

Water stress, shoot growth and storage of non-structural carbohydrates along a tree height gradient in a tall conifer

DAVID R. WOODRUFF & FREDERICK C. MEINZER

USDA FOREST SERVICE, FORESTRY SCIENCES LABORATORY, CORVALLIS, OR 97331, USA

ABSTRACT

We analysed concentrations of starch, sucrose, glucose and fructose in upper branch wood, foliage and trunk sapwood of Douglas-fir trees in height classes ranging from ~2 to ~57 m. Mean concentrations of non-structural carbohydrates (NSC) for all tissues were highest in the tallest height class and lowest in the lowest height class, and height-related trends in NSC were most pronounced in branches. Throughout a 17-month sampling period, mean values of branch NSC from the 57 m trees ranged between 30 and 377% greater than the 2 m trees. Branch NSC was inversely correlated with midday shoot water potential (Ψ_l), shoot osmotic potential at full turgor (Ψ_π) and shoot extension. Temporal fluctuation in branch NSC was inversely correlated with height, and positively correlated with midday Ψ_l , Ψ_π and shoot extension. The positive correlation between height and storage of NSC, and the negative correlation between NSC storage and shoot extension provide evidence that size-related growth decline in trees is not strongly associated with constraints on photosynthesis. The negative correlation between height and fluctuation in NSC suggests that mobilization of photosynthate in taller trees is constrained by some factor such as reductions in turgor-driven cell expansion or constraints on phloem transport.

Key-words: *Pseudotsuga menziesii*; carbon storage; drought; growth limitation; phloem transport; photosynthesis; water potential.

INTRODUCTION

Water availability is widely recognized as a primary constraint on productivity of forest ecosystems (Breshears *et al.* 2005; Bréda *et al.* 2006). Drought has been implicated as the cause of large-scale decline in temperate forests (Allen 2009; van Mantgem *et al.* 2009), and global climate change has been predicted to increase the intensity and frequency of drought in many regions (IPCC 2007), including the Pacific Northwest of the United States (Mote & Salathé 2009). The effect of water stress on plant photosynthesis has

been studied extensively and stomatal constraints on photosynthesis have become perhaps the most widely recognized physiological driver for reduced productivity under conditions of drought. However, water availability influences a number of other processes involved in tree growth, including cell expansion, phloem transport, nutrient transport and metabolic function. Each of these processes is critically important for growth and each represents a potential physiological bottleneck for productivity if water availability is limited. In order to more accurately predict tree and forest productivity under current and future climate regimes, the mechanistic relationships between drought and growth must be more fully understood.

Understanding the relationships between carbon supply and demand is important when investigating constraints on tree growth. Plants rely on both current photosynthesis and stored reserves of non-structural carbohydrates (NSC) for growth and other physiological functions. However, the extent to which trees are able to draw upon stored NSC has been questioned (Chapin, Schulze & Mooney 1990; Millard, Sommerkorn & Grelet 2007). When photosynthesis produces more carbon than is used for processes such as growth, reproduction and defence, some carbon is stored in the form of NSC. The concentrations of NSC in a tree are therefore indicative of the balance between carbon that has been fixed and the carbon that has been utilized for growth and metabolism or exuded below ground. The recurrent processes of assimilation, growth, reproduction and sometimes defence, result in periodic changes in the availability of, and demand for, mobile carbon. As such, concentrations of NSC in plants often show seasonal fluctuations (Fischer & Höll 1992; Newell, Mulkey & Wright 2002). Stored NSC potentially decouples assimilation from the processes in plants that require carbon, thereby facilitating growth and function during stressful conditions and recovery following disturbance (Iwasa & Kubo 1997). The fluctuating nature of the availability and demand for mobile carbon suggests that increased levels of NSC concentrations, particularly at any single moment, do not necessarily reflect an abundance of carbon. For example, NSC concentrations may increase as part of a regular annual cycle of accumulating reserves that later become largely depleted during leaf flush. Isolated measurements of NSC can therefore lead to an incomplete understanding of the carbon status of a tree and of the

Correspondence: D. R. Woodruff, USDA Forest Service, PNW Research Station, 3200 SW Jefferson Way, Corvallis, OR 97331, USA; e-mail: dwoodruff@fs.fed.us

temporal dynamics of mobile carbon in trees. When evaluating the extent to which a tree is carbon limited, or the relative carbon limitations in different groups of trees, it is important to evaluate storage of NSC repeatedly throughout the year, particularly before and after key phenological events.

In a given environment, water stress increases with tree height. In the absence of transpiration, the gravitational component of water potential (Ψ) leads to a xylem tension gradient of 0.01 MPa m^{-1} increase in height (Scholander *et al.* 1965); and frictional resistance during transpiration leads to an additional path-length dependent reduction in Ψ (Mencuccini & Grace 1996; Ryan *et al.* 2000). Thus, an increase in tree height is in some ways comparable with occupying drier portions of an aridity gradient. Age and size-related reductions in productivity in forest stands and individual trees following canopy closure is a well-documented phenomenon (Jarvis & Leverenz 1983; Ryan & Waring 1992; Ryan, Binkley & Fownes 1997). Much of the research investigating constraints on growth associated with increased tree age and height has focused on carbon availability. These studies have investigated mechanisms including an increased ratio of respiration to photosynthesis following canopy closure (Yoda *et al.* 1965), and constraints on photosynthetic carbon gain because of path length-related resistance to xylem water transport (Ryan & Yoder 1997). However, studies examining NSC in trees under varying conditions of water availability have provided evidence that drought-related reductions in growth are not likely to be caused by constraints on carbon availability (Körner 2006; Millard *et al.* 2007; Sala & Hoch 2009). This lack of direct evidence for carbon availability limiting growth under conditions of water stress suggests that there is a mechanism involved in growth limitation during water stress that is not directly related to carbon gain. Constraints on turgor-driven cell expansion represent a mechanism for reduced growth that is directly related to water stress but unrelated to carbon availability. Cell expansion is driven by turgor (Lockhart 1965), and turgor has also been implicated as a factor in the regulation of cell division (Kirkham, Gardner & Gerloff 1972). In the absence of osmotic adjustment, turgor in developing cells will decline proportionally with xylem water potential (Boyer 1982). In view of the relationships between tree height, xylem water potential, turgor and processes involved in cell expansion and division, it has been hypothesized that height-related trends in stem elongation and foliar morphological characteristics may occur largely because of the influence of vertical gradients in water potential on turgor (Marshall & Monserud 2003; Woodruff, Bond & Meinzer 2004; Meinzer, Bond & Karanian 2008; Woodruff, Meinzer & Lachenbruch 2008). According to this hypothesis, growth is limited by reduced cell expansion rather than carbon supply. Constraints on growth as a result of reduced turgor under moderate drought conditions are consistent with reports that the first and most sensitive response to water stress in plants is a reduction in turgor-driven expansion of cells (Hsiao 1973; Hsiao *et al.* 1976; Bradford & Hsiao 1982; Larcher 1995).

