptake by expanding tissue may increase with height and need to be taken into account when interpreting height-dependent trends in shoot expansion.

Previous research on biophysical control of leaf growth has focused mainly on herbaceous crop species. Studies on growth regulation of intact shoots of field-grown woody plants are relatively scarce (e.g., Taylor and Davies 1986, Roden et al. 1990, Metcalfe et al. 1991, McDonald et al. 1992, Taylor et al. 2003). To our knowledge, the present study represents the first attempt to assess concurrently the relative importance of more than one biophysical tissue property in regulating shoot growth of a conifer. In expanding Douglas-fir needles, turgor declines linearly with increasing height above the ground (Woodruff et al. 2004), but no consistent vertical trend in plastic extensibility was observed in our study, and the yield threshold remained low near the end of rapid needle growth (June 8). These findings extend those of Woodruff et al. (2004) for Douglas-fir and are in agreement with those of Metcalfe et

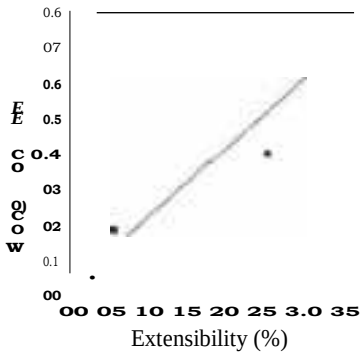

Figure 4. Relationship between mean needle elongation rate and needle plastic extensibility for the five sampling dates on which extensibility was measured.

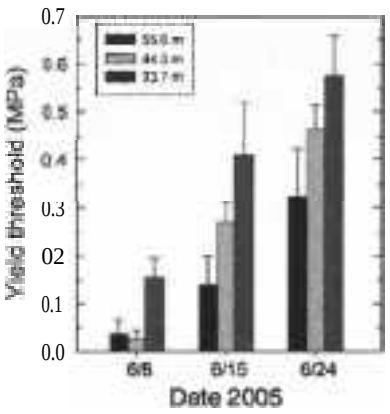

Figure 5. Mean bulk tissue yield thresholds (± SE) at the three mean sampling heights during June 2005.

al. (1991) who reported low yield thresholds and linear relationships between growth rate and turgor in rapidly expanding *Eucalyptus globulus* Labill. leaves. Nevertheless, other studies have pointed to dominant roles for plastic extensibility and yield turgor in regulating leaf expansion, especially under conditions in which water stress is rapidly or transiently imposed by altering the external osmotic environment or transpiration rate (Shackel et al. 1987, Roden et al. 1990, Lu and Neumann 1998, Hsiao and Xu 2000). Experiments in which transient water stress is rapidly imposed to study mechanisms underlying dynamic perturbations in growth rates do not create conditions analogous to the permanent vertical gradient of $'F_L$ in tall trees. In Douglas-fir, a marked vertical gradient in '1' prevails, whereas leaf expansion is completed during a brief 3-4-week period in late spring when rainfall is typically frequent, the soil is saturated, and temperatures are relatively low. Under these conditions, potential compensatory adjustments that would stabilize rates of leaf expansion with increasing height include increased plastic extensibility, reduced yield turgor and turgor maintenance by osmotic adjustment. However, plastic extensibility did not differ significantly with height, and although the yield threshold tended to be lower in the upper crown, it was not likely to have imposed a substantial limitation on elongation at any height when elongation rates were most rapid.

Although osmotic adjustment did not fully compensate for the vertical gradient in $'F_L$ (Figure 2a), it may have partially compensated for the rapid decline in plastic extensibility during the study period by generating higher turgor at all heights than would have prevailed in the absence of osmotic adjustment (Figure 3c). Nevertheless, bulk tissue W_l at the end of the study period was substantially less negative than that in mature Douglas-fir needles (Ritchie and Shula 1984, Kubiske and Abrams 1991, Woodruff et al. 2004), suggesting that turgor increases gradually during needle maturation.

Osmotic adjustment and turgor maintenance

There was a narrow time window for osmotic adjustment to have an impact on turgor-driven leaf expansion, because tissue extensibility decreased rapidly after bud break (Figure 3a). Seasonal trends in leaf osmotic potential that seem to be related to phenological cycles rather than to seasonal development of water stress have been noted in other woody species (Tyree et al. 1978, Hinckley et al. 1983, Kubiske and Abrams 1991). In Douglas-fir and other conifers, the osmotic solute content of mature leaves begins to decrease during late winter and early spring, reaching minimum values in late spring coincident with the flushing of new foliage, then increases throughout the remaining spring and summer months (Tyree et al. 1978, Ritchie and Shula 1984). The small vertical gradient in osmotic potential during rapid needle elongation was insufficient to compensate for the larger vertical gradient in $'F_L$ (Figure 2a), implying that bulk leaf turgor decreased at a rate of about 0.011 MPa $m^{-'}$ increase in height. Woodruff et al. (2004) working at the same study site reported vertical gradients of turgor in May and June from 0.009 to 0.015 MPa $m^{-'}$ depending on the year, with osmotic adjustment reducing the turgor gradient to about 0.0055 MPa m^{-1} by July, well after the needles had stopped elongating. Similarly, height-related osmotic adjustment in mature leaves of tall *Sequoia sempervirens* (D. Don) Endl. was insufficient to compensate for the vertical gradient in $'F_L$, causing turgor to decline linearly with height at a mean rate of about 0.006 MPa $m^{-'}$ (Koch et al. 2004). Extrapolation of the regression lines in Figure 2a to their intersection yields a height of about 92 m at which $'P_1 = 'P_{,,}$, implying that bulk turgor of expanding leaves would be zero. The tallest Douglas-fir tree at the study site was about 65 m and historical maximum reported heights for Douglas-fir in the study region are about 102 ± 2 m (Carder 1995). Site-specific variation around this height to an absolute maximum height of 126 m recorded for an individual found in British Columbia (Carder 1995) implies that the impact of reduced turgor on extension growth may be determined by site-specific variation in vertical gradients in osmotic and water potential.

