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2017-10

Paljakka , T , Jyske , T , Lintunen , A , Aaltonen , H , Nikinmaa , E & Hölttä , T 2017 , ' Gradients and dynamics of inner bark and needle osmotic potentials in Scots pine (Pinus sylvestris L.) and Norway spruce (Picea abies L. Karst) ' , Plant, Cell and Environment , vol. 40 , no. 10 , pp. 2160-2173 . https://doi.org/10.1111/pce.13017

http://hdl.handle.net/10138/312013
https://doi.org/10.1111/pce.13017

unspecified
acceptedVersion

Note : This is the pre-peer reviewed version of the following article:

Paljakka, T., Jyske, T., Lintunen, A., Aaltonen, H., Nikinmaa, E., & Hölttä, T. (2017). Gradients and dynamics of inner bark and needle osmotic potentials in Scots pine (Pinus sylvestris L.) and Norway spruce (Picea abies L. Karst). Plant, cell & environment, 40(10), 2160-2173.

which has been published in final form at [https://doi.org/10.1111/pce.13017]. This version includes the track changes, and track changes of deleted text have been removed for avoiding confusions to reader.

Gradients and dynamics of inner bark and needle osmotic potentials in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.)

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ABSTRACT

Preconditions of phloem transport in conifers are relatively unknown. We studied the variation of needle and inner bark axial osmotic gradients and xylem water potential in Scots pine and Norway spruce by measuring needle and inner bark osmolality in saplings and mature trees over several periods within a growing season. The needle and inner bark osmolality was strongly related to xylem water potential in all studied trees. Sugar concentrations were measured in Scots pine and they had similar dynamics to inner bark osmolality. The sucrose quantity remained fairly constant over time and position, whereas the other sugars exhibited a larger change with time and position. A small osmotic gradient existed from branch to stem base under pre-dawn conditions and the osmotic gradient between upper stem and stem base was close to zero. The turgor in branches was significantly driven by xylem water potential, and the turgor loss point in branches was relatively close to daily minimum needle water potentials typically reported for Scots pine. Our results imply that xylem water potential considerably impacts the turgor pressure gradient driving phloem

transport and that gravitation has a relatively large role in phloem transport in the stems of mature Scots pine trees.

KEYWORDS: phloem transport, osmolality, xylem, water potential, turgor pressure, tree

INTRODUCTION

Water and sugar transport systems in trees are strongly coupled, which creates a linkage between the physiological processes producing, consuming and transporting sugars and water (Christy & Ferrier 1973; Steppe et al. 2006; Hölttä et al. 2006). These components are connected to tree gas exchange and are therefore essential to understand when assessing carbon uptake, growth and mortality of trees (e.g. Nikinmaa et al. 2013; Sevanto 2014). The link of these components to environmental variables is not well known, which complicates the prediction of tree responses under changing climate conditions (Savage et al. 2015; Steppe et al. 2015). Therefore, linking phloem dynamics to tree hydraulics and the source-sink dynamics to whole tree physiology and environmental variables is an interesting and formidable challenge (Turgeon & Wolf 2009; Sevanto 2014; Netherer et al. 2015; Savage et al. 2015; Steppe et al. 2015).

Water movement in xylem is based on the cohesion tension theory and the movement of water towards lower water potential (Dixon and Joly 1894; Nobel 2005). This water flow is driven by transpiration, the rate of which plants can adjust by stomatal regulation (Cochard et al. 1996; Mäkelä et al. 1996). Photosynthetic products and other vital solutes are transported in the living and specialized sieve cells of the phloem. The sieve elements in angiosperms and sieve cells in gymnosperms are accompanied by companion and Strasburger cells, respectively, and by the parenchyma cells (Schulz 1990; van Bel 2003).

Turgor pressure is vital for living cells to maintain functionality and achieve growth (Lockhart 1965). Turgor pressure of the phloem tissue is driven by its osmotic potential and is most likely impacted by xylem water potential (Thompson & Holbrook 2003; Hölttä et al. 2006). The water potential in xylem practically consists of the pressure potential created by water since the osmotic potential in that tissue is assumed to be small (Scholander et al. 1965).

The osmotic potential (and osmolality) is driven by osmotically active solutes, e.g. carbohydrates and ions in an aqueous solution. In most tree species, sucrose is the vast majority of transported carbohydrates in phloem sap, and therefore, it is expected to have a significant effect on the changes of osmotic potential in phloem (Zimmermann 1957; Pate 1976; Turgeon & Wolf 2009).

The foundation of phloem transport theory has for long been the hydrostatic (turgor) pressure difference between the relative solute input and output locations, source and sink, which is also referred to as the 'Münch hypothesis' (Münch 1930; Kaufmann & Kramer 1967; Christy & Ferrier 1973; Knoblauch & van Bel 1998; Thompson & Holbrook 2003; Minchin & Lacointe 2005; Knoblauch & Peters 2013; An et al. 2014; Knoblauch et al. 2016). The estimations of phloem osmotic potential and xylem water potential enable the turgor pressure to be estimated since phloem tends to equilibrate rapidly with the water potential of the surrounding tissues (Thompson & Holbrook 2003). The phloem turgor pressure has been very difficult to study since direct measurements change the conditions inside the pressurized sieve cells (e.g. Sovonick-Dunford et al. 1981; van Bel 2003; Turgeon & Wolf 2009). That is why today there is still a lack of measured data on turgor pressure and a lack of support for the theory behind the phloem transport (An et al. 2014; Ryan & Asao 2014; Steppe et al. 2015), although a very few studies have successfully measured phloem turgor pressure differences directly on smaller plants (Gould et al. 2006; Knoblauch et al. 2016). Data about gymnosperms or data collected in the field conditions are especially scarce (Turgeon 2010; Knoblauch et al. 2016).

As phloem and xylem equilibrate rapidly in water potential (Molz & Klepper 1973; Sovonick-Dunford et al. 1981; Hölttä et al. 2006) it has been hypothesized that water potential in xylem impact the turgor pressure in phloem and more so higher in the tree (Sevanto et al. 2002; Woodruff et al. 2004; Hölttä et al. 2006). Low xylem water potential might hinder phloem transport by causing an accumulation of sugars that affects the phloem loading from the leaf mesophyll (Hölttä et al. 2006; Lieshce et al. 2011; Nikinmaa et al. 2013). Both the sink and source dynamics are this way connected to stomatal control and tree water relations (Paul and Foyer 2001; Nikinmaa et al. 2013).

One key question concerning phloem transport in trees is whether the turgor pressure gradient over the whole transport pathway alone, can explain phloem transport over long distances such as those that are found in taller trees. It is not known how plants could regulate the turgor gradient over long distances (Thompson 2006; Ryan and Asao 2014). Mencuccini & Hölttä (2010) predicted, based on the scaling between plant height and phloem transport rates from different studies that the turgor pressure varies with plant height. This mechanism could also explain the long-distance phloem transport with the aid of structural adjustments along the transport pathway from canopy to roots. The phloem conduit lumen size has been found to impact phloem conductivity significantly (Jensen et al. 2012; Woodruff 2014). Several studies have reported gradual changes in conduit size from the stem apex towards the tree base (Rosner et al. 2001; Petit & Crivellaro 2014; Woodruff 2014; Jyske & Hölttä 2015). The ratio of phloem conductivity to xylem conductivity has also been found to decrease towards the base of the tree (Jyske & Hölttä 2015). These studies indicate that structural adjustments do exist as a function of tree height and they are bound to affect the turgor pressure gradient through the phloem and xylem conductivity. It is also possible that leakage and retrieval of sugars along the phloem transport pathway is aiding in maintaining a sufficient turgor gradient especially in larger trees (Thompson and Holbook 2003; Epron et al. 2016).

Axial osmotic pressure gradients have been studied and reported since the 1930s to find support for the Münch hypothesis in natural conditions (Münch 1930; Zimmermann 1960; Kaufmann & Kramer

1967; Hammel 1968; Rosner et al. 2001). Axial osmotic gradients can be expected to counteract the xylem water potential gradient and maintain a turgor pressure gradient from the source to sink. In addition, the osmotic gradient may be expected to be steeper near the apex since the conduit size at the apex is smaller for both xylem and phloem (Anfodillo 2006; Woodruff 2014; Petit & Crivellaro 2014; Jyske & Hölttä 2015). The phloem transport velocities and allocation of sugars in trees are also affected by seasonality (Dannoura et al. 2011). The sink strength of growing tissue is dependent both on temperature and stage of phenological development, which theoretically should impact upon the magnitude and perhaps even the direction of the turgor gradient. The previous studies on axial osmotic gradients have almost exclusively detected decreasing osmotic gradients from leaves to the stem base. However, an osmotic gradient translates to a turgor pressure gradient only when it is larger than the xylem water potential gradient (Kaufmann & Kramer 1967). Some support for a turgor pressure gradient has been obtained from studies involving osmotic pressure measurements in angiosperms but with differing methods (Hammel 1968; Rogers & Peel 1975). Temporal studies of phloem pressure have been conducted at least on white ash (*Fraxinus americana* L.) (Lee 1981). The osmotic gradients of conifers have been studied mainly using Norway spruce (*Picea abies* Karst.) (Rosner et al. 2001). Temporal studies of water and sugar content in the phloem have also been carried out on Norway spruce and spatial studies on Douglas-fir (*Pseudotsuga menziesii*) (Gall et al. 2002; Woodruff et al. 2004; Woodruff 2014). Osmotic gradient studies on Scots pine (*Pinus sylvestris* L.) are scarce or have not been carried out. Temporal estimations of turgor pressure *in situ* also seem to be missing with Scots pine.

Our aim in this study was to measure the osmolality gradients in Scots pine and Norway spruce to find preconditions of turgor gradient driving phloem transport in conifers. We measured osmolality in needles and inner bark of branches, upper stem and stem base in mature trees. The osmolality in Scots pine and Norway spruce saplings were also measured in needles, stem bark and coarse roots. Additionally, we measured xylem water potential and estimated its dynamics with point

dendrometer measurements to understand how the water potential equilibrium is maintained between xylem and inner bark. Water and solute contents as well as sugars were examined to understand the drivers of osmotic potential and turgor pressure.