For this study, we examined growth, water potential and tissue water relations of shoots, plus concentrations of NSC in foliage, small branches and trunk tissues at the tops and other portions of Douglas-fir trees of different heights. This work was done in order to investigate interrelationships between growth, water relations and storage of non-structural carbohydrates along a gradient of tree height. We sampled multiple times throughout a 17-month period in order to examine temporal variations in carbon balance. Tracking NSC in trees of different ages and sizes can provide information about the growth constraints of trees at different stages in their ontogeny. As explained previously, height influences tissue water relations in such a way as to function as a surrogate for an aridity gradient. As such, assessing NSC along a height gradient provides insight into how changes in climate conditions may influence source-sink relationships and overall growth in trees. Because phenology is largely driven by environmental cues that may change dramatically with predicted changes in climate because of increasing levels of atmospheric carbon dioxide, the phenological component of this study was included to provide additional insight into the potential impacts of climate change on forest productivity.

MATERIALS AND METHODS

Field site and sampling

Four stands, each containing Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) trees of a different height class, were located within 3.1 km of each other in the Wind River Basin of south-western Washington State, USA. Access to tree tops in the tallest sampling height class was facilitated by a 75-m tall construction tower crane at the Wind River Canopy Crane Research Facility (WRCCRF). Periodic dieback of the tops of some of the old growth trees within the WRCCRF stand suggested that these trees were close to their maximum height for this site. Tree tops in the two intermediate height classes were accessed by non-spur climbing and the lowest height class was accessible from the ground. The Pacific maritime climate of the region is characterized by wet winters and dry summers. Mean annual precipitation in the region is about 2.2 m, much of which falls as snow, with a dry season from June to September. Mean annual temperature is 8.7°C , with a mean of 0°C in January and 17.5°C in July. The soils are well drained and of volcanic origin (Shaw *et al.* 2004). Low precipitation between June and September ($\sim 119 \text{ mm}$) typically leads to drought conditions in the upper portion of the soil profile. However, soil water remains accessible to Douglas-fir roots at depths greater than about 1 m throughout the summer dry period (Warren *et al.* 2005; Meinzer, Warren & Brooks 2007).

Branch samples 40 to 70-cm long were collected from sun-exposed locations within 5 m of the tops of three trees per site at mean sampling heights of 2.0 (SE = 0), 19.2 (SE = 0.20), 35.8 (SE = 0.69) and 56.5 (SE = 2.4) m. Core samples (5 mm) of the outermost 6 years of sapwood were

collected at 1.3 m height. Immediately following collection, all samples were placed into sealed plastic bags and on ice in a cooler. Samples were always collected between late morning and early afternoon. Samples were collected nine times between May 2009 and October 2010.

Tissue water relations

Pressure–volume analyses (Tyree & Hammel 1972) were conducted on shoots c. 10 to 15-cm long. These samples were excised from branch samples that were collected early in the morning before significant transpirational water loss, sealed in plastic bags with moist paper to prevent desiccation and then stored in a refrigerator within 1–4 h of excision. Pressure–volume curves were initiated by first determining the fresh mass of the shoot, and then measuring shoot water potential (Ψ_i) with a pressure chamber (PMS Instrument Company, Corvallis, OR, USA). Alternate determinations of fresh mass and Ψ_i were repeated during slow dehydration of the shoot on the laboratory bench until values of Ψ_i 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.

Shoot water potential

Minimum shoot water potential was measured with a pressure chamber (PMS Instrument Company, Albany, OR, USA) on shoots approximately 10 to 15-cm long, excised from the larger 40 to 70-cm long samples collected within 5 m of the tops of three trees per site. It was not possible to collect samples from all of the different height classes on the same day so water potential measurements were limited to periods of clear weather between 1200 and 1500 h. Water potential measurements were taken during spring (May and June) and summer (August and September). Both spring and summer measurements are reported in the Results section; however, the spring time measurements are not presented in the figures. Minimum shoot water potential was measured immediately after sampling.

Chemical analyses

Samples were stored in a freezer before being microwaved for 90 s to stop all enzymatic activity, oven dried for 72 h at 65°C and ground to a fine powder. Dried and ground samples of needles, branch xylem and trunk sapwood were analysed for content of sucrose, glucose + fructose, starch and total NSC. Water was added to the powdered samples and NSC was extracted from the solutions by heating them in steam for 1.5 h. The concentration of free glucose was determined photometrically on a 96-well microplate photometer after enzymatic conversion of glucose to gluconate-6-phosphate (Multiskan FC, Thermo Scientific, Waltham, MA, USA). Photometric analysis was based on absorbance of samples at 340 nm in solution with reference to the absorbance of a glucose reference solution. Samples were

analysed both before and after enzymatic treatments of sucrose digestion by invertase for 45 min and starch digestion by amyloglucosidase overnight. Glucose + fructose content was determined from photometric analysis of sample solutions with no enzymatic treatment. Sucrose content was determined by subtracting the glucose + fructose content from the photometric analysis of glucose concentration of sample solutions following invertase enzyme treatment. Total NSC content was determined by adding the sucrose content to the photometric analysis of glucose content of sample solutions following the amyloglucosidase enzyme treatment. Starch content was determined by subtracting the glucose content of sample solution following invertase enzyme treatment from total NSC content. All NSC values are presented as percent dry matter, and fluctuation of NSC is presented as the difference between the maximum and minimum levels, divided by the maximum level, for the entire 17-month period of sampling.

