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Research paper

The impacts of water stress on phloem transport in Douglas-fir trees

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Despite the critical role that phloem plays in a number of plant functional processes and the potential impact of water stress on phloem structural and phloem sap compositional characteristics, little research has been done to examine how water stress influences phloem transport. The objectives of this study were to develop a more accurate understanding of how water stress affects phloem transport in trees, both in terms of the short-term impacts of water stress on phloem sap composition and the longer-term impacts on sieve cell anatomical characteristics. Phloem sieve cell conductivity (k_p) was evaluated along a gradient of tree height and xylem water potential in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees in order to evaluate the influence of water stress on phloem transport capacity. The Hagen–Poiseuille equation was used with measurements of sieve cell anatomical characteristics, water content of phloem sap, non-structural carbohydrate content of phloem sap and shoot water potential (Ψ_l) to evaluate impacts of water stress on k_p. Based on regression analysis, for each 1 MPa decrease in mean midday Ψ_l, sieve cell lumen radius decreased by 2.63 μm MPa^{-1}. Although there was no significant trend in sucrose content with decreasing Ψ_l, glucose and fructose content increased significantly with water stress and sieve cell relative water content decreased by 13.5% MPa^{-1}, leading to a significant increase in sugar molar concentration of 0.46 mol l^{-1} MPa^{-1} and a significant increase in viscosity of 0.27 mPa s MPa^{-1}. Modeled k_p was significantly influenced both by trends in viscosity as well as by water stress-related trends in sieve cell anatomy.

Keywords: drought, growth limitation, non-structural carbohydrates, *Pseudotsuga menziesii*, tree height.

Introduction

Phloem is an integral part of the vascular system of plants and, in addition to transporting nutrients, it is responsible for the translocation of defensive and signaling compounds throughout vascular plants (van Bel 2003). Much of the carbon that is assimilated in photosynthetic tissues is transported away from these sources in the phloem as sugars in order to meet the demands of the non-photosynthesizing components of the plant. In spite of the range of important functions that phloem performs in plants, very little research has been done to investigate its vulnerability to water stress, and as such relationships between environmental stress and phloem physiology have not been well characterized.

Phloem transport is controlled by a number of factors which are influenced by water availability and as such it is likely to be strongly influenced by water stress. The pressure flow hypothesis for phloem transport (Münch 1927) proposes that sugars are loaded into sieve cells, which reduces the phloem osmotic potential near source tissues. A hydrostatic pressure gradient results from the influx of water that is osmotically drawn in from adjacent xylem, leading to a hydrostatic pressure-driven mass flow through an interconnected network of sieve cells to sink tissues where the sugars are unloaded. The removal of sugars at sink locations increases solute potential in these regions, resulting in the release of water back into the xylem and a lower hydrostatic pressure than in the regions where sugars are loaded. Phloem sap viscosity influences phloem sieve cell conductivity (k_p) and viscosity is largely determined by the relative concentrations of water and solutes, such as sugars within the phloem sap. With increased water stress, the growth of sieve

elements during cell development is likely to be reduced due to the decline in turgor-driven cell expansion (Hsiao 1973). This reduction in cell expansion can affect sieve cell anatomical characteristics such as lumen diameter, which has a potentially large impact on phloem transport (van Bel 2003).

Water stress increases with tree height as a result of both gravitational and frictional resistance to water transport. 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 and Grace 1996, Ryan et al. 2000). An increase in tree height is therefore comparable to occupying drier portions of an aridity gradient. The hydraulic status of phloem tissue is linked to that of the xylem due to the exchange of water between the two as described in the Münch hypothesis (Münch 1927), and a Ψ equilibrium is often assumed between the two tissues in models of phloem transport (Daudet et al. 2002, Thompson and Holbrook 2003, Hölttä et al. 2006). Examining phloem tissue along a tree height gradient within comparable soil and climate conditions thus provides an opportunity to examine the effects of water stress on phloem characteristics that dictate the capacity for phloem transport.

Both sieve cell structural characteristics and phloem sap compositional characteristics were hypothesized to be impacted by chronic water stress in ways that would lead to reductions in k_p. Turgor drives cell expansion (Lockhart 1965) and unless altered by osmotic adjustment, the turgor of cells in the apical and vascular meristems will decline proportionally with xylem Ψ (Boyer and Silk 2004). As such, phloem tissue that develops under prolonged conditions of enhanced water stress will be less expanded (i.e., smaller) than that which has developed under lesser degrees of water stress, thus resulting in greater constraints on phloem transport capacity. Because phloem sap viscosity is substantially influenced by the relative concentrations of solutes and water, I hypothesized that the sap within phloem tissue experiencing greater levels of water stress would have higher viscosity, therefore leading to further reductions in k_p. For this study we measured a number of anatomical and compositional characteristics of phloem sieve cells and phloem sap from small branches of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees of different heights and under different levels of sustained water stress. These characteristics included tissue water relations of phloem, phloem sieve cell lumen diameter, sieve pore depth and concentrations of different sugars in phloem tissues. I then modeled the impacts of water stress on k_p using the Hagen–Poiseuille equation.