METHODS

Measurement site and plant material

The measurements of the mature Scots pine were carried out at the Station for Measuring Forest Ecosystem – Atmosphere Relations (SMEAR II) in southern Finland (61° 51' N, 24° 17' E, 181 m above sea level) (Hari et al. 2013). The mean growing season at that location is from the end of April to mid-October (Vanhatalo et al. 2015). Mean annual rainfall is approximately 700 mm (Pirinen et al. 2012). The forest stand is dominated by 50-year-old Scots pine trees and it represents a typical managed sub-xeric heath forest of Finland. The mean height of the trees was 18 m and the mean diameter at breast height was 18.5 cm during the sampling periods in 2014 and 2015. The mean soil depth is approximately 0.5 m above the bed rock (Vanhatalo et al. 2015). Measurements of the mature Scots pine were carried out in the early autumn of 2011, in July and September 2014 and in June 2015. The mean temperatures over these measurement periods and some other environmental variables are shown in Table 1. Measurements of the mature Norway spruce were conducted during the growing season in 2012 in a spruce dominated forest site in Haapastensyrjä, Southern Finland (60° 4' N, 24° 3' E, 120 m above sea level). The spruce trees were 30 years old and rooted from cuttings (clone no. 255) planted in a fertile former agricultural land (Jyske et al. 2015). The sun rises approximately at 4:00, 4:30 and 6:30, and sets around 23:00, 22:30 and 20:00 in June, July and mid-September, respectively, in Southern Finland (University of Helsinki Almanac Office).

Scots pine saplings were collected in the beginning of May 2012 from a forest opening that is situated close to SMEAR II station in a similar vegetation type to mature Scots pine trees in this study. The initial height of the saplings was about 40 cm. The saplings were carefully dug up with a shovel,

avoiding damage to the roots, and planted into pots of 16.5 cm in diameter and 18.0 cm in depth. The soil used for planting was taken from the growing site. Planted saplings were kept in the greenhouse during the winter and outside, under a transparent roof, during summer. Norway spruce saplings were obtained from a greenhouse experiment in Haapastensyrjä, Loppi, Southern Finland, during the growing season of 2012. The saplings were grafted in 2009 by using cuttings (clones E330, E2833, and K8051) of mother trees originating from central Finland. The mean height of the grafts was 63 ±11 cm (from the top to the grafting point). The saplings had been grown outside until the autumn of 2011, and then planted in pots. The potted grafts were stored over winter in a plastic-covered greenhouse in ambient temperature but without snow cover. The roots in the pots were covered with moss to avoid frost damage. The potted grafts were transferred into greenhouse on the 15th of March 2012 into two departments. One department had temperature elevated to +1^{o}C above the ambient (outdoors) conditions; the temperature was always kept above freezing point. The other department had temperature +4^{o}C above the ambient. The temperature differentiation started on 10th April.

Stem and xylem diameter

The stem and xylem diameter variations were measured by linear displacement transducers (LVDT, Solartron AX/5.0/S, Solartron Inc. West Sussex, UK). Both the stem and xylem sensors were attached side-by-side to a metal frame around the tree through holes and tightened with a screw at 1.5 m height. The xylem sensor rested against a screw that was attached to the xylem, whereas the stem sensor rested against the bark where the rhytidome had been removed (e.g. Mencuccini et al. 2013). The data received of the diameter changes occurred at a one-minute frequency.

Bark and needle sampling

The numbers and timings of sample collections are shown in Table 2. The sample positions are illustrated in Figure 1. The osmolality and water content samples of mature Scots pine from the needles, the branches, the upper stem and the stem base were collected at 7:00 and 15:00 resulting in two samples per sampling position per day for five consecutive days in 2011. In total 6–8 samples per position for two consecutive days were collected between 9:00 and 19:30 with approximately three hours between the samplings in July and September in 2014. The sampling procedure in June 2015 was in other ways similar except that an additional sample was collected at 7:00, which amounted to between 7–10 samples per position for two consecutive days. The measurements of the Scots pine were carried out on two mature trees and the sampling involved only one tree per measurement period. A tree with (LVDT) diameter measurements was used for the sample collections in July 2014 and June 2015. Samples taken from August to September in 2011 and in September 2014 were collected from the tree without the diameter measurements. The needle and branch samples were collected from the canopy that was exposed to light at 16 m height. Branches of a mean length of 40 cm were first cut from the tree and samples were immediately collected. The stem samples were collected from the upper stem, which located at the lower canopy, and from the stem base, 10–12 m and 1.5–2.5 m in height, respectively. Bark samples including tissues of the cambium and the cork cambium were detached using a scalpel after the cork was removed. We henceforth refer to these samples as ‘inner bark’ in this study. The sample dimensions varied from 0.5 to 1 cm in width and 1.5–2 cm in length. The samples were immediately placed in 5 ml cryo tubes (Nunc, Thermo Scientific, Massachusetts, US), which were then frozen in liquid nitrogen. Samples for the osmolality analysis were stored in a freezer at -80 °C. Two bark pieces with similar dimensions that were adjacent to osmolality samples were collected for water content and sugar concentration measurements. The sugar concentration samples were collected only in July and September 2014. All the sugar concentration samples were first frozen and stored in -80 °C. The needle samples for water content were collected from the same group of needles in the same branch as the osmolality

198 samples. One sample collection, included osmolality, sugar and water content samples, took
199 approximately 45 to 60 minutes.

200

201 Samples were taken from two mature Norway spruce trees that were 23 meters in height.
202 Collections were carried out once every month between 13:00 and 15:00 from May to August in
203 2012. The sampling heights of the needles and the branches were the same and both varied from 13
204 to 17 m. The upper stem and stem base heights were similar to those with Scots pine being 11–13 m
205 and 1.3 m, respectively. In addition, samples from the stem at the ground level were collected.
206 Sampling of all positions were carried out in July and August. All samples in August were taken at
207 predawn. At other times samples were collected only from upper stem and stem base. Only
208 osmolality and water potential was measured for Norway spruce.

209 The data of Scots pine saplings has already been published previously (Aaltonen et al. 2017). Samples
210 from Scots pine saplings were collected from needles, inner bark of stem and coarse roots with a
211 scalpel. This procedure, carried out in August 2013, was similar to sampling of mature trees. The
212 stem samples were collected from the middle of the stem. Samples were placed in cryo tubes and
213 stored in liquid nitrogen until measuring. Stem inner bark samples from Norway spruce saplings were
214 collected from 6 individuals each time, which represented one clone per treatment (i.e., one graft
215 each of clone E330, E2833, and K805 from both greenhouse departments) once or twice a month
216 from April to August and once on October. Needles and inner bark of roots were collected in mid-
217 May, mid-June, late July, and mid-October with the stem bark sampling. The samples were collected
218 between 12:00 and 15:00. The sampling locations for needles, stem inner bark, and root inner bark
219 were within the upper 1/3 of the crown, between the whorls of years 2010 and 2011, and the upper
220 coarse roots, respectively. After sampling, the outer bark was immediately removed and all samples
221 placed into cryo tubes, frozen with dry ice (−78°C), and then stored in −80°C until analysis.

222

The osmolality analyses

The sap from inner bark and needles was extracted using a method similar to those previously described by Irvine et al. (1998) and Devaux et al. (2009). We measured the mean osmolality of the needles and inner bark of samples that had undergone the freezing and mechanical sap extraction. The samples were kept at room temperature for 15 to 30 min inside cryotubes to thaw before the sap extraction. After this, a fresh cut was made in the bark samples with a scalpel. Each needle sample included five pairs of needles cut in two. Needle and bark samples were set in silica-based membrane collection tubes (GeneJET Plasmid Miniprep Kit, Thermo Scientific, Massachusetts, USA) the fresh cut downwards against the membrane. Sap was extracted with a centrifuge (Heraeus Fresco 17 Centrifuge, Thermo Scientific, Massachusetts, USA) in 14000 g 10 min prior to osmolality measurement (Devaux et al. 2009). The extracted liquid was immediately moved to osmometer tubes with a pipette and measured with a freezing point osmometer (Osmomat 030 cryoscopic osmometer, Gonotec, Berlin, DE). The exposure to room air was avoided during the sample preparation. Samples were ready for osmometer measurement within an hour from thawing.

The osmolality value was measured as the mean of two repetitions in mature Scots pine in 2011 and 2014. At other times and in other studied trees, only one repetition was used. The cell membranes are expected to be damaged and to have released their contents into the apoplast after rapid freezing in liquid nitrogen (Steponkus 1984; Fuller 2004). The mechanically extracted sap from the needle and inner bark samples therefore consists of a mixture of phloem sap and sap from the surrounding cells and from the apoplast. The phloem cells in particular are expected to die as a result of this kind of procedure (Ball 2004). However, the advantage of mechanical sap extraction with a centrifuge is that no solvents are added, and the results describe actual circumstances in the samples (Devaux et al. 2009). The unit of osmolality measurement is molar concentrations of solutes per mass of water and is expressed as mol kg^{-1}. Osmolality is temperature independent because it is related to mass and not the volume (Lord 1999).

248

249 *Effects of sampling procedure on results describing the phloem dynamics*

250 The inner bark samples, consisting of tissues from the cambium to cork cambium, are a
251 heterogeneous unit and the actual sieve cells, through which the phloem transport takes place, is
252 only a small part of the unit. Measurements targeted accurately at the conducting phloem are
253 difficult to execute in practice. They contain a large risk of error due to the evaporation from samples
254 that occurs during the protocol. Therefore, our approach is less precise but is also supported by
255 previous studies (Rosner et al. 2001; Thompson & Holbrook 2003). The osmotic potential values
256 obtained from the conductive phloem when measured alone, have been reported to be higher but
257 they follow similar dynamics to those obtained in the conducting and non-conducting phloem when
258 measured in combination (Rosner et al. 2001). The *in situ* osmolality seems not to be very different
259 between these two compartments in Norway spruce (Rosner et al. 2001).

260 *Water content of tissue*

261 The fresh weight for water content was measured immediately after sample collection. Then, the
262 samples were stored in a freezer in -20 °C for less than three weeks before they were dried in an
263 oven at 80 °C for 72 hours, after which their dry weight was measured. The water content (WC) of
264 samples was calculated as

265 $$WC = (FW - DW)/DW \quad (1)$$

266 where FW is fresh weight and DW is dry weight.

267

268 *Relative water content*

269 The turgid weight of samples was measured for relative water content calculations. The turgid
270 weight was measured after the samples had been saturated in closed tubes with milli-Q water in a

cold room at 5 °C for 48 hours. Samples were preserved in a cold room at 5 °C for 24 hours before the saturation procedure began. Before weighing, the extra water on the surface of samples was carefully wiped with a tissue. The relative water content (RWC) was calculated as

$$RWC = (FW - DW) / (TW - DW) \quad (2)$$

where TW is the turgid weight.

Osmolality at full saturation

The relative water content (RWC) was used for calculating the osmolality at full saturation as follows (Takami et al. 1981):

$$osMol_{full\ saturation} = osMol_{in\ situ} * RWC \quad (3)$$

where $osMol_{in\ situ}$ is the *in situ* osmolality in mol kg^{-1}.