Growth analyses

Shoot extension growth was determined from samples collected at the same locations as those used for the NSC assays. Secondary, non-terminal shoots were selected in all height classes in order to minimize tree damage and to avoid bias from hormonal influences because of relative sampling position of shoots on branches. Shoot annual extension increments were determined with calipers by measuring the 2010 growth internode from bud scar to bud scar.

Soil moisture content

Soil volumetric water content (θ) was quantified using multi-sensor, frequency domain capacitance probes (Paltineanu & Starr 1997). These probes contained six capacitance sensors (EnviroSCAN, Sentek Pty Ltd, Adelaide, Australia) capable of quantifying small changes in θ ($\pm 0.003\%$). Each probe was installed into a ~ 6 cm diameter PVC access tube, with sensors located at depths of 20, 30, 40, 50, 60 and 100 cm. The sensors measured changes in the soil dielectric constant within a ~ 10 cm $\times$ 10 cm sphere of influence. Water content was estimated based on applying a calibration equation to the scaled frequency output of the capacitance sensors. Soil volumetric water content was scanned by a data logger every 20 s and averages were recorded every 30 min (CR10X2M; Campbell Sci. Inc., Logan, UT, USA). The data shown represent values averaged across all six of the measurement depths, with the different depths weighted according to the portion of the vertical profile that they represent.

Statistical analyses

Data were pooled per tree and analysed using regression analysis (PROC REG: Statistical Analysis Software, SAS, Cary, NC, USA). In most cases, data points represent means and standard deviations of three branches per tree. Shoot

growth values represent means of between three and four shoots and osmotic potentials for the 2 m height class are from only one pressure volume curve per tree.

RESULTS

Chemical analyses

Starch represented the largest component of NSC in all three tissue types, in each of the four height classes during all of the sampling sessions. Sucrose was the second most abundant component in branches and foliage, followed by glucose and fructose (Fig. 1). NSC fluctuated seasonally, particularly in branches and foliage, such that peak concentrations of branch and foliage NSC occurred during spring and early summer, with reductions occurring during fall and winter (Fig. 1). NSC concentrations were highest in foliage, consistent with its function of assimilating carbon, synthesizing sugars and the higher concentration of live cells where NSC can be converted and stored. On a dry matter basis, the combined symplast solute content (N_s/DM) of

sucrose, glucose and fructose in foliated shoots ranged from 0.057 to 0.28 mOsmol g^{-1} . Total symplast solute content of Douglas-fir foliated shoots in a former study ranged from ~0.55 to 1.1 mOsmol g^{-1} (Woodruff *et al.* 2004). NSC concentrations were lower in branches than in foliage, but higher than in trunks (Fig. 1). The higher concentration of NSC in branches relative to trunks is likely associated with their proximity to foliage where sugars are synthesized and exported.

NSC concentrations in branches typically followed a height-related pattern in which the highest concentrations occurred in the tallest height class and the lowest concentrations occurred in the lowest height class (Fig. 2). Height-related trends were most pronounced during the fall, and least pronounced during the spring. During 2009, there was not a significant height-related trend in branch NSC in May ($P = 0.093$, Fig. 2a). Mean branch NSC concentrations increased with height by 0.074% m^{-1} during June ($P = 0.0073$, Fig. 2b); by 0.086% m^{-1} during July and August ($P < 0.0001$, Fig. 2c); and by 0.1% m^{-1} during September 2009 ($P < 0.0001$, Fig. 2d). Regardless of the sampling time,