Much research has been done to examine the impacts of water stress on a number of different physiological processes in plants, especially growth and gas exchange. There is currently a great deal of interest in determining the mechanisms involved in drought-related tree mortality. As phloem is responsible for the transport of carbon from sources to sinks in a solution of water (phloem sap), any advancement in our understanding of how water stress impacts phloem transport should be relevant to improving our understanding of the role of carbon starvation vs dehydration in tree death. This study examines how tree height and associated water stress influences key phloem characteristics that influence phloem transport capacity: sieve cell anatomical characteristics and phloem sap composition. It also provides information about the relative contributions of changes in each of these characteristics toward the impact of water stress on k_p. Developing a more accurate understanding of how water stress influences the capacity for phloem transport in trees represents a potentially substantial advancement in our understanding of the impacts of drought on plant physiology, vigor and mortality. This information may allow modelers to more accurately characterize current and future impacts of drought on forest productivity, carbon storage in forest ecosystems, tree resilience to insect attacks and tree species migration.

Materials and methods

Field site and sampling

Four stands, each containing Douglas-fir 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 (latitude N 45°49'13.76" and longitude W 121°57'06.88"). 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). 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 ~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 (~119 mm) typically leads to drought conditions in the upper portion of the soil profile. Branch samples 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 = 0.00), 19.2 (SE = 0.20), 35.8 (SE = 0.69) and 56.5 m (SE = 2.4). Immediately following collection, all samples were placed in sealed plastic bags and on ice in a cooler and later stored in a freezer. Samples were always collected between late morning and early afternoon. Samples for analyses of moisture content, non-structural carbohydrates (NSCs) and shoot water potential (Ψ_l) were collected during May 2009 and samples for anatomical analyses were collected on 30 March and 19 April 2011. Values of Ψ_l from 2009 were used as an explanatory variable for anatomical and phloem sap compositional variables during 2011 because spring time soil

moisture content is very consistent at this site from year to year. Between the beginning of March and the end of May soil moisture content was consistently ~30% during 2009, 2010 and 2011 (Figure 1). All samples were collected from trees in stands within a very limited geographic range, and only from sun-exposed tops of trees so as to avoid any potentially confounding factors such as variability in climate, soil characteristics or irradiance/shading on measured characteristics.

Soil moisture content

Soil volumetric water content (θ) was quantified using multi-sensor, frequency domain capacitance probes (Paltineanu and Starr 1997). These probes contained six capacitance sensors (EnviroSCAN, Sentek Pty Ltd, Adelaide, Australia) capable of quantifying small changes in θ (±0.003%). Each probe was installed into an ~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 an ~10 × 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 (Figure 1) 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.

Shoot water potential and phloem water content

Shoot water potentials were measured only during clear weather using a pressure chamber (Model 600, PMS Instrument Company, Albany, OR, USA) on shoots ~10–15 cm long collected within 5 m of the tops of three trees per site between 1200 and 1500 h. Regression analyses of water stress (Ψ_l) and phloem anatomical and biophysical characteristics were examined using Ψ data collected during the spring (when soil moisture content is high) due to the strong association between phloem cell development and high soil moisture content (Figure 1; Alfieri and Evert 1968, Marcati et al. 2008, Prislan et al. 2013). Phloem tissue water content was determined by taking a fresh weight and a subsequent dry weight of a thin (<1 mm) layer of inner phloem section directly adjacent to the cambium. Sieve cell water content was measured for the determination of sieve sap viscosity. Sieve cell water content (S_w) was estimated according to Eq. (1) using phloem tissue water content (P_w) and published values of xylem cell fibre saturation (F_s) as values for sieve cells are not available (Panshin and De Zeeuw 1970):

$$S_w = P_w(1 - F_s) \quad (1)$$

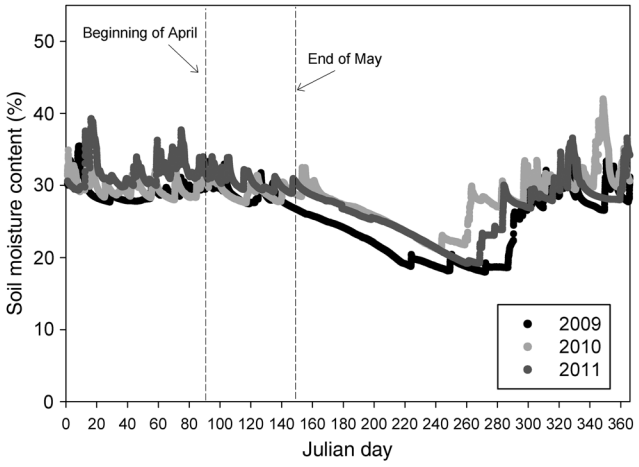

Figure 1. Values of mean daily θ integrated across six soil depths of (20, 30, 40, 50, 60 and 100 cm) for the years 2009, 2010 and 2011. The beginning and end of the time period when sampling and measurements were conducted is highlighted by the vertical dashed lines. The relatively consistent soil moisture content of ~30% during April and May across the years when sampling was done illustrates the validity of using Ψ values from one year as indicative of water stress for another.