Sugar concentrations

The soluble sugar concentrations in the needle and inner bark samples were measured at the Natural Resources Institute Finland laboratory. The samples were freeze-dried and milled into powder in a ball mill while frozen. About 50 mg of the powder was weighed into glass test tubes. The soluble sugars were extracted with an 80% aquatic ethanol solution to which meso-erythritol (Sigma) was added as an internal standard. The tubes were placed in an ultrasonic water bath for 45 minutes, and then eluted overnight. The suspension was then shaken and centrifuged at 3000 rpm for 3 min. A 450 µl volume of the supernatant of each sample was pipetted into a separate autosampler vial for three times (three repetitive subsamples). The samples were dried with N_2 flow, placed into a vacuum oven for at least 15 min, and then silylated with 0.5 ml of N-trimethylsilyl imidazole (TMSi in pyridine, Sigma-Aldrich) for at least two hours or overnight, and analysed by gas chromatography–

mass spectrometry (GC–MS; Agilent Hewlett-Packard 6890 GC, fitted with a Zebron ZB-SemiVolatiles column (30 m 9 0.25 mm i.d. 90.25 lm df) and Hewlett-Packard 5973 MSD, EI-MS 70 eV), in which helium was used as the carrier gas (flow 1.5 ml/min). The chromatographic conditions were as follows: an initial temperature of 110 °C; rate of temperature increase 10 °C min^{-1}; a final temperature of 320 °C that was maintained for 14 min; an injector temperature 260 °C, and a split ratio 1:20. The MS-interface temperature was 300 °C and the ion source temperature was 230 °C. The results were calculated using an internal standard and the following external standards: D-fructose (Merck), myo-inositol (Merck), D-glucose (BDH AnalaR, VWR International Ltd., Poole, UK), sucrose (BDH AnalaR), D-raffinose pentahydrate (Fluka), and D-pinitol (Sigma-Aldrich). The results per each sample were calculated as the arithmetic mean of three repetitive subsamples. The sugar concentrations (g_{sugars} / $g_{dry\ weight}$) are calculated to sugar osmolality as

$$\text{sugar osmolality} = \text{sugar concentration} / M / WC \quad (4)$$

where M is the molar mass of the sugar in question and the fresh and dry weights are measured from the same bark sample as the osmolality.

A recent study by Quentin et al. (2015) demonstrated that it is very difficult to obtain reliable absolute values for sugar contents as the results from sugar analysis were shown to vary considerably from one method to another, and from one lab to another. Therefore, we focus here only on the temporal and spatial variation of sugar contents all carried out with the same method in the same lab.

Stem and needle water potential

Stem water potential was measured by sealing the needles of mature trees in small aluminum foil bag for 20 min to equilibrate with the xylem water potential (Scholander et al. 1965; Boyer 1967). Samples were taken for water potential measurements just before each collection of osmolality

samples. The water potential measurement was performed immediately after the sampling cycle using Pump-Up Chamber (PMS Instrument Company, Albany, US). The stem water potential values are the mean of three to five repetitions collected around the canopy that is exposed to light. The time lag between the sampling of stem water potential and osmolality from the needles, branches, upper stem and stem base were on average 5, 7, 20 and 45 minutes, respectively. The water potential in Scots pine saplings was measured as the mean of three individual needles for each sapling that were not equilibrated with xylem water potential and therefore represented the needle water potential. Needles were collected from the saplings around 12:00 and each needle was measured immediately. In Norway spruce saplings, the water potential was measured from branch tips c. 5 cm in length. These samples were collected from the same branch from which the needles for osmolality were collected. The branch tips were sealed by gently wrapping them in aluminum foil inside plastic zipper bags at a minimum of 20 minutes before collection, thus representing the stem water potential. Water potential was measured in a similar way as described above. Water potential of mature Norway spruce represented the needle water potential.

Turgor pressure

The turgor pressure was estimated using the assumptions of xylem and phloem equilibrium

$$\Psi_{xylem} = \Psi_{phloem} \quad (5)$$

where Ψ_{xylem} is the total water potential in xylem and Ψ_{phloem} is the total water potential in phloem. With this assumption, the turgor pressure can be estimated as

$$\Psi_{Pph} = \Psi_{\pi p} + \Psi_{Px} \quad (6)$$

where Ψ_{Pph} is the pressure potential in phloem, $\Psi_{\pi p}$ is the osmotic potential in phloem and Ψ_{Px} is the pressure potential in xylem (e.g. Sovonick-Dunford et al. 1981). The xylem osmotic potential is assumed to be zero in this study. Osmolality is converted into osmotic potential as follows

$$\Psi_{\pi p} = RTc \quad (7)$$

where $\Psi_{\pi p}$ is the osmotic potential (MPa), R is the universal gas constant 8.314 (J mol^{-1} K^{-1}), T is the air temperature (K) at the time of sample collection and c is osmolality (mol g^{-1}).

Statistical analyses

Linear regression analysis was used for statistical testing. The statistical testing was performed using R (v. 3.2.1, 2015, R Foundation for Statistical Computing, Vienna, Austria). Results were considered to be significant when the p-value was lower than 0.5.

RESULTS

The coupling of needle and inner bark osmolality with water potential was clearly seen in both saplings and mature Scots pine. Osmolality correlated with the stem water potential in needles, in branches and the upper stem in mature Scots pine (Fig. 2A). The connection with stem water potential was diminished at the stem base. Osmolality was also found to be a function of water potential in saplings in both Scots pine and Norway spruce. The relationship between osmolality and needle water potential in Scots pine saplings was slightly weaker in comparison to the mature trees (Fig. 2C). In contrast, the correlation with stem water potential was strong in needles and in stem bark in Norway spruce saplings (Fig. 2E). The correlation diminished towards the lowest sampled tree positions in all the studied trees, stem base in mature trees and roots in saplings. The low number of observations prevented firm conclusions about the relationship between osmolality and stem water potential in mature Norway spruce.

The osmolality gradient between higher sample positions and the stem base was significantly correlated with water potential in Scots pine (Fig. 2B and 2D). The gradient between the stem positions diminished when the stem water potential was approximately -0.2 MPa, which approximately corresponded to pre-dawn water potential in the mature Scots pine as soil water potential was very close to zero during all periods (not shown). A small osmotic gradient remained between the branches and stem base at the pre-dawn water potential.

Inner bark osmolality at the two stem positions was negatively correlated with the xylem diameter change measured at the stem base in Scots pine (Fig. 3). Needle osmolality was similarly negatively correlated with xylem diameter but this correlation was much weaker than those of the stem positions (not shown). The correlation between the xylem diameter and inner bark osmolality was the strongest in the stem base where the xylem diameter was measured.

When the daily dynamics of osmolality were examined, osmolality was almost always the highest in the needles and always the lowest at the stem base (Fig. 4). The changes in osmolality were the most consistent from the end of August to September when osmolality increased towards the afternoon for all the sample positions (Fig. 4C & 4D). The distance between needles and bark at the branch sampling position was less than 20 cm while the difference in osmolality was as large as amongst the other positions. This indicates a large transport resistance in xylem and/or phloem over this short distance. The gradient between needles and branch was often close to zero or reversed, which indicated that branch osmolality was sometimes larger than that in the needles. Moreover, the gradient between branches and upper stem seemed to have a large daily variation and was occasionally close to zero or even reversed.

The mean osmolality was the highest in the needles and decreased gradually towards the stem base in every measurement period in mature trees of both species and in the Scots pine saplings (Fig. 5A, 5D and 5E). The Norway spruce saplings had a less clear pattern as it often had higher values in the roots than in the stem (Fig. 5D). The mean water content mainly showed an opposite pattern to that of osmolality (Fig. 5C). The mean water content was high and very similar in both stem positions in July.

Stem water potential and branch turgor pressure correlated at all times. The correlation was highly significant in July and September. The regression line of branch turgor pressure and stem water potential approached zero turgor between -1.7 and -1.6 MPa in all the measurement periods (Fig. 6). The slope of the regression between the turgor pressure and stem water potential differed in July compared to other measurement periods. The mean estimated turgor pressure in Scots pine branches was 0.3, 0.5 and 0.7 MPa for June, July and September, respectively.

The osmolality is a function of the amount of water and the concentration of osmotically active solutes; therefore, we compared osmolality with these two components. Neither water content nor relative water content correlated with osmolality, except in the needles in June for water content (not shown). The mean relative water content decreased gradually from needles to stem base, with only small change in levels of given sample position between the sampling periods. The osmolality at full saturation was used to assess the influence of osmotically active solutes that drive the variation in osmolality (Fig. 7). Osmolality at full saturation varied with the *in situ* osmolality with similar significant regressions between inner bark samples and with highly significant regression in needles. Osmolality at full saturation, i.e. changes in the molar amount of osmotically active substances, explained 36% to 50% of the changes *in situ* osmolality in the inner bark samples and more than 90% in the needles. The mean osmolality at full saturation

showed a much more distinct pattern between the positions compared to mean *in situ* osmolality (Fig. 5A and 5B).

The calculated osmolality of sucrose in the inner bark was axially less variable within a tree compared to those of glucose, fructose and pinitol (Fig. 8A). The ratio of sucrose to monosaccharides decreased from the stem base towards the branch. The amounts of pinitol in the samples were in similar or larger quantities compared to glucose in all bark positions (not shown). The calculated total sugar osmolality was in strong correlation with the measured osmolality (Fig. 8B).

DISCUSSION

Dynamics of inner bark and needle osmolality and water potential

Osmolality is clearly associated with water potential in mature Scots pine and in saplings of Scots pine and Norway spruce. The exceptions to this are the lowest measurement points, i.e. stem base in mature Scots Pine and the roots in saplings of both species. However, the stem base osmolality positively correlate with the xylem diameter changes, which reflect the water potential in the xylem (Irvine and Grace 1997). These results suggest that xylem water potential is driving a significant portion of inner bark osmolality dynamics. Modelling studies suggest that the daily dynamics of transpiration are most likely to influence phloem turgor and its gradients and thus phloem sugar transport (Hölttä et al. 2006; Steppe et al. 2006; Nikinmaa et al. 2014; Steppe et al. 2015). Moreover, De Schepper & Steppe (2010) and De Swaef et al. (2013) combined existing models of coupled xylem and phloem transport with measured stem diameter data, and reported estimations for osmotic pressures in phloem that support the strong interaction between xylem and phloem. Additionally, Mencuccini et al. (2013) and Chan et al. (2016) estimated, based on stem diameter change measurement and a hydraulic model, the osmotic concentration in Scots pine trees to peak during

436 the afternoon and be at its lowest when transpiration ceased. The examples of modelling and data
437 analyses cited above indicate that models with coupled xylem and phloem transport seem to predict
438 the osmotic concentration in phloem realistically, but they ~~had~~ have not been validated against
439 measured data. On the contrary, an MRI study by Windt et al. (2006)
440 conducted on smaller herbaceous plants and poplar saplings showed rather constant phloem
441 transport velocities during daytime while the transporting phloem area was found to vary diurnally. It
442 must be emphasized that measured value of phloem osmotic potential in the present study is for the
443 bulk tissue, and the osmotic potential of sieve cells involved in phloem transport could differ from
444 this. Nevertheless, changes in xylem water potential were shown to affect the osmolality of the bulk
445 phloem tissue, and at least the changes in total water potential can be expected to change in a
446 similar way in the sieve cells as in the other cell types in the phloem (Thompson & Holbrook 2003).
447 The osmotic potential of the needles in our study also describe a heterogeneous bulk since majority
448 of the needle is e.g. mesophyll and transfusion tissue, and xylem and phloem tissues represent only
449 the minority (Liesche et al. 2011). However, also the needle osmolality follows xylem water potential
450 dynamics.