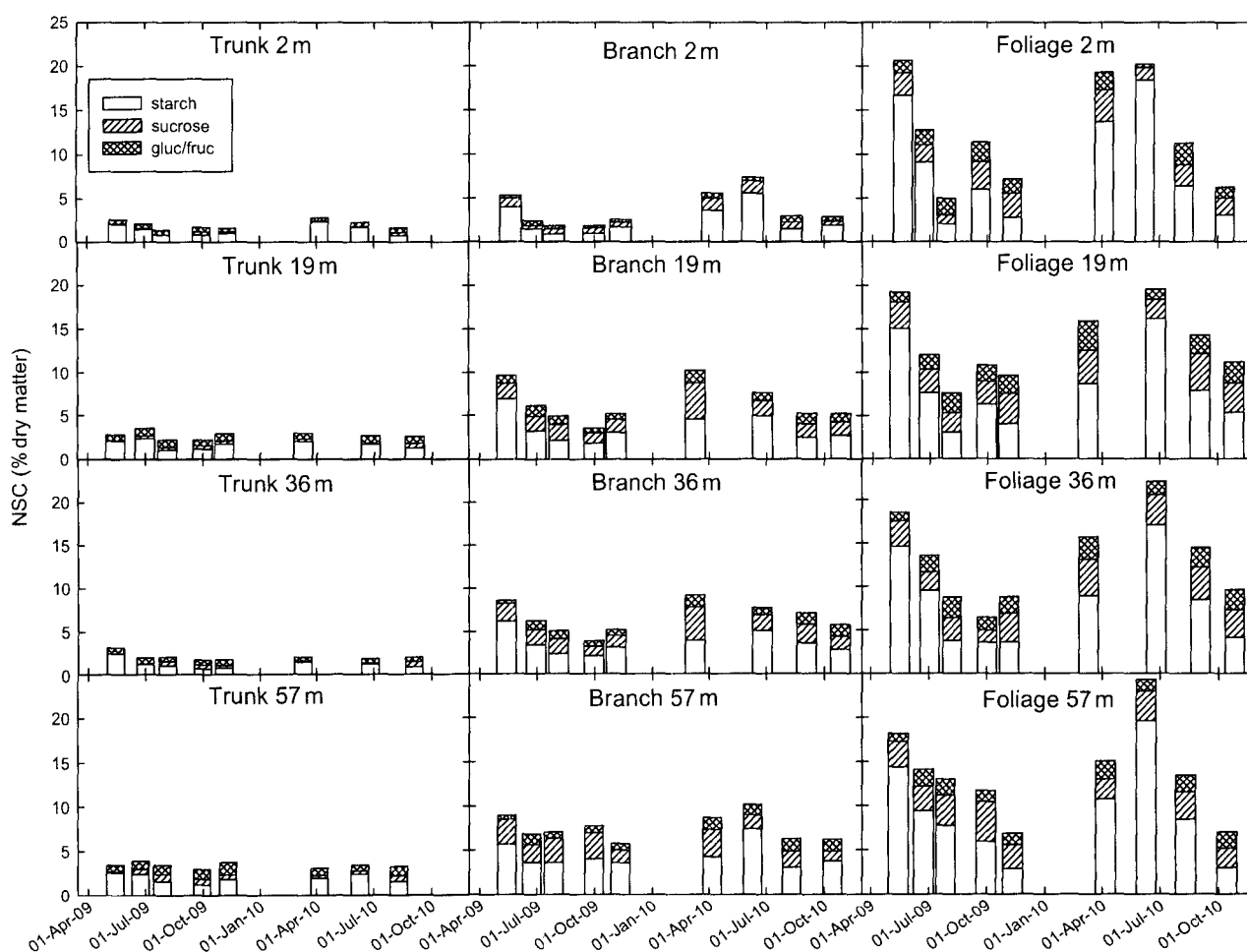


Figure 1. Percent dry matter concentrations of starch, sucrose and combined glucose and fructose in trunks, branches and foliage of four height classes of trees. Samples were collected over a 17-month period from May of 2009 until August of 2010 for trunks, and until November of 2010 for branches and foliage.

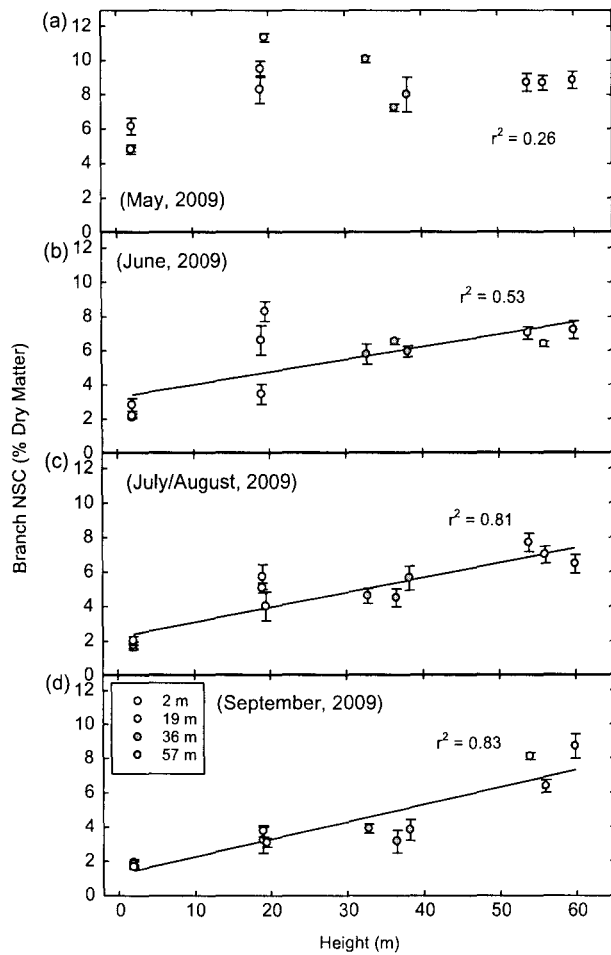


Figure 2. Percent dry matter concentrations of total non-structural carbohydrates (NSC) plotted against height for branch samples collected in May of 2009 (a), June of 2009 (b), July and August of 2009 (c) and September of 2009 (d). The progressively increasing slope in the relationship between branch total NSC and height from May until September illustrates the dynamic nature of the link between height and storage of NSC.

however, mean branch NSC was always greater in the tallest height class than in the lowest height class (Fig. 3a). Consistent with the observed seasonal shift in height-related trends in NSC, the degree to which branch NSC fluctuated over time was significantly correlated with tree height. Mean total fluctuation of branch NSC over the 17-month sampling period decreased by 0.5% for every 1 m increase in height ($P < 0.000013$, Fig. 3b).

Water relations

Mean midday Ψ_l decreased by 0.011 MPa, and 0.016 MPa m^{-1} for measurements taken during spring and summer ($r^2 = 0.67$, $P = 0.001$, $r^2 = 0.79$, $P = 0.0001$, respectively; data not shown). Both branch NSC and fluctuation in branch NSC were significantly correlated with Ψ_l ($P = 0.0072$, $P = 0.00044$, respectively; Fig. 4). Consistent with the vertical trend in Ψ_l , mean osmotic potential at full

turgor (Ψ_π) decreased by 0.012 MPa m^{-1} ($P = 0.00006$, Fig. 5, insert). When Ψ_π was employed as an integrated measure of height-related trends in water stress, correlations between Ψ_π and July/August NSC content ($P < 0.000001$; Fig. 5a) and fluctuation in branch NSC ($P = 0.00018$; Fig. 5b) were substantially stronger than those observed for Ψ_l .

Shoot growth

Shoot extension growth showed significant changes along gradients of height and shoot water potential and was also significantly correlated with patterns of Ψ_π and NSC. Shoot extension growth was negatively correlated with height ($P = 0.0006$, Fig. 6a) and positively correlated with midday Ψ_l ($P = 0.028$, Fig. 6b). Shoot extension was negatively correlated with shoot NSC ($P = 0.00014$, Fig. 6c) and positively correlated with Ψ_π ($P = 0.0001$, Fig. 6d).