Phloem anatomical characteristics

Sieve cell lumen radius and sieve cell wall thickness were examined from phloem tissue samples collected at the phloem–cambium interface, from 3- to 5-year-old shoots collected at the tops of trees along a height and water stress gradient. Both sieve cell lumen diameter and wall thickness were measured from cross-sectional microscopy images. Sieve cell lumen radius was measured due to the potentially substantial impact of conduit radius on k_p due to the conductivity of transport cells being strongly determined by lumen size (see 'Phloem sap viscosity and phloem sieve cell conductivity' section). In addition to lumen radius, the conductivity of cells linked more or less in series is also influenced by the structural characteristics of the connections between individual cells. As such, sieve cell wall thickness was measured to provide an estimate of the depth of sieve cell pores. Because intercellular pores traverse the width of sieve cell walls, measurements of cell wall thickness were considered a reasonable means of estimating the depth of the pores from one cell to the next. Whole phloem tissue conductivity is likely to be influenced by additional factors that impact the transfer of phloem sap between adjacent sieve cells such as sieve pit pore characteristics (Thompson and Holbrook 2003). In addition, the flow rate of phloem sap is determined by additional factors that influence phloem movement such as the distance, as well as the pressure differential, between source and sink locations. Although the majority of the phloem tissue directly adjacent to the cambium in *P. menziesii* is composed of sieve cells (~80–85% based on microscopic images, data not shown), parenchyma cells are also present, thereby making the proper identification of sieve cells necessary for anatomical analyses.

Pseudotsuga menziesii sieve cells are block-shaped to nearly round with light-colored cell walls. Sieve cells were only un-collapsed (i.e., functional) in a relatively narrow strip several cells wide near the cambium, beyond which they were partially to completely crushed. Sections of phloem tissue were made by hand sectioning of fresh tissue with a fresh razor blade. Very thin cross-sectional slices of fresh phloem tissue were immersed in glycerin on a slide and then imaged. Images of anatomical characteristics of un-collapsed sieve cells adjacent to the cambium were created using a compound microscope and video camera with ×20 objectives and total magnifications of ×200. Dimensional measurements were done using the ImageJ image analysis software (Abramoff et al. 2004).

Chemical analyses

Phloem sap sugar content was characterized in order to examine how changes in NSCs with water stress may influence phloem sap viscosity. Phloem sap sugar content was estimated by analyzing both the water content and the percent dry weight of different sugars of phloem tissue. Although in theory phloem sap may also be influenced by changes in salt concentrations, as opposed to observed significant changes in sugar concentrations with water stress (Sala and Hoch 2009, Woodruff and Meinzer 2011), there is no known evidence of changes in salt concentrations along height or water stress gradients in coniferous trees. Phloem tissue samples from the phloem–cambium interface were analyzed for the content of sucrose and glucose + fructose following the methods described for NSC analyses in Woodruff and Meinzer (2011). 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 analyzed 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. All NSC values are presented as percent dry matter.

Phloem sap viscosity and phloem sieve cell conductivity

Phloem sap viscosity (u_p, mPa s) was calculated based on the following equation from Chirife and Beura (1997):

$$u_p = a\exp(EX) \quad (2)$$

where a represents an empirically derived multiplier, E is an empirically derived parameter related to the free energy activation for viscous flow per mole solute and X is calculated as

$$X = \frac{m}{55.51 + m} \quad (3)$$

where m equals solution molality (moles of solute per kg of water, assuming a constant temperature of 20 °C). Molar concentration of sugars were determined using the percent dry weight NSC data and sieve cell water content from Eq. (1), both normalized by tissue dry weight. Phloem sieve cell conductivity (k_p, $m^4\ s^{-1}\ MPa^{-1}$) was calculated based on the Hagen–Poiseuille equation:

$$k_p = \frac{\pi D^4}{128\mu_p} \quad (4)$$

where D is sieve cell diameter (m). k_p is a modeled estimate of the conductivity of individual phloem sieve cells and thus should not be considered equivalent to whole phloem tissue conductivity. However, as tissue conductivity represents the cumulative conductivity of its individual conduits, k_p is highly indicative of whole phloem tissue conductivity.

Viscosity declines with increasing temperature leading to potentially substantial impacts on viscosity and conductivity where extreme temperature gradients exist. For this study a constant phloem sap temperature of 20 °C was used for all locations. Although temperature can vary substantially along a height gradient in a forest at any given time, on average there was only a 1° difference in the mean annual temperature between micrometeorological stations at 2 and 70 m heights within the old growth stand at the Wind River site (Wind River Research Station micrometeorological data archives), where sampling and measurements were done for this study. Given that samples were collected along a slightly smaller height gradient, and samples were collected from the tops of trees in different nearby stands of varying height as opposed to along varying heights along a single canopy height gradient, variability in temperature between different sampling locations for this study is likely to have negligible effects on viscosity.