451 The weaker correlation between stem water potential and osmolality measured at the stem base
452 might be because of time lag. Time lag in xylem diameter changes between the upper stem and stem
453 base has been reported to be approximately 30 minutes for a similar distance in the same species at
454 the same site as our measurements (Sevanto et al. 2002). Further, the bark water potential has a
455 time lag with xylem water potential (Pfautsch et al. 2015). However, the time lag in our sampling
456 procedure (see Materials and Methods) more or less cancels out the time lag between the xylem
457 water potential and osmolality for different tree positions when comparing the dynamics of stem
458 water potential and bark osmolality.

459 The relation between the osmolality gradients and the stem water potential is evident in mature
460 Scots pine. When the stem water potential approached pre-dawn conditions, there was only a small

osmolality gradient between branch and stem base. If the turgor gradient is to be driving the phloem transport, then the osmotic potential gradient plus gravitational potential would need to exceed the xylem water potential gradient (Taiz et al. 2015). Our Scots pine trees were c. 18 meters in height, thus the gravitational potential is close to 0.2 MPa at the canopy. Our results indicate that at pre-dawn conditions the osmotic gradient between the upper stem and stem base is zero, which would leave a driving force ~0.2 MPa for phloem transport because of gravity. Therefore, in pre-dawn and rainy conditions the gravitational potential may have a significant role in phloem transport in the stem (Hölttä et al. 2006).

The gravitation could also compensate for some of the resistance that increases with the increasing axial pathway length in taller trees (Hölttä et al. 2013; Ryan & Asao 2014). Small phloem pressure gradients have also been predicted in previous studies (Thompson 2006). Recently, a study by Knoblauch et al. (2016) showed that turgor pressure difference between source and sink was up to 1.65 MPa in 14 meter high herbaceous vine. However, Turgeon (2010) reported that trees tend to have lower turgor gradients compared to herbaceous plants. Additionally, the resistance to phloem sap flow may be expected to be larger in conifers judging from the small size of the sieve cells in relation to sieve tubes in angiosperm trees (Schulz 1992; Jensen et al. 2012; Liesche et al. 2015).

Turgor pressure in branches

Branch turgor pressure followed stem water potential very closely especially in July and September in Scots pine. The trend of regression in our study indicate that inner bark turgor pressure estimates for branches approach zero turgor between stem water potentials -1.7 to -1.6 MPa in all the measurement periods. Generally, stomatal control has been reported to be sensitive in Scots pine (Irvine et al. 1998; Zweifel et al. 2007) to preserve the needle water potentials above -1.5 MPa to prevent widespread xylem embolism in a mature Scots pine stand (Irvine et al. 1998). Although, in

very dry conditions needle water potentials less than -2 MPa have been reported (e.g. Poyatos et al. 2013). Salmon et al. (2015) reported xylem vulnerability curves for Scots pine where the observed embolism increased considerably at needle water potentials below -1.5 MPa. Our results are in agreement with these observations and a loss of turgor seems to occur below this threshold in branches unless osmoregulation prevents it (Morgan 1984; Nikinmaa et al. 2014). The turgor pressure calculated with stem water potential might be a slightly rough estimate because of the radial time lag between the xylem and phloem (Sovonick-Dunford et al. 1981; An et al. 2014). However, we are not aware of any turgor estimates reported for Scots pine branches in any previously published studies.

Axial osmotic gradients and turgor pressure gradients

The mean osmolality values during the daytime were the highest in the needles and the lowest in the stem base in all measurement periods in mature trees and saplings of both species. The spruce saplings differed from other studied trees by having a high osmolality in the stem and the roots in relation to the needles at low water potentials. Decreasing axial osmotic gradients towards the stem base have been reported before, but those studies for the most part have been conducted on Norway spruce or other species than pine trees (Zimmermann 1957; Kaufmann & Kramer 1967; Hammel 1968; Rosner et al. 2001). The axially increasing xylem resistance can be assumed to affect the axial osmolality gradient because xylem and phloem are thought to equilibrate rapidly (Thompson & Holbrook 2003; Hölttä et al. 2006). Furthermore, the decreasing xylem water potential towards the apex has been proposed to be a partial explanation for the decrease in conducting cell size towards the apex (Woodruff 2014). Structural properties of the conducting tissue of both the xylem and phloem are reported to vary axially in conifers (Petit & Crivellaro 2014; Jyske & Hölttä 2015). The decreasing conduit size towards the apex, in both xylem and phloem, is in accordance with our finding of increasing osmotic potential gradient towards the apex (Jensen et al. 2012). The

daily dynamics of osmolality in the canopy positions of mature Scots pine momentarily overlapped in June and July, and the gradients were the most consistent with osmolality decreasing towards the stem base in both autumn periods. This could indicate to seasonal differences, and the brief overlapping of branch and upper stem osmolality that phloem transport could temporarily reverse the direction towards the needles during daytime (Hölttä et al. 2006; Nikinmaa et al. 2014). Turgor profiles for the whole tree would be possible with our osmolality measurements, if the xylem water potentials along the tree axial gradient were also known. It can be estimated from our results that the mean xylem water potential in the stem base would have to be higher than -0.8 MPa in June and July, and higher than -0.6 MPa in September for the mean turgor gradient of inner bark to be in the direction towards the roots and not towards the branch during the daytime.

Osmolality values compared to literature

The osmolality of the needles is similar in magnitude as that reported earlier by Irvine et al. (1998) for mature Scots pine, who reported values between 0.6 and 0.9 mol kg^{-1}. Fu et al. (2011) reported mean leaf osmolality in species considered as passive loaders to be c. 0.8 mol kg^{-1}, which is similar to the mean osmolality in our dataset for July and September. The mean osmolalities in the upper stem and stem base in Scots pine are higher than that previously reported for Norway spruce. Rosner et al. (2001) reported *in situ* upper stem osmolalities from c. 0.4 to 0.5 mol kg^{-1} for the conductive and the non-conductive phloem, respectively, in Norway spruce in July. Kraemer (1953) reported osmolality c. 0.3 to 0.5 mol kg^{-1} in the stem in Norway spruce.

Influence of water and solutes on osmolality

In theory, both an increase in transpiration and photosynthesis should affect the osmolality in phloem because the transpiration decreases the water content and photosynthesis increases the amount of sugars. Our osmolality data was also compared against photosynthetically active radiation (PAR) but no clear association there was seen (not shown). Relative water content decreased from the tree top to base. This is reasonable as the turgor pressure is also expected to follow the same trend. Water content per dry weight shows an opposite pattern, but in addition to water status, it reflects also the anatomical properties of the tissue (Rosner et al. 2001).

Significant temporal and spatial patterns have been reported for water status in the vertical profile of trees (Rosner et al. 2001; Gall et al. 2002; Woodruff 2014). Additionally, in study by Lintunen et al. (2016) the water content had a significant role in generating the osmolality levels of branch inner bark in different latitudes around Europe. Osmolality at full saturation explain *in situ* osmolality with 30% to 50% regression in branches and stem positions when all the sampling periods are examined together in our study. The relationship between the *in situ* osmolality and osmolality at full saturation in the needles is different in June compared to other studied periods, which could be due to active shoot growth during that time (Hansen & Beck 1994).

Phloem osmotic concentration has been reported to be mainly driven by its sugar concentrations, especially the sucrose concentration (Pate 1976; Rennie & Turgeon 2009). The mean sucrose concentration remained temporally and spatially constant compared to monosaccharides at all the sampling positions in our study. The relation of sucrose to the sum of glucose, fructose and pinitol increased from the branches towards the stem base. These observations indicate that the variations in inner bark osmolality are driven more by the monosaccharides and pinitol than by sucrose. Additionally, Woodruff (2014) reported that the sugar content increased as the result of increases in monosaccharides when the water potential decreased in Douglas-fir. Thus, sucrose had no significant spatial trend unlike that for the monosaccharides. Other studies have also found sugar

concentrations to increase with decreasing water potential (e.g. Weatherley et al. 1959). The measured osmolality strongly correlated with the total sugar osmolality calculated from the sugar concentrations in a daily scale, in both July and September. Devaux et al. (2009) reported that total sugar contents changed on a monthly scale and that significant daily variations could not be observed in phloem sap of maritime pine. However, our sugar concentrations represent the sugar content of the bulk needle and inner bark in addition to the phloem sap.

CONCLUSIONS

Our results indicate that xylem water potential considerably influences the needle and inner bark osmotic potentials and their gradients in addition to branch turgor pressures. These findings are in line with theory and the models that emphasize a close relationship between xylem and phloem transport in trees. Gravitation seems to have a considerable effect on the driving force of phloem transport in mature trees. This seems to be especially the case in the stem where the osmotic concentration gradients and predicted pre-dawn turgor pressure gradients were found to be very small. The axial osmotic gradients from the needles to stem base are evident in all studied periods but seem to be coherent at the whole tree level in the autumn. Both water and sugars seem to be significantly involved in the changes in osmolality in the short term. Our data on sugar concentrations, although limited, suggest that sucrose concentration remains rather constant on both the temporal and spatial scales whereas monosaccharide concentrations vary much more.

ACKNOWLEDGEMENTS

This work was supported by the Academy of Finland grants #268342 and #272041. We are grateful to Hanna el-Khouri for conducting the sugar analyses in Natural Resources Institute Finland, and Laura

585 Nikinmaa for helping with the data collection. We also want to thank Yann Salmon for useful
586 thoughts in interpretation of results.

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588 **REFERENCES**

589 Aaltonen H., Lindén A., Heinonsalo J., Biasi C., Pumpanen J. (2016) Effects of prolonged drought stress on
590 Scots pine seedlings carbon allocation. Tree physiology 1-10.

591 An, F., Cahill, D., Rookes, J., Lin, W. & Kong, L. (2014) Real-time measurement of phloem turgor pressure in
592 hevea brasiliensis with a modified cell pressure probe. Botanical Studies 55(1): 1.

593 Anfodillo, T., Carraro, V., Carrer, M., Fior, C., & Rossi, S. (2006) Convergent tapering of xylem conduits in
594 different woody species. New Phytologist, *169*(2), 279-290.

595 Ball, M. C., Canny, M. J., Huang, C. X., & Heady, R. D. (2004) Structural changes in acclimated and
596 unacclimated leaves during freezing and thawing. Functional Plant Biology, 31(1), 29-40.