Soil moisture content

Values of mean daily θ integrated across six soil depths between 20 cm and 1 m showed that θ varied seasonally

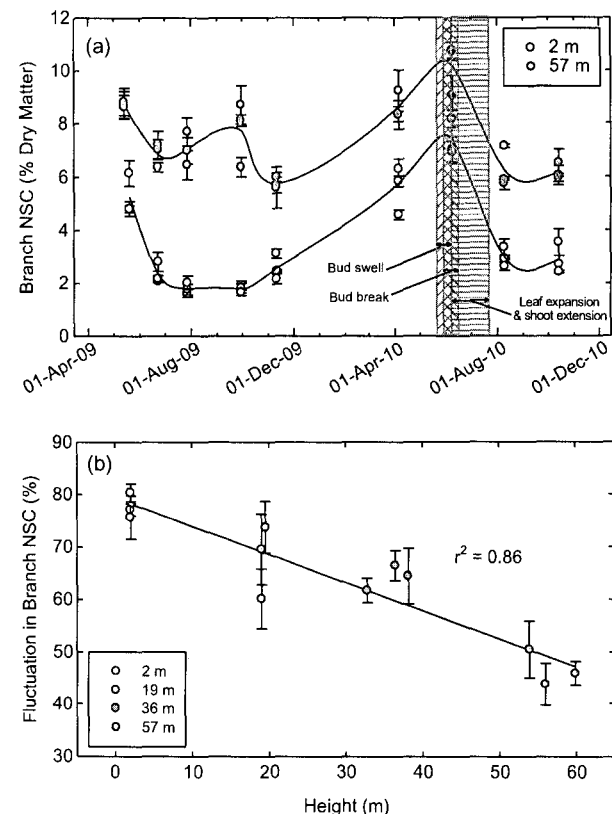


Figure 3. Percent dry matter of branch non-structural carbohydrates (NSC) in the 2 and 57 m height classes plotted over a 17-month sampling period. Shaded areas indicate periods of time during which key phenological events occurred (a). Fluctuation in branch NSC over the 17-month sampling period for trees in the four height classes (b). Error bars represent standard errors of three branches per tree.

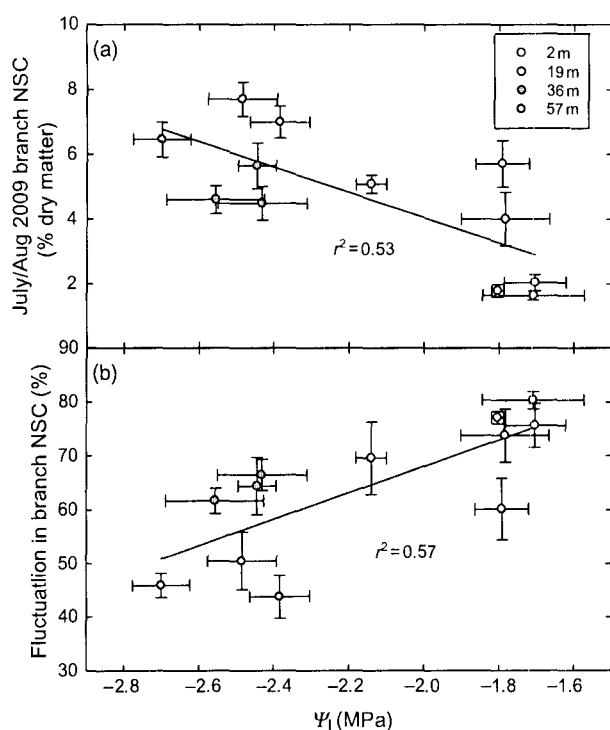


Figure 4. Percent dry matter of shoot non-structural carbohydrates (NSC) (a) and fluctuation in branch NSC over the 17-month sampling period (b), plotted against midday shoot water potential for four height classes of trees measured during August and September. Error bars represent standard errors of three branches per tree.

with minimum summer values ranging from ~22 to ~25%, and maximum winter values between ~36 and 37% (Fig. 7). Values of θ began to steadily decline in mid-May of 2009, and early June of 2010; reaching seasonal minimum values in early August of 2009 and late August of 2010. Values began to rapidly increase again in mid-October of 2009 and mid-September of 2010, coinciding with the onset of fall rains. In the 2-m trees, seasonal trends in branch wood NSC appeared to closely track those of θ ($r^2 = 0.43$, $P = 0.00015$), but in the 57-m trees this relationship was less apparent ($r^2 = 0.07$, $P = 0.18$).

DISCUSSION

Tree height and water stress

The observed trends of Ψ_l and NSC in branches along a vertical gradient (Fig. 2) resulted in a strong positive correlation between height-related increases in shoot water stress and the accumulation of non-structural carbohydrates. There was also a strong vertical trend of decreasing shoot extension with increasing height (Fig. 6). The coordination of water stress, decreased shoot extension and increased storage of non-structural carbohydrates contrasts with a number of studies which have proposed that constraints on tree growth associated with water stress are primarily associated with carbon limitation (Yoder *et al.*

1994; Mencuccini & Grace 1996; Ryan & Yoder 1997; McDowell *et al.* 2002; Delzon & Loustau 2005). The positive correlation between height and storage of NSC provides evidence that size-related growth decline in trees is not necessarily caused by constraints on assimilation of carbon. Many plant processes are affected by even mild levels of water stress, and because of the integrated nature of plant physiological processes, most primary effects of water stress on specific processes are followed by secondary and tertiary effects. Based on an extensive review of the literature on plant responses to drought, Hsiao (1973) developed a comparison of the sensitivity of selected plant physiological and biophysical processes to water stress. This analysis indicated that cell expansion, cell wall synthesis and protein synthesis are substantially more sensitive to water stress than stomatal conductance or CO_2 assimilation. A mechanism that could explain reduced growth with increased tree size that is independent of carbon assimilation or carbon availability is the reduction in cell expansion because of height-related reductions in cell turgor. It was previously hypothesized that vertical gradients in water potential and turgor were largely responsible for observed height-related trends in