Statistical analyses

Data were pooled per tree and analyzed using regression analyses. In most cases data points represent means and standard errors of three branches per tree, with at least three independent measurements per branch. Shoot water potential values used in anatomical and biochemical regression analyses are derived from the linear regression of height to midday Ψ_l measured during the spring so as to diminish the error associated with temporal variability in climatic and soil conditions.

Results

Soil moisture content

Values of mean daily θ integrated across six soil depths between 20 cm and 1 m showed that although θ varied substantially over the course of a year, it varied little between Julian day 90 (the beginning of April) and Julian day 150 (the end of May), and it varied little across years for this time period. During this 2-month period, the average θ for 2009, 2010 and 2011 were 28.6, 29.2 and 30.5%, respectively (Figure 1). The low variability in θ across this 2-month time period and across these years illustrates the highly consistent soil moisture values across the time periods when Ψ_l measurements and phloem tissue samplings were conducted.

Water relations

Midday Ψ_l declined linearly with tree height decreasing by 0.011 MPa for each 1 m increase in tree height during the spring, and by 0.015 MPa during the summer ($r^2 = 0.67$, $P = 0.001$ and $r^2 = 0.75$, $P = 0.0003$, respectively; data not shown). Not surprisingly, there was a reduction in the water content of phloem tissue with decreasing Ψ_l. The mean phloem tissue water content of shoot samples collected from the tops of the shortest and the tallest trees was 60.8% (SE = 1.0%) and 49.2% (SE = 0.6%), respectively (Table 1); with relative water content decreasing by 19% for each 1 MPa reduction in Ψ_l (springtime, $r^2 = 0.81$, $P < 0.0001$; Figure 2). Although water content analyses were done on phloem tissue that also contained parenchyma cells, sieve cells represented the majority of the phloem tissue samples based on microscopic observations (~80–85%, data not shown). Owing to the relatively small amount of parenchyma cells, they were assumed to have little impact on the reported values of water content for sieve cells. The same applies to NSC content of the analyzed phloem tissue.

Anatomy

Sieve cell lumen radius decreased with increasing water stress with the mean lumen radius declining by 2.63 μm MPa^{-1} ($r^2 = 0.57$, $P < 0.005$; Figure 3a). The mean sieve cell lumen radius of shoot samples collected from the tops of the shortest and the tallest trees was 9.8 μm (SE = 0.5) and 7.8 μm (0.33), respectively (Table 1), resulting in an ~21% decrease in lumen radius due to the increasing water stress associated with the gravitational and path-length resistance of an ~55 m gradient in tree height. Although there was no observed trend in sieve cell pore depth with water stress (Figure 3b), there may be other sieve cell pit anatomical characteristics that influence phloem transport capacity which were not analyzed in the present study, such as sieve cell pit diameter, which could vary along a gradient of water stress.

NSCs and viscosity of phloem sap

Contrary to expectations, there was no significant trend in total sugar content and sucrose content in phloem tissue with increasing water stress ($P = 0.33$ and $P = 0.11$, respectively, Figure 4). Glucose and fructose, however, showed a significant increase in percent dry weight of phloem tissue with increasing water stress ($P = 0.01$, Figure 4).

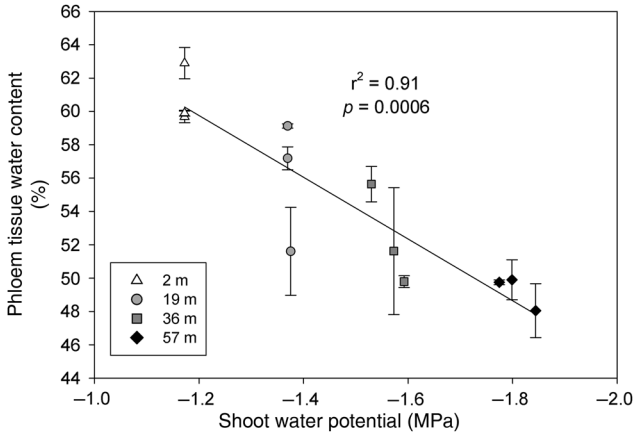

Figure 2. Relative water content of phloem samples in relation to Ψ_l for Douglas-fir (*P. menziesii*) trees obtained within 1–5 m of the tops of trees along a height gradient. The observed linear relationship shows the degree to which the water status of the phloem tissue and that of the xylem are linked.

Table 1. Sample height, midday Ψ_l (from regression analysis), phloem tissue water content, sieve cell lumen radius, sieve cell pore depth, viscosity and phloem sieve cell conductivity.