The gradual osmotic changes in developing Douglas-fir leaves contrast with the rapid, water stress-induced osmotic adjustment in roots of herbaceous species that allows partial recovery of turgor and contributes to resumption of growth (Hsiao and Xu 2000). Water-stress-induced osmotic adjustment is somewhat slower in leaves than in roots, but can eventually lead to complete turgor maintenance (Michelena and Boyer 1982, Van Volkenburgh and Boyer 1985). However, turgor maintenance in leaves may not restore growth to prestress rates if tissue extensibility and yield turgor have changed (Roden et al. 1990, Lu and Neumann 1998). The rapid decline and slow recovery of osmotic solute content in conifer foliage concurrent with seasonal phenological cycles of bud swelling and flushing and maturation of new foliage is suggestive of significant physiological constraints on the availability and supply of solutes to generate turgor pressures sufficient to drive expansive growth at maximal rates. Scarcity of organic solutes such as sugars is likely to have a synergistic negative impact on growth because of their dual roles as osmotica and raw material for cell wall synthesis. For example, when buds swell in Douglas-fir, sugars are metabolized more rapidly than they are produced and therefore tend to be limited (Krueger and Trappe 1967, Billow et al. 1994). Billow et al. (1994) found that sugar concentrations in Douglas-fir foliage *nearly* doubled between May and October. Inorganic osmotica such as potassium, unless they are sequestered in compartments from which they can be rapidly released and translocated to developing foliage, must be taken up from the soil and moved in the transpiration stream to the upper canopy, a process that would require at least 2 weeks in the tallest Douglas-fir trees at the study site (Meinzer et al. 2006). As nearly all needle expansion was confined to a period of 3 weeks or less, the soil reservoir was unlikely to have been a significant source of solutes driving short term osmotic adjustment. The nature of the constraints on osmotic adjustment in expanding Douglas-fir leaves remains speculative until the sources and identities of osmotic solutes are characterized. Nevertheless, our results indicate that constraints on the availability of solutes for osmotic adjustment increase with increasing tree height.

Phenology

Douglas-fir leaves quickly reached their maximum size between May and June, during which time tissue plastic extensibility dropped precipitously and bulk tissue $\grave{f}_n$ was about 0.7 MPa less negative than the value of -2.5 MPa typical for mature current-year needles in summer (Woodruff et al. 2004). Although we made no detailed observations of vegetative bud phenology, two independent lines of evidence pointed to increasingly delayed bud break with increasing height, which could lead to height-dependent temporal displacement of the periods of most active leaf expansion. First, needle plastic extensibility at 34.4 m declined to a stable minimum value by June 1 and did not decrease significantly between June 1 and the last measurement date on June 15, whereas needle extensibility at 56.8 m decreased significantly ($P = 0.02$) between June 1 and 15 (Table 1). Second, the mean yield threshold for June 8, 15, and 24, after elongation had essentially ceased, was 0.2 MPa greater at 34.4 m than at 56.8 m ($P = 0.03$), implying that phenological development was more advanced lower down. Delayed phenological development with increasing height may have mitigated the impact of reduced turgor on leaf expansion by displacing leaf expansion toward periods of higher temperatures somewhat later in spring. Consistent with this hypothesis, McDonald et al. (1992) observed that extension rates of *Salix viminalis* L. leaves were greater during the day when temperature was higher and turgor lower than at night when the temperature was lower and turgor higher. In our study, mean maximum air temperature was 13.2 °C during the week centered on the first sampling date on May 18 and 16.1 °C during the week centered on the June 8 sampling date. Thus, an interaction between phenology, osmotic adjustment and turgor in determining leaf expansion as a function of height seems probable. The causes of the vertical gradient in timing of phenological development are unknown, but turgor is likely to be involved.

Conclusion

It is likely that biophysical constraints on stem and leaf extension in Douglas-fir are similar. A previous study conducted on Douglas-fir demonstrated a highly significant linear decline in annual branch elongation with increasing height between 13.5 and 56.5 m (Woodruff et al. 2004). Therefore, our results may provide insights into the underlying causes of the well-known decline in height growth rates as Douglas-fir trees increase in size. In the absence of height-related trends in extensibility and, probably, yield turgor of growing tissue, future work should focus on environmental and physiological factors that limit osmotic adjustment along a height gradient during the relatively brief interval in spring when developing tissues are plastic and elongation is likely to be a linear function of turgor. This approach would provide further insights into the mechanisms governing site-specific rates of height growth and maximum heights. It is not known whether biophysical limitations on extension growth are similar among coniferous species, but Douglas-fir's status as one of the tallest tree species on earth makes it an appropriate model for studying the nature of limits on tree height.

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