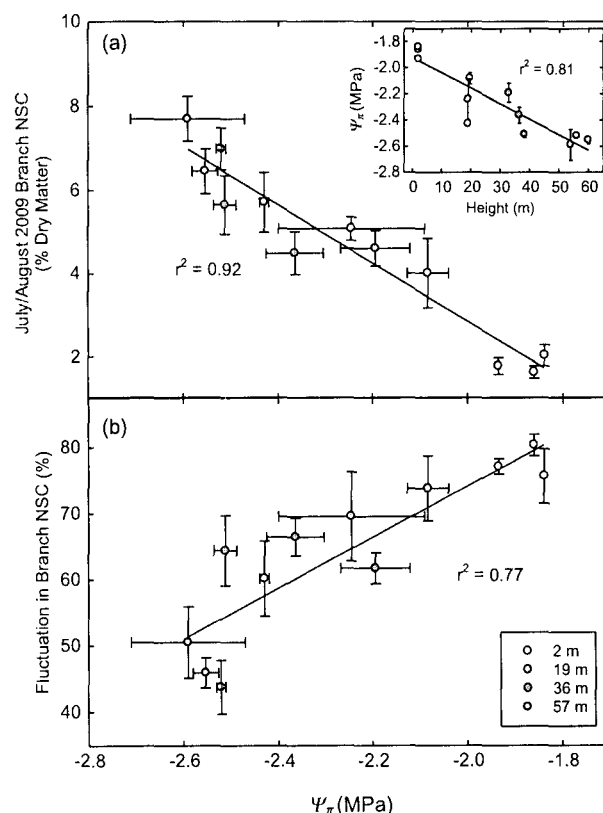


Figure 5. Percent dry matter of shoot non-structural carbohydrates (NSC) (a) and fluctuation in shoot NSC over the 17-month sampling period (b), plotted against shoot osmotic potential at full turgor (Ψ_π) for four height classes of trees. Shoot osmotic potential (Ψ_π) plotted against height (insert in Fig. 5a). Error bars represent standard errors of three branches per tree. [Corrections added after online publication (18 August 2011): Figure x-axis label and legend were amended.]

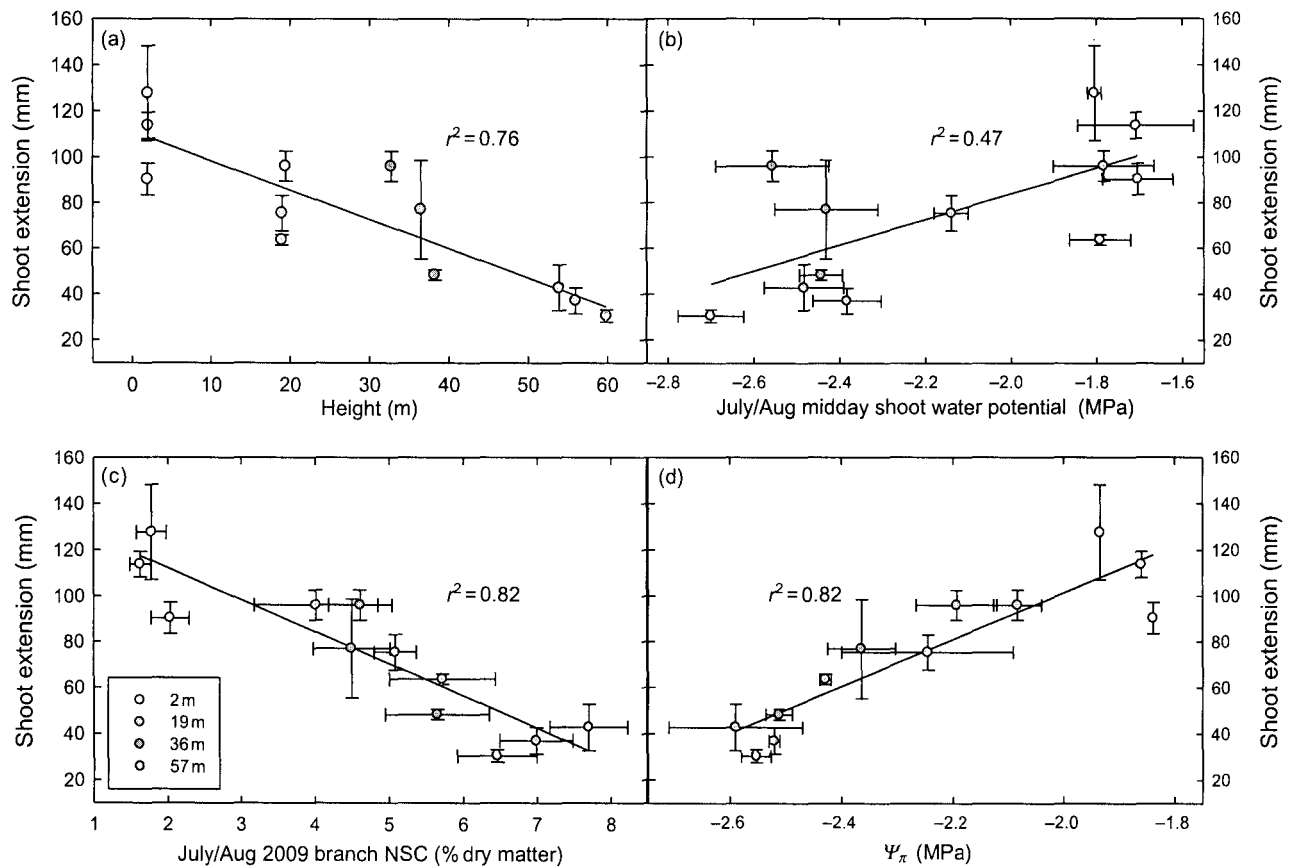


Figure 6. Shoot extension growth during 2010 plotted against sampling height (a), August/September midday shoot water potential (Ψ_i) (b), percent dry matter of shoot non-structural carbohydrates (NSC) (c) and shoot osmotic potential at full turgor (Ψ_π). Error bars represent standard errors of three branches per tree.