597 Boyer, J. (1967) Leaf water potentials measured with a pressure chamber. Plant Physiology, *42*(1), 133-137.

598 Chan, T., Hölttä, T., Berninger, F., Mäkinen, H., Nöjd, P., Mencuccini, M. & Nikinmaa, E. (2016) Separating
599 water-potential induced swelling and shrinking from measured radial stem variations reveals a cambial growth
600 and osmotic concentration signal. Plant, Cell & Environment 39(2): 233-244.

601 Christy, A. L., & Ferrier, J. M. (1973) A mathematical treatment of Munch's pressure-flow hypothesis of phloem
602 translocation. Plant Physiology, 52(6), 531-538.

603 Cochard, H., Bréda, N. & Granier, A. (1996) Whole tree hydraulic conductance and water loss regulation in
604 Quercus during drought: evidence for stomatal control of embolism? 53(2-3): 197-206.

605 Dannoura, M., Maillard, P., Fresneau, C., Plain, C., Berveiller, D., Gerant, D. et al. (2011) In situ assessment of
606 the velocity of carbon transfer by tracing 13C in trunk CO2 efflux after pulse labelling: Variations among tree
607 species and seasons. New Phytologist 190(1): 181-192.

608 De Schepper, V. & Steppe, K. (2010) Development and verification of a water and sugar transport model using
609 measured stem diameter variations. Journal of experimental botany 61(8): 2083-2099. 10.1093/jxb/erq018 [doi].

610 Devaux, M., Ghashghaie, J., Bert, D., Lambrot, C., Gessler, A., Bathellier, C. et al. (2009) Carbon stable isotope
611 ratio of phloem sugars in mature pine trees throughout the growing season: Comparison of two extraction
612 methods. Rapid Communications in Mass Spectrometry 23(16): 2511-2518.

613 De Swaef, T., Driever, S. M., Van Meulebroek, L., Vanhaecke, L., Marcelis, L. F., & Steppe, K. (2013)
614 Understanding the effect of carbon status on stem diameter variations. *Annals of botany*, *111*(1), 31-46.

615 Dixon, H. H. & Joly, J. (1894) On the ascent of sap. Proceedings of the Royal Society of London 57(340-346):
616 3-5.

617 Epron, D., Cabral, O. M. R., Laclau, J. P., Dannoura, M., Packer, A. P., Plain, C., ... & Gérant, D. (2016) In situ
618 13CO2 pulse labelling of field-grown eucalypt trees revealed the effects of potassium nutrition and throughfall
619 exclusion on phloem transport of photosynthetic carbon. Tree physiology, 36(1), 6-21.

620 Fu, Q., Cheng, L., Guo, Y. & Turgeon, R. (2011) Phloem loading strategies and water relations in trees and
621 herbaceous plants. Plant Physiology 157(3): 1518-1527. 10.1104/pp.111.184820.

622 Fuller, B. J. (2004) Cryoprotectants: The essential antifreezes to protect life in the frozen state. CryoLetters
623 25(6): 375-388.

624 Gall, R., Landolt, W., Schleppi, P., Michellod, V. & Bucher, J. B. (2002) Water content and bark thickness of
625 Norway spruce (Picea abies) stems: Phloem water capacitance and xylem sap flow. Tree
626 physiology 22(9): 613-623.

627 Gould, N., Thorpe, M. R., Koroleva, O., & Minchin, P. E. (2005) Phloem hydrostatic pressure relates to solute
628 loading rate: a direct test of the Münch hypothesis. Functional Plant Biology, 32(11), 1019-1026.

629 Hammel, H. T. (1968) Measurement of turgor pressure and its gradient in the phloem of oak. Plant Physiology
630 43(7): 1042-1048.

631 Hansen, J. & Beck, E. (1994) Seasonal changes in the utilization and turnover of assimilation products in 8-year-
632 old Scots pine (Pinus sylvestris L.) trees. Trees 8(4): 172-182.

633 Hari, P., Nikinmaa, E., Pohja, T., Siivola, E., Bäck, J., Vesala, T., & Kulmala, M. (2013) Station for measuring
634 ecosystem-atmosphere relations: SMEAR. In Physical and Physiological Forest Ecology (pp. 471-487). Springer
635 Netherlands.

636 Hölttä, T., Vesala, T., Sevanto, S., Perämäki, M. & Nikinmaa, E. (2006) Modeling xylem and phloem water
637 flows in trees according to cohesion theory and ~~münch~~ Münch hypothesis. Trees 20(1): 67-78.

638 Hölttä, T., Kurppa, M. & Nikinmaa, E. (2013) Scaling of xylem and phloem transport capacity and resource
639 usage with tree size. Frontiers in plant science 4496. 10.3389/fpls.2013.00496.

640 Irvine, J. & Grace, J. (1997) Continuous measurements of water tensions in the xylem of trees based on the
641 elastic properties of wood. Planta 202(4): 455-461.

642 Irvine, J., Perks, M. P., Magnani, F. & Grace, J. (1998) The response of Pinus sylvestris to drought: Stomatal
643 control of transpiration and hydraulic conductance. Tree Physiology 18(6): 393-402.

644 Jensen, K. H., Liesche, J., Bohr, T. & Schulz, A. (2012) Universality of phloem transport in seed plants. Plant,
645 Cell & Environment 35(6): 1065-1076.

646 Junninen, H., Lauri, A., Keronen, P., Aalto, P., Hiltunen, V., Hari, P., Kulmala, M. (2009) Smart-SMEAR: on-
647 line data exploration and visualization tool for SMEAR stations. Boreal Environment Research 14, 447–457.

648 Jyske, T. & Hölttä, T. (2015) Comparison of phloem and xylem hydraulic architecture in Picea abies stems. New
649 Phytologist 205(1): 102-115.

650 Jyske T., Suuronen, J-P., Pranovich, A., Laakso, T., Watanabe, U., Kuroda K., Abe, H. (2015) Seasonal variation
651 in formation, structure, and chemical properties of phloem in Picea abies as studied by novel microtechniques.
652 Planta 242: 613–629.

653 Kaufmann, M. R. & Kramer, P. J. (1967) Phloem water relations and translocation. Plant Physiology 42(2): 191-
654 194.

Knoblauch, M. & Peters, W. S. (2013) Long-distance translocation of photosynthates: A primer. Photosynthesis Research 117(1-3): 189-196.

Knoblauch, M. & van Bel, A. J. (1998) Sieve tubes in action. The Plant Cell 10(1): 35-50.

Knoblauch, M., Knoblauch, J., Mullendore, D. L., Savage, J. A., Babst, B. A., Beecher, S. D. et al. (2016) Testing the Münch hypothesis of long distance phloem transport in plants. eLife 5e15341.

Kraemer, G. (1953) Die kritischen grenzen der brutbaumdisposition für borkenkäferbefall an fichte (Picea excelsa L.)*). Zeitschrift für Angewandte Entomologie 34(4): 463-512.

Lee, D. (1981) Synchronous pressure-potential changes in the phloem of Fraxinus americana L. Planta 151(4): 304-308.

Liesche, J., Martens, H. J. & Schulz, A. (2011) Symplasmic transport and phloem loading in gymnosperm leaves. Protoplasma 248(1): 181-190.

Liesche, J., Windt, C., Bohr, T., Schulz, A., & Jensen, K. H. (2015) Slower phloem transport in gymnosperm trees can be attributed to higher sieve element resistance. *Tree physiology*, *35*(4), 376-386.

Lintunen, A., Paljakka, T., Jyske, T., Peltoniemi, M., Sterck, F., Von Arx, G., … & Gebauer, R. ~~et al.~~ (2016) Osmolality and non-structural carbohydrate composition in the secondary phloem of trees across a latitudinal gradient in Europe. Frontiers in Plant Science, 7.

Lockhart, J. A. (1965) An analysis of irreversible plant cell elongation. Journal of theoretical biology 8(2): 264-275.

Lord, R. C. (1999) Osmosis, osmometry, and osmoregulation. Postgraduate medical journal 75(880): 67-73.

Mäkelä, A., Berninger, F. & Hari, P. (1996) Optimal control of gas exchange during drought: Theoretical analysis. Annals of Botany 77(5): 461-468.

Mencuccini, M. & Hölttä, T. (2010) The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. New Phytologist 185(1): 189-203.

678 Mencuccini, M., Hölttä, T., Sevanto, S. & Nikinmaa, E. (2013) Concurrent measurements of change in the bark
679 and xylem diameters of trees reveal a phloem-generated turgor signal. New Phytologist 198(4): 1143-1154.

680 Minchin, P. E. H., & Lacointe, A. (2005) New understanding on phloem physiology and possible consequences
681 for modelling long-distance carbon transport. New Phytologist, 166(3), 771-779.

682 Molz, F. J., Klepper, B. & Browning, V. D. (1973) Radial diffusion of free energy in stem phloem: An
683 experimental study. Agronomy Journal 65(2): 219-222.

684 Morgan, J. M. (1984) Osmoregulation and water stress in higher plants. Annual review of plant physiology,
685 35(1), 299-319.

686 Münch, E. (1930) Stoffbewegungen in der ~~pflanze~~Pflanze.

687 Netherer, S., Matthews B., Katzensteiner K., Blackwell E., Henschke P., Hietz P., Pennerstorfer J., Rosner S.,
688 Kikuta S., Schume H., Schopf A. (2015) Do water limiting conditions predispose Norway spruce to bark beetle
689 attack? New Phytologist. 205(3): 1128-1141. DOI: 10.1111/nph.13166.

690 Nikinmaa, E., Hölttä, T., Hari, P., Kolari, P., Mäkelä, A., Sevanto, S. & Vesala, T. (2013) Assimilate transport in
691 phloem sets conditions for leaf gas exchange. Plant, Cell & Environment 36(3): 655-669.

692 Nikinmaa, E., Sievanen, R. & Holtta, T. (2014) Dynamics of leaf gas exchange, xylem and phloem transport,
693 water potential and carbohydrate concentration in a realistic 3-D model tree crown. Annals of botany 114(4):
694 653-666.

695 Nobel, P. S. (2005) Physiochemical and Environmental Plant Physiology, 3rd Edn. New York, NY: WH
696 Freeman and Company.

697 Pate, J. S. (1976) Nutrients and metabolites of fluids recovered from xylem and phloem: significance in relation
698 to long-distance transport in plants. Transport and transfer processes in plants, 253-281.

699 Paul, M. J. & Foyer, C. H. (2001) Sink regulation of photosynthesis. Journal of experimental botany 52(360):
700 1383-1400.

Petit, G. & Crivellaro, A. (2014) Comparative axial widening of phloem and xylem conduits in small woody plants. Trees 28(3): 915-921.

Pfautsch, S., Renard, J., Tjoelker, M. G. & Salih, A. (2015) Phloem as capacitor: Radial transfer of water into xylem of tree stems occurs via symplastic transport in ray parenchyma. Plant Physiology 167(3): 963-971. 10.1104/pp.114.254581 [doi].