Height (m)	Spring midday Ψ_l (MPa)	Phloem tissue water content (%)	Sieve cell lumen radius (μm)	Sieve cell pore depth (μm)	Viscosity (mPa s)	k_p ($m^4\ s^{-1}\ MPa^{-1}$)
2 (0.0)	–1.18	60.8 (1.0)	9.8 (0.5)	6.6 (0.38)	2.05 (0.012)	1.78×10^{-12} (1.1×10^{-13})
19.2 (0.2)	–1.38	56.0 (2.3)	8.3 (0.34)	5.9 (0.41)	2.13 (0.0.019)	8.85×10^{-13} (7.1×10^{-14})
35.8 (1.6)	–1.57	52.3 (1.7)	8.9 (0.42)	6.9 (0.45)	2.17 (0.0.012)	1.16×10^{-12} (9.6×10^{-14})
56.6 (1.8)	–1.80	49.2 (0.6)	7.8 (0.33)	6.3 (0.6)	2.23 (0.0.011)	6.62×10^{-13} (6.8×10^{-14})

Values in parentheses are standard errors.

The observed trends in phloem sugar content and water content produced subsequent patterns in phloem sap sugar molarity and viscosity. There was no significant trend in sucrose molar concentration with Ψ_l ($P = 0.32$; Figure 5a). The molar concentration of sucrose + glucose and fructose, however, did increase significantly with decreasing Ψ_l ($P = 0.00004$, $r^2 = 0.83$; Figure 5b). Consistent with the relationship between Ψ_l and sucrose molar concentration, the viscosity of the phloem sap did not correlate with water stress when only considering sucrose within the phloem sap ($P = 0.32$, $r^2 = 0.1$; Figure 5c). However, when considering fructose and glucose along with sucrose as active constituents within the phloem sap, phloem sap viscosity increased significantly, and was very highly correlated with decreasing Ψ_l ($P = 0.00016$, $r^2 = 0.77$; Figure 5d). Along the vertical sampling gradient over which spring time midday Ψ_l declined by 0.63 MPa, estimates of phloem sap viscosity increased by 8%, from a mean value of 2.05, to a mean of 2.23 mPa s (Figure 5d). (Note that the units for viscosity are millipascal seconds (mPa s) and that this is not a typo for megapascal seconds (MPa s) or megapascals per second (MPa s^{-1}).) The observed increase in phloem sap viscosity was attributable to a 19% reduction in the relative water content of the phloem tissue (Figure 2), and a 95% increase in phloem sap molar concentration of sugars from 0.305 to 0.595 mol l^{-1} (Figure 5b).

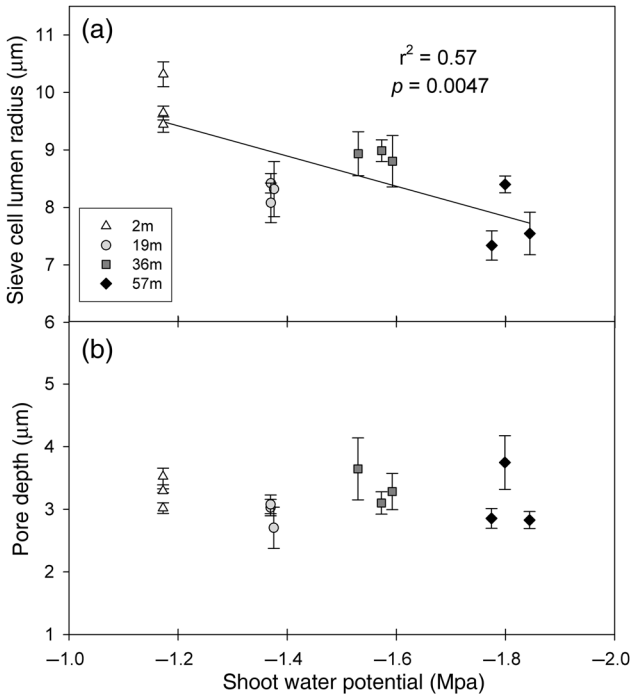

Figure 3. Sieve cell lumen radius (a) and sieve cell pore depth (b) in relation to Ψ_l for Douglas-fir (*P. menziesii*) phloem sieve cells from samples along a gradient of water stress. The relationship between Ψ_l and sieve cell lumen size illustrates the relationship between water availability and the turgor-driven expansion of cells that transport phloem sap.

k_p

Using the observed trends in sieve cell anatomical characteristics and phloem sap composition with increasing water stress, the effect of water stress on k_p was evaluated by means of the Hagen–Poiseuille equation. As calculated based on Eq. (4), k_p decreased significantly with increasing water stress (see below); k_p decreased significantly with decreasing Ψ_l based on