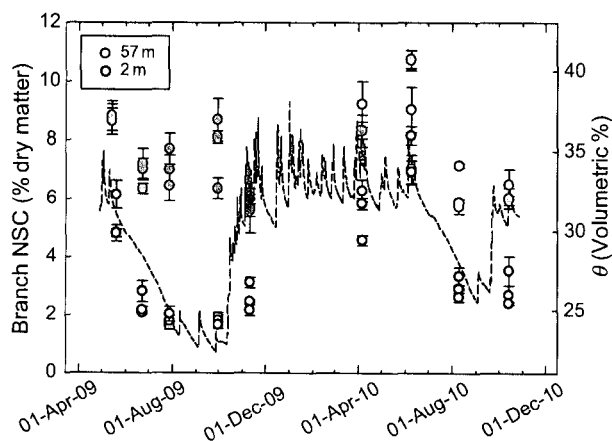


Figure 7. Percent dry matter of shoot non-structural carbohydrates (NSC) for the 2 and 57 m height classes (left y-axis) and percent soil volumetric moisture content (θ) averaged across eight sampling locations between soil depths of 20 to 100 cm (right y-axis) plotted over a 17-month-long sampling period.

stem elongation and foliar morphological characteristics (Marshall & Monserud 2003; Woodruff *et al.* 2004, 2008; Meinzer *et al.* 2008). This hypothesis implies that growth is reduced by constraints on the sink strength of expanding tissues as opposed to carbon availability.

A number of other studies have found evidence suggesting that growth of mature trees is often not limited by carbon supply (Körner 2003, 2006; Würth *et al.* 2005; Millard *et al.* 2007; Sala & Hoch 2009). Würth *et al.* (2005) analysed concentrations of NSC in mature tropical trees during dry and moist seasons and found that NSC reserves remained high throughout the year, and increased during the dry season. Sala & Hoch (2009) were the first to investigate NSC in trees of different heights as a means to investigate mechanisms for size-related reductions in productivity in individual trees and stands following canopy closure. They analysed concentrations of NSC in the upper third portions of the crowns of Ponderosa pine trees ranging in height from approximately 5 to 40 m. They found that NSC increased with tree height and this vertical trend was more pronounced in trees at a dry site than in those at a moist site. Although a positive correlation has been shown between concentrations of NSC and seasonally dry conditions (Würth *et al.* 2005), tree height (Sala & Hoch 2009), and tree height and water stress (this study), this does not

preclude the possibility of severe prolonged water stress leading to the depletion of carbon reserves. This could occur, for example, following a threshold where reductions in photosynthesis eventually outpace reductions in respiration, as modelled by McDowell (2011).

The seasonal patterns of accumulation and depletion of NSC (Figs 1 and 7) are strongly influenced by phenological events such as the development of foliage and xylem. Foliar buds of Douglas-fir at the WRCCRF site swelled in May and bud break occurred in late May to early June. Needle expansion and shoot extension occurred throughout June and stopped during July (Fig. 3a). Forests in the Pacific Northwest experience annual droughts during the summer months during which soil moisture is progressively depleted from early June until rains return in the fall (Waring & Franklin 1979; see also Fig. 7 in this study). The pronounced increase in the slope and strength of the relationship between height and storage of NSC throughout the growing season (Fig. 2) further emphasizes the relationship between moderate water stress and increased storage of non-structural carbohydrates. As soil moisture becomes more depleted during the summer months, taller trees that are subjected to greater gravitational effects and greater hydraulic resistance because of increased path length become increasingly water stressed. There was disparity in the degree of coupling of temporal variations in NSC with θ in this study, with the NSC of branches in the 2 m trees tracking θ in a relatively consistent manner throughout the year, and the branches of the 57-m trees maintaining a relatively high level of NSC, despite pronounced seasonal fluctuations in θ (Fig. 7). Stomatal control of photosynthesis in isohydric plants is tightly coordinated with Ψ_l and therefore soil water potential (Ψ_s) (Tardieu & Simonneau 1998). As such, the patterns of θ and NSC in Fig. 7 for younger trees suggest a relatively tight coupling between the storage of carbohydrates, and depletion of stored carbohydrates, with stomatal control of photosynthesis. The lack of a strong association between seasonal patterns in θ and branch NSC in the taller trees suggests a weaker connection between stomatal control of photosynthesis and accumulation and depletion of carbohydrates.

Reduced accessibility of stored non-structural carbohydrates has been proposed as an explanation for the accumulation of carbon reserves. Mechanisms for reduced accessibility of NSC include compartmentalization in tissues where they cannot be retrieved (Chapin *et al.* 1990; Millard *et al.* 2007), insufficient enzymatic access to the inner nucleus of starch granules (Srichuwong & Jane 2007) and constraints on their transport in phloem because of water stress (Sala & Hoch 2010). NSC concentrations were significantly greater in the taller height classes for each of the three tissue types that were sampled, particularly during late summer (Fig. 2). Although branch wood NSC and fluctuation in branch wood NSC were significantly correlated with Ψ_l (Fig. 4), these were both more strongly correlated with Ψ_π (Fig. 5). As a measure of the solute content of a tissue, Ψ_π provides a more integrated measure of the water relations of shoots and foliage compared with

instantaneous measures of Ψ_l . Reductions in Ψ_π are indicative of sustained water stress and solute adjustment to offset the effects of limited water availability on cell turgor (Osonubi & Davies 1978; Turner & Jones 1980). The most consistent and pronounced height-related trend in storage of non-structural carbohydrates occurred in small branches (Fig. 1). As the link between the source of photosynthates (foliage) and the vast majority of the potential reservoir for storage of NSC (trunk), small branches represent a key indicator for constraints on the delivery of photosynthate from its source to the rest of the tree. The negative correlation between height and annual fluctuation in NSC (Fig. 3) suggests that the accumulation of NSC may be related to constraints on phloem transport. There are a number of factors associated with water stress that could result in constraints on the movement of photosynthates. These include reductions in sink strength; increases in phloem viscosity; changes in phloem conduit anatomy such as reductions in sieve element lumen areas because of reduction in turgor-driven cell expansion; and increased path length relative to pressure gradients between sources and sinks. As originally advanced by Münch (1927), the pressure flow hypothesis proposes that transport in the phloem is driven by a pressure gradient that is created by the active loading and unloading of phloem at sources and sinks. Flow is also influenced by the viscosity of the phloem, which is largely determined by the relative concentrations of water and sugars. With decreased phloem relative water content, viscosity will increase and the capacity for flow will be reduced. Hölttä *et al.* (2006) modelled phloem viscosity as a function of physiologically possible solute concentrations and found that this can vary the resistance of phloem sap flow by several orders of magnitude. With increased water stress, the expansion of sieve elements during cell development will also be reduced because of the decline in turgor-driven cell expansion. The Hagen–Poiseuille equation describes fluid transport through conduits and has been used to describe phloem transport (J_v) in sieve elements (van Bel & Hafke 2005):