Pirinen, P., Simola, H., Aalto, J., Kaukoranta, J., Karlsson, P. & Ruuhela, R. (2012) Tilastoja suomen ilmastosta 1981–2010. The Finnish Meteorological Institute 1.

Poyatos, R., Aguadé, D., Galiano, L., Mencuccini, M., & Martínez-Vilalta, J. (2013) Drought-induced defoliation and long periods of near-zero gas exchange play a key role in accentuating metabolic decline of Scots pine. New Phytologist, 200(2), 388-401.

Quentin A.G., Pinkard E.A., Ryan M.G., Tissue D.T., Baggett L.S., Adams, H. D., et al. (2015) Non-structural carbohydrates in woody plants compared among laboratories. TreePhysiol. 35,1146–1165. doi:10.1093/treephys/tpv073.

Rennie, E. A. & Turgeon, R. (2009) A comprehensive picture of phloem loading strategies. Proceedings of the National Academy of Sciences of the United States of America 106(33): 14162-14167. 10.1073/pnas.0902279106 [doi].

Rogers, S. & Peel, A. (1975) Some evidence for the existence of turgor pressure gradients in the sieve tubes of willow. Planta 126(3): 259-267.

Rosner, S., Baier, P. & Kikuta, S. B. (2001) Osmotic potential of Norway spruce [Picea abies (L.) karst.] secondary phloem in relation to anatomy. Trees 15(8): 472-482.

Ryan, M. G. & Asao, S. (2014) Phloem transport in trees. Tree physiology 34(1): 1-4. 10.1093/treephys/tpt123 [doi].

Salmon, Y., Torres-Ruiz, J. M., Poyatos, R., Martinez-Vilalta, J., Meir, P., Cochard, H., & Mencuccini, M. (2015) Balancing the risks of hydraulic failure and carbon starvation: a twig scale analysis in declining Scots pine. Plant, cell & environment, 38(12), 2575-2588.

Savage, J. A., Clearwater, M. J., Haines, D. F., Klein, T., Mencuccini, M., Sevanto, S. et al. (2015) Allocation, stress tolerance and carbon transport in plants: How does phloem physiology affect plant ecology? Plant, Cell & Environment.

Scholander, P. F., Bradstreet, E. D., Hemmingsen, E. A., & Hammel, H. T. (1965) Sap pressure in vascular plants. *Science*, *148*(3668), 339-346.

Schulz, A. (1990) Conifers. In: Behnke, H. D., & Sjolund, R. D. (Eds.). (2012). Sieve elements: comparative structure, induction and development. Springer Science & Business Media.

Schulz, A. (1992) Living sieve cells of conifers as visualized by confocal, laser-scanning fluorescence microscopy. *Protoplasma*, *166*(3), 153-164.

Sevanto, S., Vesala, T., Perämäki, M. & Nikinmaa, E. (2002) Time lags for xylem and stem diameter variations in a ~~scots~~ Scots pine tree. Plant, Cell & Environment 25(8): 1071-1077.

Sevanto, S. (2014) Phloem transport and drought. Journal of experimental botany 65(7): 1751-1759. 10.1093/jxb/ert467.

Sovonick-Dunford, S., Lee, D. R. & Zimmermann, M. H. (1981) Direct and indirect measurements of phloem turgor pressure in white ash. Plant Physiology 68(1): 121-126.

Steponkus, P. L. (1984) Role of the plasma membrane in freezing injury and cold acclimation. Annual Review of Plant Physiology 35(1): 543-584.

Steppe, K., De Pauw, D. J., Lemeur, R., & Vanrolleghem, P. A. (2006) A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. Tree physiology, 26(3), 257-273.

Steppe, K., Sterck, F. & Deslauriers, A. (2015) Diel growth dynamics in tree stems: Linking anatomy and ecophysiology. Trends in plant science 20(6): 335-343.

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015) *Plant physiology and development*. Sinauer Associates, Incorporated.

Takami, S., Turner, N. C., & Rawson, H. M. (1981) Leaf expansion of four sunflower (Helianthus annuus L) cultivars in relation to water deficits. I. Patterns during plant development. Plant, Cell & Environment, 4(5), 399-407.

Thompson, M. V. (2006) Phloem: The long and the short of it. Trends in plant science 11(1): 26-32.

Thompson, M. & Holbrook, N. (2003) Scaling phloem transport: Water potential equilibrium and osmoregulatory flow. Plant, Cell & Environment 26(9): 1561-1577.

Turgeon, R. & Wolf, S. (2009) Phloem transport: Cellular pathways and molecular trafficking. Annual review of plant biology 60207-221.

Turgeon, R. (2010) The puzzle of phloem pressure. Plant Physiology 154(2): 578-581. 10.1104/pp.110.161679 [doi].

van Bel, A. J. (2003) The phloem, a miracle of ingenuity. Plant, Cell & Environment 26(1): 125-149.

Vanhatalo, A., Chan, T., Aalto, J., Korhonen, J., Kolari, P., Hölttä, T. et al. (2015) Tree water relations can trigger monoterpene emissions from Scots pine stems during spring recovery. Biogeosciences 12(18): 5353-5363.

Weatherley, P., Peel, A. & Hill, G. (1959) The physiology of the sieve tube Preliminary experiments using aphid mouth parts. Journal of experimental botany 10(1): 1-16.

Windt, C. W., Vergeldt, F. J., De Jager, P. A., & Van As, H. (2006) MRI of long-distance water transport: a comparison of the phloem and xylem flow characteristics and dynamics in poplar, castor bean, tomato and tobacco. Plant, Cell & Environment, 29(9), 1715-1729.

Woodruff, D., Bond, B. & Meinzer, F. (2004) Does turgor limit growth in tall trees? Plant, Cell & Environment 27(2): 229-236.

Woodruff, D. R. (2014) The impacts of water stress on phloem transport in Douglas-fir trees. Tree physiology 34(1): 5-14. 10.1093/treephys/tpt106 [doi].

Zimmermann, M. H. (1960) Transport in the phloem. Annual Review of Plant Physiology 11(1): 167-190.

Zimmermann, M. H. (1957) Translocation of organic substances in trees. I. ~~the~~ The nature of the sugars in the sieve tube exudate of trees. Plant Physiology 32(4): 288-291.

Zweifel, R., Steppe, K., & Sterck, F. J. (2007) Stomatal regulation by microclimate and tree water relations: interpreting ecophysiological field data with a hydraulic plant model. Journal of Experimental Botany, 58(8), 2113-2131.

Table 1. Environmental variables at the measurement site of the Scots pine stand

	August 2011	July 2014	September 2014	June 2015
Monthly mean air tempreature, °C	15.2	18.6	10.1	11.9
Mean air temperature in the measurement period, °C	10.3	23.2	12.7	9.4
Maximum air temperature in the measurement period, °C	16.5	29.3	18.3	14.5
Monthly precipitation, mm	83.1	44.1	22.1	81.5
Volumetric soil water content in the measurement period (depth 2-36 cm), $m^3\ m^{-3}$	0.279	0.211	0.213	0.222

790 The mean monthly air temperature and precipitation for the site was obtained from the Finnish
791 meteorological institute weather archives. Other variables are calculated with the data from Smart
792 Smear data service (Junninen et al. 2009).

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805 **Table 2.** Number of samples collected in measurement periods

	Scots pine mature trees 2011 Aug. 29th-Sep. 2nd tree A	2014 Jul. 22nd-23rd tree B	2014 Sep. 11th-12th tree A	2015 Jun. 16th-17th tree B	Norway spruce mature trees 2012 May-Aug. tree A and B	Scots pine saplings 2013 Aug.	Norway spruce saplings 2012 May-Aug.
Needles	10	7	6	9	4	31	10
Branches	10	8	6	9	4		
Upper stem	10	6	6	8	6	33	31
Stem base	10	8	6	8	6		
Ground level					4		
Roots						31	4
Water potential	10	8	6	9	6	33	32
Sugar concentration		3-4 per pos.[b]	3-4 per pos.[b]				
Water content	osm. nr[a]	osm. nr[a]	osm. nr[a]	osm. nr[a]			
Relative water content		osm. nr[a]	osm. nr[a]	osm. nr[a]			

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807 [a] The number of samples was the same as the collected osmolality samples.

808 [b] Number of the sugar samples per position during the sampling period.

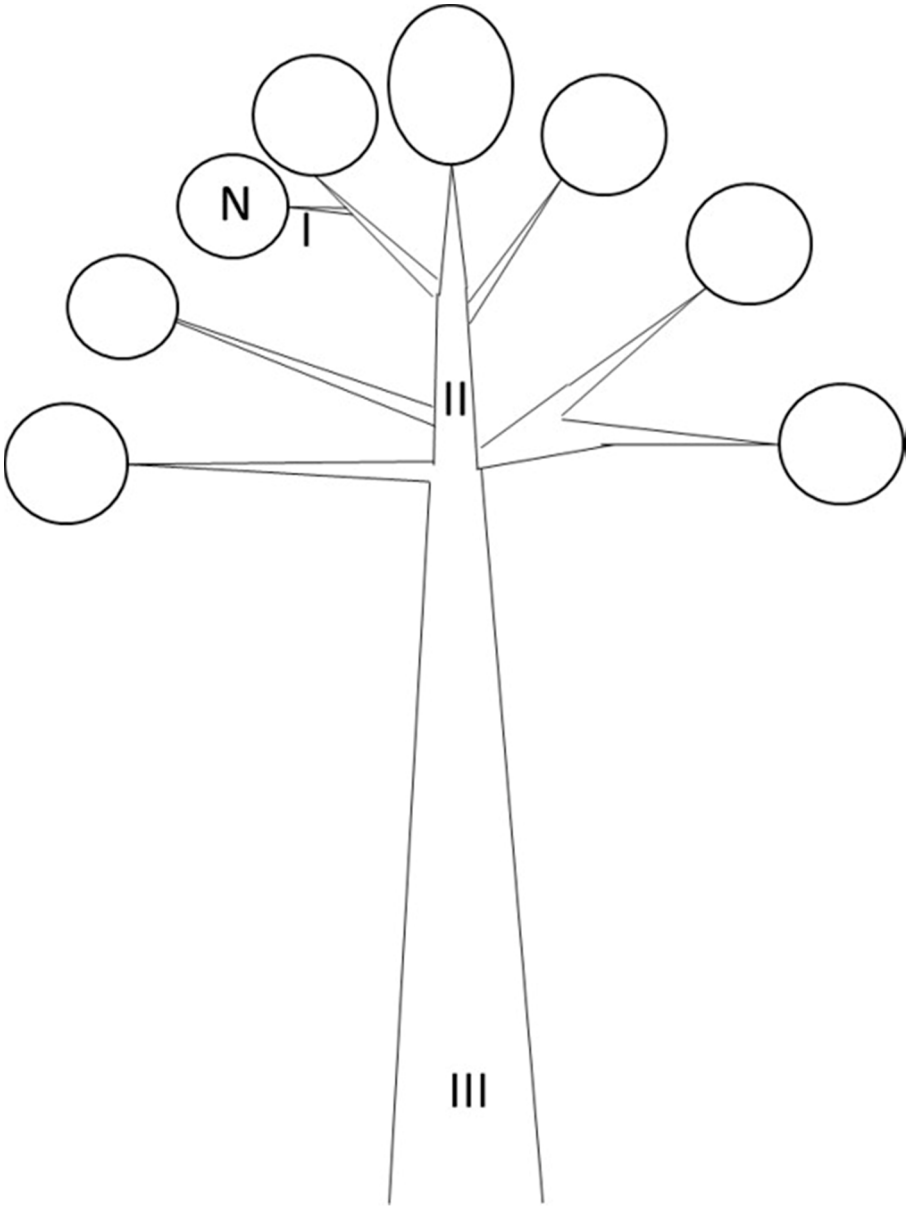

Figure 1. Illustration of sampling positions of mature Scots pine where N, I, II and III stand for needles, branches, upper stem and stem base, respectively.