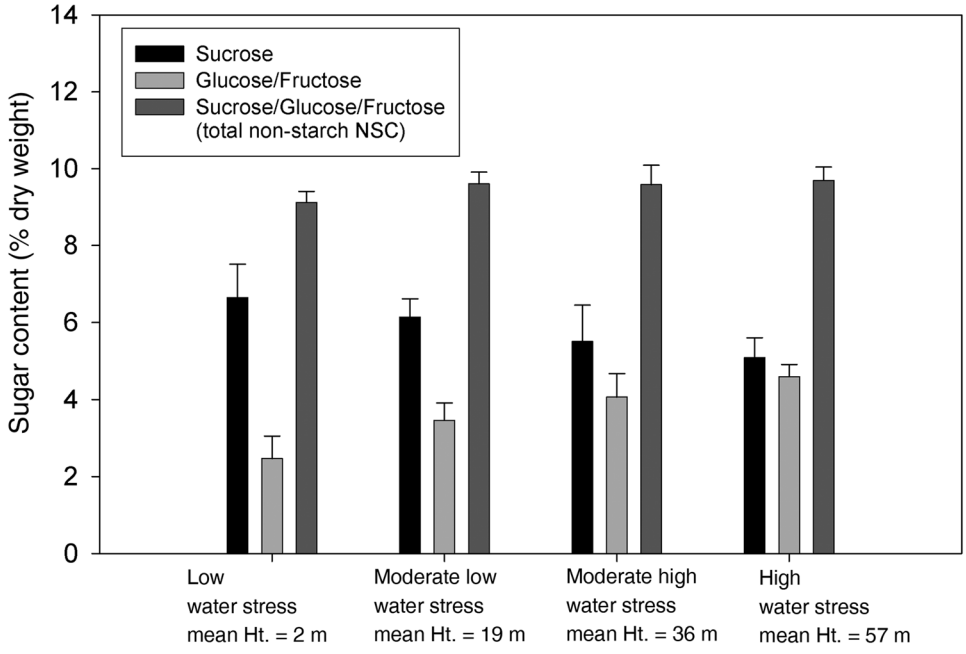

Figure 4. Sucrose, glucose and fructose and sucrose, glucose and fructose content in phloem tissue of Douglas-fir (*P. menziesii*) trees from samples along a gradient of water stress.

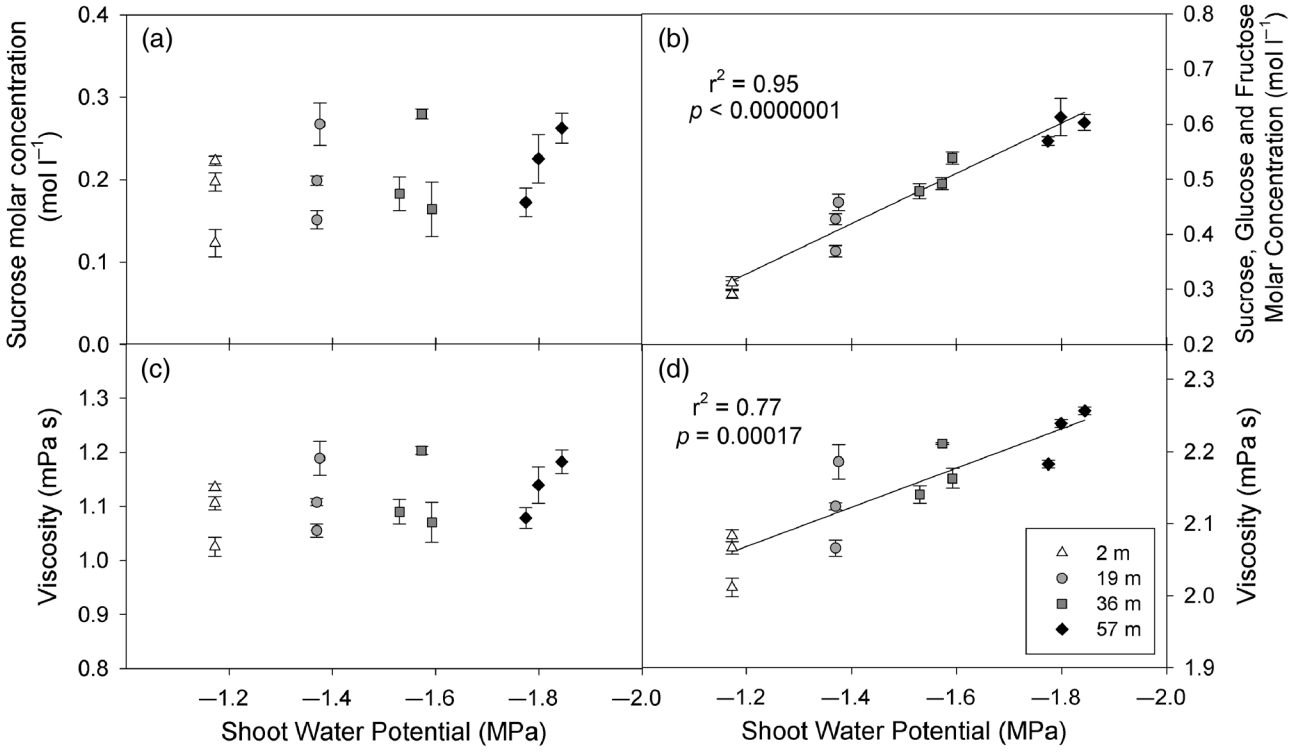

Figure 5. Phloem sap sugar molar concentration (a and b) and viscosity of phloem sap (c and d) for phloem tissue of Douglas-fir (*P. menziesii*) trees at four different levels of water stress. Panels a and c indicate sugar molar concentration and phloem sap viscosity (respectively) assuming only sucrose as a translocated sugar in the phloem sap. Panels b and d indicate sugar molar concentration and phloem sap viscosity (respectively) assuming glucose, fructose and sucrose as translocated sugars in the phloem sap. The strong linear trends in panels a and d compared with b and c highlight the contribution of glucose and fructose to viscosity with increasing water stress.