$$J_v = \Delta \Psi_p \pi r^4 / 8 \eta L \quad (1)$$

where Ψ_p = turgor, r = conduit lumen radius, η = phloem sap viscosity and L = the distance between source and sink. Reductions in conduit area have a substantial impact on conduit transport capacity as illustrated by the fourth power relationship between r and J_v . Furthermore, any reduction in Ψ_p because of water stress at source sites will reduce the pressure gradient from source to sink locations, thereby directly limiting J_v . As the capacity of phloem tissue to transport sugars in solution is diminished, the likelihood of the build up of NSC near source locations increases.

Cell growth involves the movement of water and solutes into the cell to generate sufficient turgor pressure to sustain irreversible growth as cell walls relax and expand with the cleaving of hemicellulose tethers within the wall (Harold 1990). The cleaving of these wall components and the subsequent demand for the synthesis of wall components are the

origin of sink strength for carbohydrates during plant growth. In the absence of sufficient water availability for turgor-driven cell expansion, the sink strength for the use of assimilated carbon is reduced or eliminated. As sink strength is reduced, assimilated carbon is either stored as sugars or converted to starch for storage, thereby resulting in an accumulation of NSC. The accumulation of NSC can also impact photosynthetic capacity through feedback inhibition (Neales & Incoll 1968). Studies involving experiments where sugar concentrations have been artificially elevated near source locations through girdling or continuous lighting have discovered a co-occurring reduction in photosynthetic parameters including maximum photosynthesis, carboxylation rate and electron transport rate (Layne & Flore 1995; Myers, Thomas & DeLucia 1999; Johnsen *et al.* 2007). The down-regulation of genes associated with photosynthesis has been linked to sugar concentration in *Arabidopsis* (Cheng, Moore & Seemann 1998) and other species (Jang & Sheen 1994); and starch concentration has also been linked to a down-regulation of photosynthesis in three species of conifers (Equiza, Day & Jagels 2006).

It has been hypothesized that NSC, particularly sucrose, may play a role in sensing (Secchi & Zwieniecki 2011) and reversal (Bucci *et al.* 2003) of xylem embolism. Contact cells are parenchyma cells that are in direct contact with xylem conduits via pits (Alves *et al.* 2001), suggesting a direct exchange of compounds between these parenchyma cells and xylem conduits (Czaninski 1977; Sauter & Van Cleve 1992; Alves *et al.* 2001). One functional role that has been proposed for this exchange is the refilling of embolized conduits (Sperry *et al.* 1987; Hacke & Sauter 1996; Utsumi *et al.* 1998; Ewers *et al.* 2001; Clearwater & Goldstein 2005; Cobb, Choat & Holbrook 2007). A number of authors have proposed the occurrence of seasonal refilling of xylem cells during spring or winter in order to repair cells that have been embolized by freeze-thaw activity (Sperry *et al.* 1987; Hacke & Sauter 1996; Utsumi *et al.* 1998; Ewers *et al.* 2001; McCulloh *et al.* 2011). Diel patterns of loss and recovery of hydraulic conductance in leaves, however, have been identified in a number of species (Bucci *et al.* 2003; Johnson *et al.* 2009, 2011), suggesting that the refilling of embolized xylem conduits may occur on a daily basis. One proposed mechanism for the refilling of embolized cells involves the hydrolyzing of starch into sugars, especially sucrose, in xylem parenchyma and the loading of the sugars into the embolized xylem, resulting in a sufficient reduction in xylem solute potential for water flow into the embolized conduits (Canny 1997). If this mechanism for hydraulic repair of embolized conduits was in fact a common occurrence, it could indicate a positive adaptive role for elevated concentrations of NSC in branches and foliage, particularly in tissues that are prone to experiencing greater levels of tension and cavitation within their xylem.

CONCLUSIONS

Globally, forests cover about 7% of earth's surface and about 30% of the world's land surface, and they represent the

largest store of terrestrial carbon (Birdsey *et al.* 2007). On the basis of above ground volume, Douglas-fir is the dominant tree species in the United States with nearly twice the volume of the next most common species, loblolly pine (Smith *et al.* 2002). Despite the importance of forest ecosystems in sequestering atmospheric carbon, methods that are currently used to simulate tree growth are based on assumptions regarding mechanistic relationships between climate parameters and growth that are not necessarily realistic. These methods are often predicated on a carbon balance approach in which productivity is primarily determined by the degree to which parameters such as daytime vapour pressure deficit, soil moisture and temperature limit photosynthesis. Recent research, however, implies that growth of trees subjected to certain environmental stresses may not be primarily carbon limited. The co-occurrence of reduced shoot growth and increased storage of NSC observed here suggests a scenario in which it is not exclusively photosynthesis or carbon that is the limiting factor for growth, but also the ability of the tree to 'utilize' the carbon that has been assimilated. These findings suggest that increasing drought severity may lead to scenarios in which photosynthesis is less of a key determinant of productivity than other factors such as cell turgor, long-distance transport of assimilates or changes in carbon allocation associated with reduced sink strength of growing shoot tips. By examining the impacts of water stress on growth and storage of non-structural carbohydrates, this work aims to provide insights into the impacts of water stress on carbon balance and the role of stored carbon in responses to stress. Further research is needed to examine the extent to which increased concentrations of NSC are related to reductions in cell expansion and sink strength versus constraints on the capacity for the delivery of photosynthates. Additional research is also needed to further explore the potential role of NSC in the refilling of embolized xylem cells.

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