91x121mm (150 x 150 DPI)

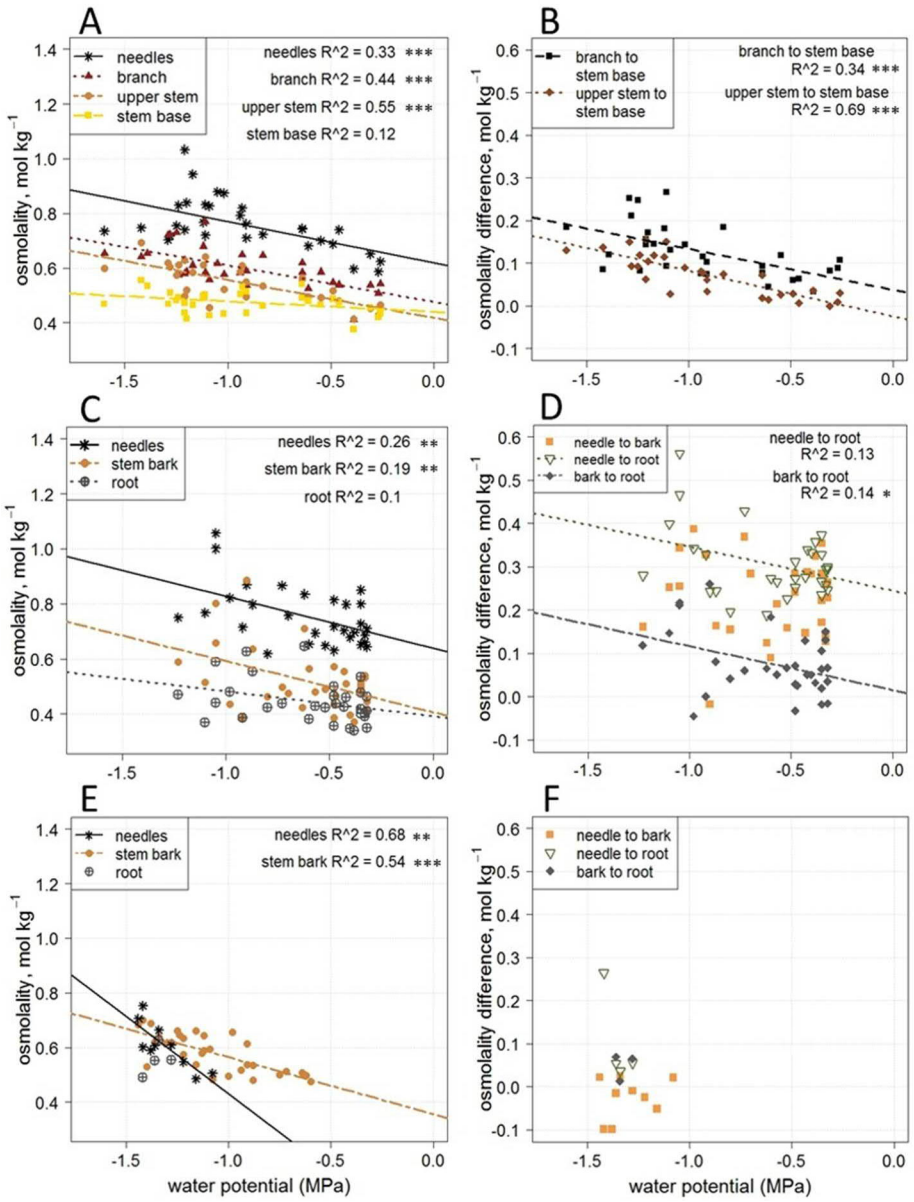

Figure 2. Osmolality in situ (A, C, E) and osmolality difference (B, D, F) as a function of stem water potential in mature Scots pine (A, B) and Norway spruce saplings (E, F) and as a function of needle water potential in Scots pine saplings (C, D). Statistically tested p-value is in symbols; *, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$. The linear regression equations of osmolality and water potential are in mature Scots pine (A) y=0.62-0.15x (needles); y=0.48-0.13x (branch); y=0.42-0.14x (upper stem); y=0.44-0.04x (stem base); and in Scots pine saplings (C) y=0.64-0.19x (needles); y=0.41-0.19x (stem bark); y=0.39-0.09x (root); and in Norway spruce saplings (E) y=-0.14-0.57x (needles); y=0.35-0.21x (stem bark). The linear regression equations of osmolality difference and water potential are in mature Scots pine (B) y=0.04-0.1x (branch to stem base); y=-0.02-0.11x (upper stem to stem base); and in Scots pine saplings (D) y=0.25-0.1x (needle to root); y=0.02-0.1x (bark to root).

147x189mm (148 x 150 DPI)

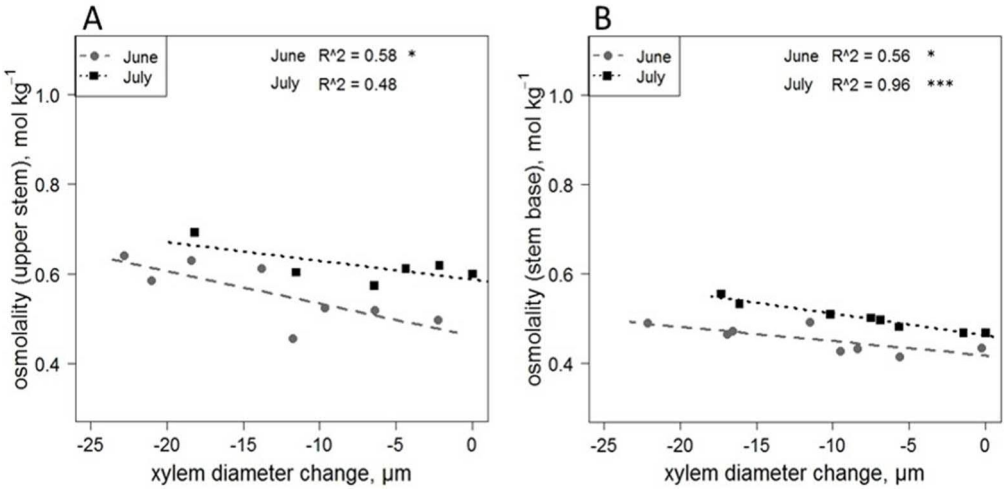

Figure 3. Osmolality in situ of stem positions (A & B) and xylem diameter change measured in 1.5 m height in mature Scots pine in July 2014 and June 2015. Statistically tested p-value is in symbols; *, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$.

225x106mm (98 x 101 DPI)

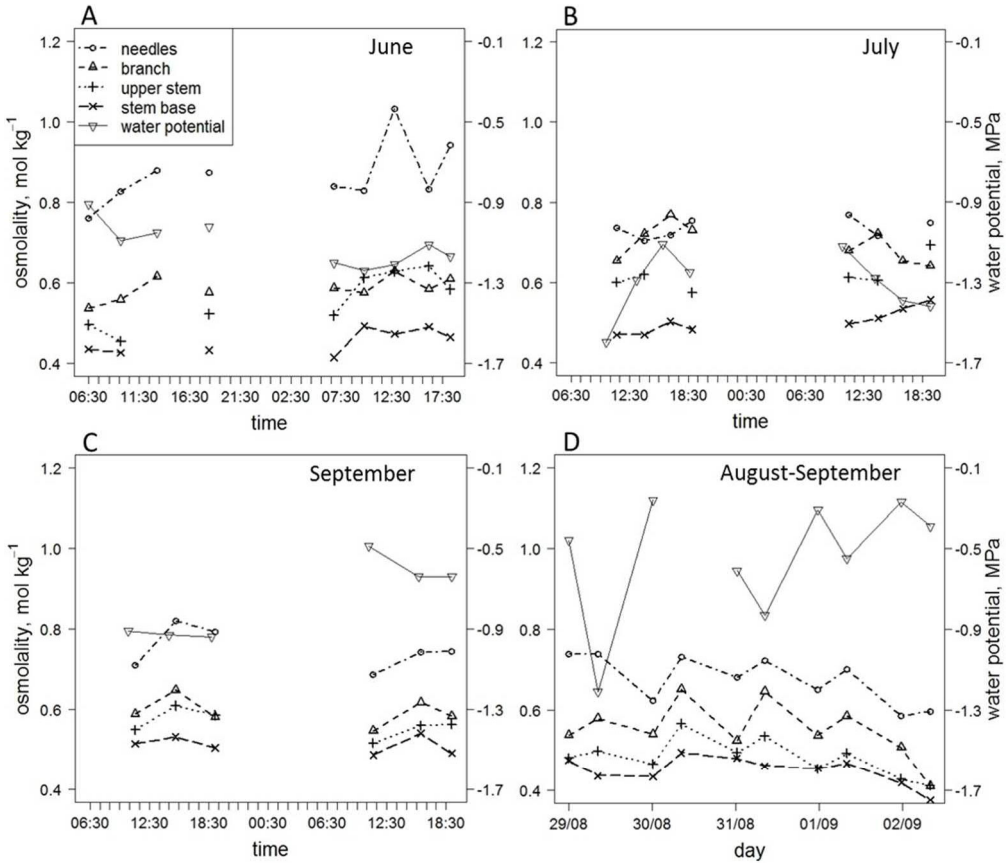

Figure 4. Osmolality in situ in needles, branches, upper stem and stem base, and stem water potential in daytime in Scots pine in June 2015 (A), July (B) and September (C) 2014, and early autumn 2011 (D). In figure D, the measurement points at the date ticks are measured around 7 a.m. whereas points between the ticks are measured around 3 p.m.