sucrose, glucose and fructose as active phloem constituents ($P = 0.0032$, $r^2 = 0.60$; Figure 6), as well as when considering just sucrose alone as an active phloem sap constituent ($P = 0.0034$, $r^2 = 0.59$; data not shown). The mean values of k_p in the least and most water-stressed groups were 1.78×10^{-12} and 6.62×10^{-13} $m^4 s^{-1} MPa^{-1}$, respectively; representing a 63% reduction in k_p.

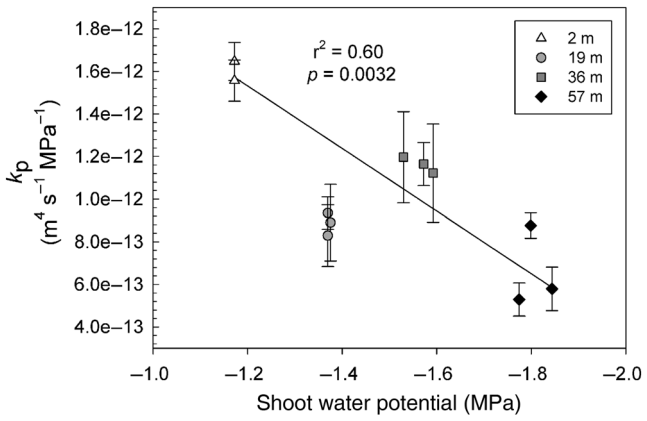

Figure 6. Phloem sieve cell conductivity (k_p) modeled using a modified version of the Poiseuille equation for fluid transport for (*P. menziesii*) phloem sieve cells from samples along a gradient of water stress. The observed trends in the modeled values of k_p suggest that tree height and associated water stress lead to significant constraints on phloem transport capacity.

Discussion

Modeling k_p using measured anatomical and phloem sap compositional parameters obtained along a gradient of sustained water stress provided evidence that tree height and associated water stress lead to significant constraints on phloem transport capacity. These constraints appeared to be more highly influenced by changes in sieve cell structural characteristics than by phloem sap composition characteristics associated with increased viscosity.

The observed decline in midday Ψ_l was consistent with that which has been found in previous studies examining changes in characteristics, including water stress, along a height gradient in Douglas-fir trees (Woodruff et al. 2007, 2008, 2010, Domec et al. 2008, Woodruff and Meinzer 2011) and the water content of phloem tissue in this study also followed a linear trend comparable to the decline in water Ψ (Figure 2). Reductions in sieve cell lumen area with decreasing Ψ_l were consistent with the dependence of cell development on turgor-driven cell wall expansion (Hsiao et al. 1976, Nonami and Boyer 1989) and with trends in turgor with increased gravitational and path-length

resistance (Woodruff et al. 2004). The fourth power relationship between conduit diameter and transport efficiency, as described by the Hagen–Poiseuille equation (Eq. (4)), illustrates how even small changes in the diameter of sieve cells can have a very substantial impact on k_p. Because the water necessary for cell expansion may come from the phloem as well as from the xylem (Boyer and Silk 2004), the observed reduction in conductivity of the phloem due to constraints on cell expansion may in turn further limit future cell development and expansion because it represents not only a nutrient supply limitation but also a potential hydraulic limitation imposed upon the system that supplies expanding cells. The interrelationship between water availability and cell expansion leads to a scenario of positive reinforcement in which water stress leads to constraints on the delivery of water, which in turn leads to greater water stress, and so on.

The modeling conducted in this study involved estimating conductivity of individual sieve cells based on the conduit lumen diameter. In some studies of anatomical and transport characteristics in plants, conduit length has also been shown to play a substantial role in transport capacity because the longer the conduit, the fewer end-wall crossings must be made per unit length, and so the greater the end-wall conductivity (Hacke et al. 2004, Sperry et al. 2006). However, an analysis was done for the present study that involved incorporating values of sieve cell length that varied with Ψ_l and a more complex phloem transport model based on the Hagen–Poiseuille equation that includes sieve cell length as a variable (Thompson and Holbrook 2003), and this analysis resulted in virtually identical values for k_p and identical trends of k_p with water stress, thus illustrating the prevalent role of conduit diameter over conduit length in influencing phloem transport capacity.

The observed changes in sugar molar concentration with water stress were due to both the direct effect of decreased water content, as well as an increase in the sugar concentration of the phloem sap; and particularly the increase in the amount of simpler fructose and glucose molecules with an associated decrease in sucrose (Figure 4). This observed decrease in sucrose and increase in glucose and fructose content with increasing water stress suggests a possible adaptive mechanism for influencing the osmotic potential and turgor of phloem sap. In a study by Cernusak et al. (2003) sugar content of phloem sap was shown to increase in response to water stress. In that study, phloem sap molar concentration increased from ~0.5 to ~0.8 mol l^{-1} with a corresponding decrease in daytime Ψ_l from approximately –1.0 to –2.5 MPa; as well as an integrated reduction in $\delta^{13}C$ of phloem sap sugar from ~22 to ~12‰. Although many earlier sources suggest that sucrose and raffinose are the overwhelmingly predominant sugars transported in the phloem and that studies showing an abundance of reducing sugars such as glucose and fructose occur only as experimental artifacts (Ziegler 1975), more recent research has provided evidence that many plant families translocate appreciable amounts of hexoses in their phloem, with some translocating over 80% of carbohydrates in the form of hexoses (van Bel and Hess 2008). With regard to the lack of raffinose in the analyses for the current study, although raffinose can represent a substantial portion of the sugars transported in the phloem of some angiosperms, it does not represent a significant component of phloem sap in conifers (Smith and Zavarin 1960, Barras and Hodges 1969, Gall et al. 2002). Sucrose, glucose and fructose were the only sugars detected by paper chromatography in the phloem tissues of *Pinus taeda* by Barras and Hodges (1969), which was in close agreement with Smith and Zavarin (1960), who found glucose, fructose and sucrose, and only a small amount of raffinose in the inner bark of eight conifers. Gall et al. (2002) also found that along with sucrose, glucose and fructose concentrations were substantial in phloem tissues and phloem exudates and raffinose concentrations were negligible.

The explanatory variables examined in this study which impact phloem transport capacity fall under two general categories: those which influence viscosity and those which influence sieve cell anatomical properties. Substituting constant values for these explanatory factors provides a means to evaluate the relative extent to which they influence k_p. When a constant viscosity value of 3.0 mPa s is used for all treatments, a decline in mean k_p of ~60% is observed that is entirely attributable to changes in sieve anatomical characteristics with increasing water stress. Substituting a constant value of 1×10^{-5} m for the sieve cell lumen radius provides a decline in k_p of ~8%, which is entirely attributable to trends in phloem sap viscosity. These relative contributions of viscosity and anatomical characteristics to changes in k_p when the other factor is held constant suggests that, along the water stress gradient represented in this study, reductions in sieve cell lumen size had a substantially greater impact on modeled estimates of k_p than did changes in phloem sap sugar composition. These two variables, viscosity and sieve cell anatomy, are factors that are affected on different time scales. Whereas phloem sap viscosity can be influenced by alterations in sap content that can occur within a relatively short time frame such as changes in water availability, variability in photosynthate abundance or the conversion of stored carbohydrates to soluble sugars, sieve cell anatomical properties are determined during the development of the cells, and once the cells are formed, these factors are changed less easily. As such, water stress during phloem cell development can have a more sustained impact on k_p, and the influence of increased viscosity on k_p could potentially be alleviated relatively quickly if water availability increases.

Drought has been predicted to become more common in the future in many regions of the world as a result of a number of different factors including higher temperatures, greater variability in precipitation and earlier snowmelt (IPCC 2007, US GCRP 2009). In the absence of increased evapotranspiration, higher temperatures will also lead to greater vapor pressure deficit.

With more intense and more frequent incidences of drought, the importance of understanding the mechanisms involved in how water stress impacts physiological processes in trees such as phloem transport becomes more critical. This research represents advancements in our understanding of how water stress influences phloem transport that involves actual measured anatomical and sap compositional characteristics of phloem sieve cells. Further advancements in the area of phloem transport research could be achieved by developing methods for the accurate measurement of the transport rate in situ, and by scaling up from the cellular level to the whole tree and whole stand level. Although the importance of phloem transport is most commonly considered in the context of the delivery of carbohydrates for growth and metabolism, there is a growing understanding that phloem regulates a vast array of essential processes in plants and the limitation of the translocation of carbohydrates for growth and metabolism is only a portion of the story of how phloem transport is affected by water stress. Understanding how water stress influences phloem transport is important when investigating not only constraints on tree growth, but also tree mortality. Constraints on the ability of phloem to transport carbohydrates from storage areas to sites where they can be metabolized could inhibit the use of stored reserves, thereby leading to carbon limitation or potentially carbon starvation (Shigo 1985, McDowell and Sevanto 2010). Reduced capacity for phloem transport could also exacerbate water stress in trees if compounds cannot be translocated where they are needed for osmotic refilling of embolized vessels (Bucci et al. 2003, Nardini et al. 2011) or the solute-mediated regulation of cell turgor (Turner and Jones 1980, Morgan 1984). To develop a more comprehensive understanding of how plants may be affected under future climate regimes, future research could focus on the ways in which water stress compromises each of these critical phloem-mediated processes.

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Conflict of interest

None declared.

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