218x185mm (131 x 133 DPI)

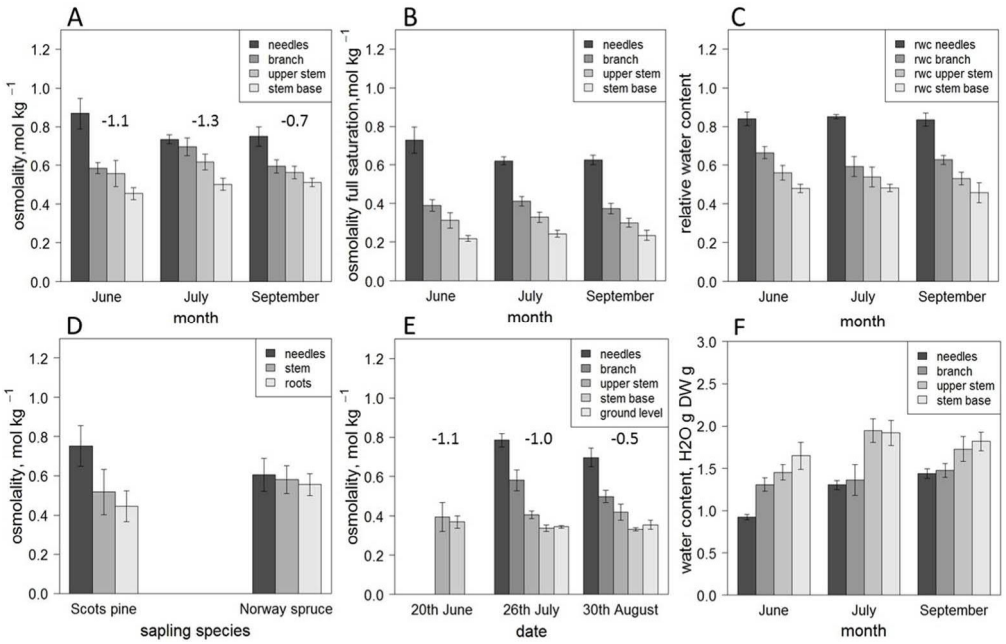

Figure 5. Mean of osmolality in situ (A), osmolality at full saturation (B), mean relative water content (C) and mean water content (F) in mature Scots pine. Osmolality in situ in Scots pine and Norway spruce saplings (D) and in mature Norway spruce (E). The mean stem water potential (MPa) during the measurement period is shown as values in figures A and E. The error bars are standard deviations.

262x163mm (129 x 132 DPI)

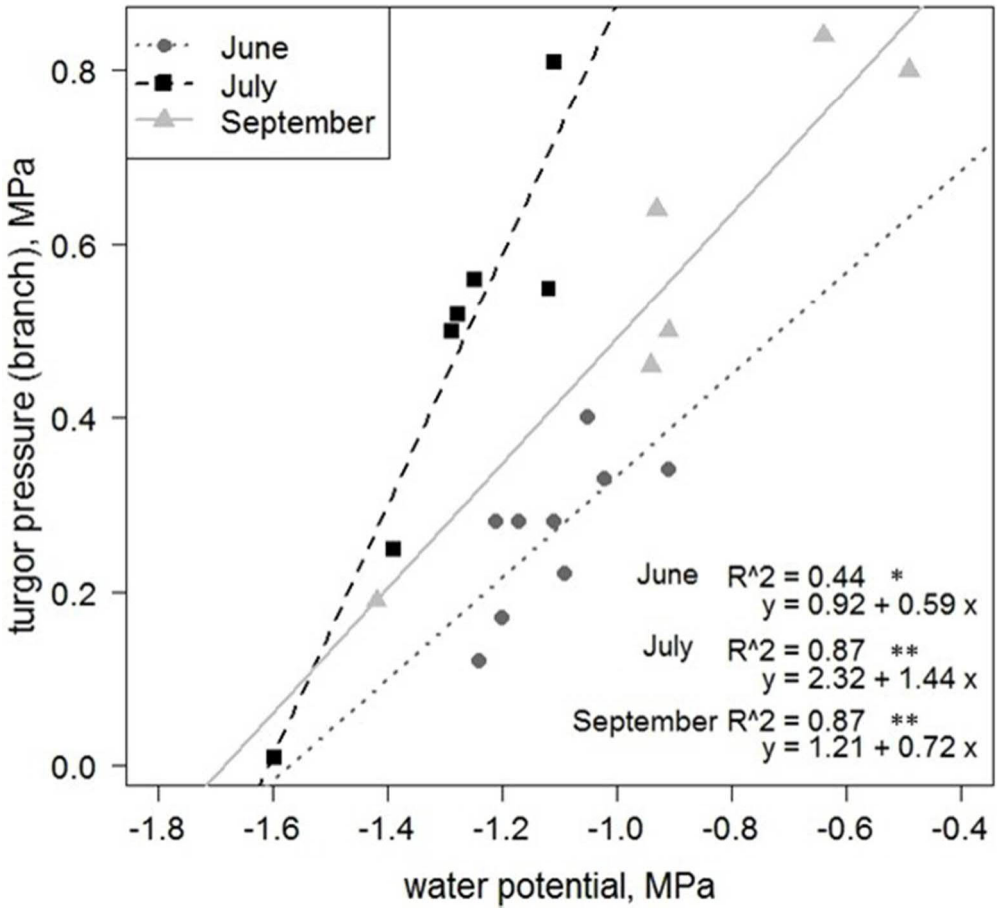

Figure 6. Inner bark turgor pressure estimate in Scots pine branches in July and September 2014, and in June 2015 as a function of stem water potential. Statistically tested p-value is in symbols; *, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$.

145x132mm (104 x 104 DPI)

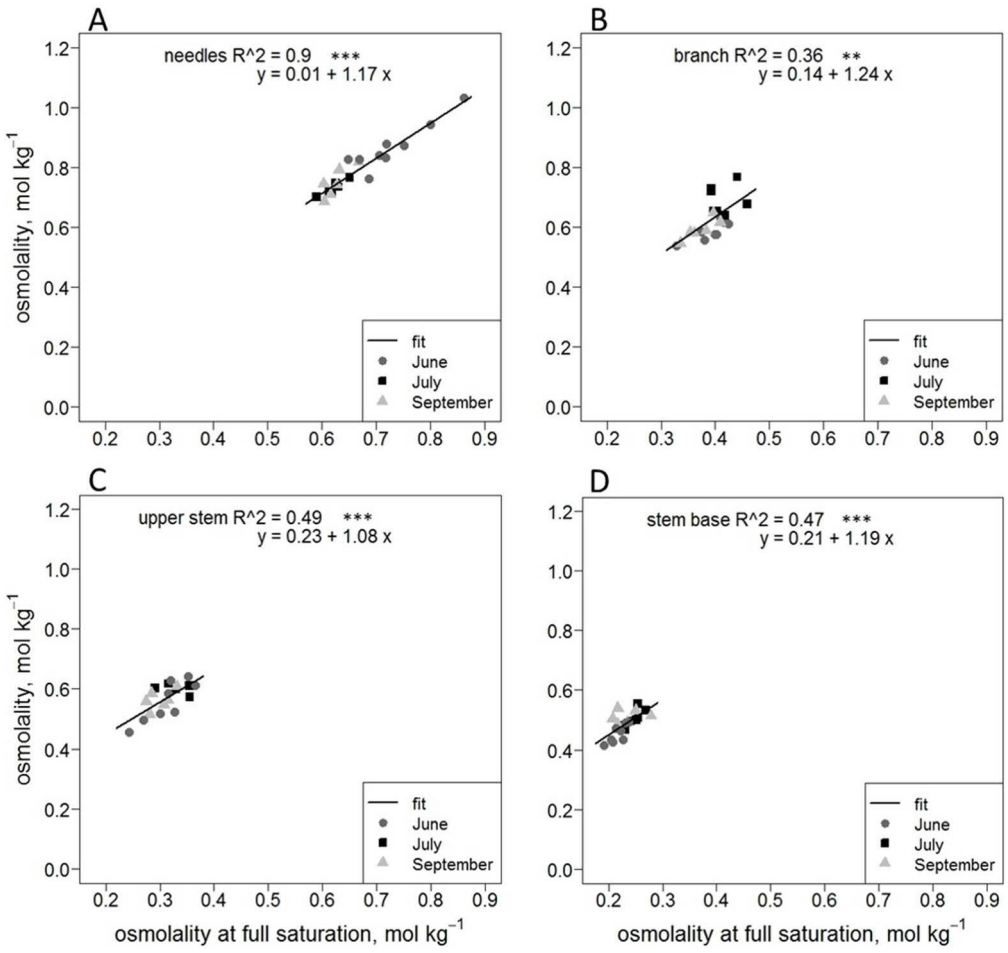

Figure 7. Osmolality in situ and osmolality at full saturation in needles (A), branches (B), upper stem (C) and stem base (D) in mature Scots pine in July and September 2014, and in June 2015. Statistically tested p-value is in symbols; *, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$.

185x173mm (147 x 149 DPI)

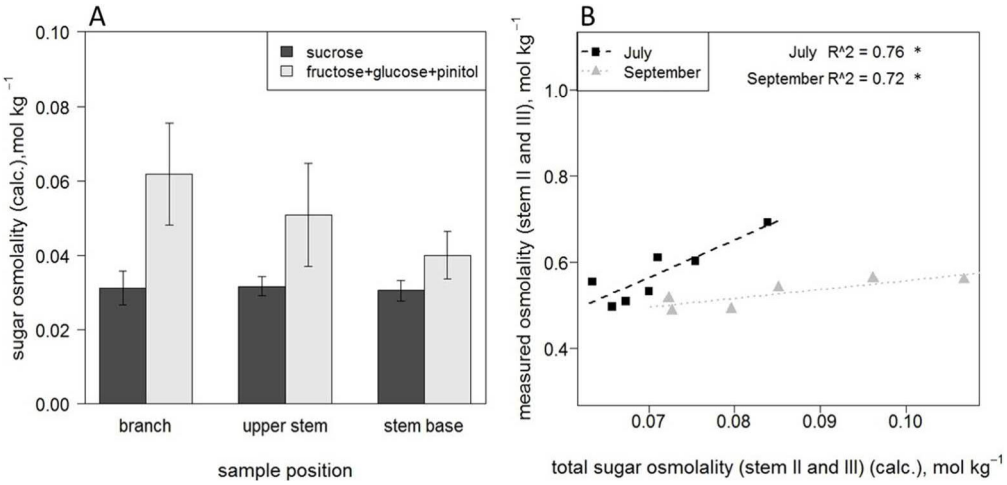

Figure 8. The mean of calculated sucrose compared to sum of fructose, glucose and pinitol in branches, in upper stem and stem base (A) in mature Scots pine. Together all these four sugars represented 97 % or more of the total sugar osmolality. The measured osmolality in situ as the function of calculated total sugar osmolality in the stem positions (B). The error bars are standard deviations (Fig. 8A). The statistically tested p-value is in symbols (Fig. 8B); *, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$.

250x117mm (128 x 131 DPI)