Dissertationes Forestales 302

Tree water transport mediating the changing environmental conditions to tree physiological processes

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Academic dissertation

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TIIVISTELMÄ

Puun johtosolukot yhdistävät puun fysiologiset prosessit ja puun kasvun kasvupaikan resurssien saatavuuteen. Vettä liikkuu maasta pitkin mantopuuta, eli ksyleemisolukkoa, jopa 100-metristen puiden latvukseen. Kuoressa sijaitsevaa sokereita ja muita tärkeitä yhdisteitä kuljettavaa nilasolukkoa pitkin yhteyttämistuotteet liikkuvat lehdistä puun aineenvaihdunnallisiin prosesseihin, varastoihin, kasvuun ja puun puolustukseen. Veden saatavuuden heikentymisen seurauksena puu sulkee lehtien ilmarakoja. Tämä samalla laskee puun yhteytystuotosta, koska yhteyttämisessä tarvittavat vesi ja hiilidioksidi siirtyvät molemmat ilmarakojen kautta lehtien ja ympäröivän ilman välillä. Nilakuljetuksen saa aikaiseksi hydrostaattiset paine-erot, turgorpaine-erot, mitkä muodostuvat kuljetussolukkoihin, esimerkiksi puun latvuston ja juuriston välille. Turgorpaine-erot muodostuvat nilan osmolalisuuden, eli osmoottisesti aktiivisten yhdisteiden pitoisuuden, ja ksyleemin vesipotentiaalin yhteisvaikutuksesta. Puut ovat sopeutuneet paikallisiin olosuhteisiin perinnöllisten tekijöiden avulla sekä mukautuen rakenteellisesti kasvun avulla. Nopeisiin ympäristön muutoksiin puut sopeutuvat fysiologisilla vasteilla. Tässä väitöskirjassa tarkastellaan puun fysiologisia vasteita, kuten nilasolukon osmolalisuutta, puun vesipotentiaalia ja lehtien ilmarakojen aukioloastetta eli ilmarakokonduktanssia, ja kuinka ne ovat kytköksissä muuttuviin ympäristöolosuhteisiin. Väitöskirjan tutkimukset keskittyvät metsämäntyyn ja –kuuseen, ja ne ovat pääosin kenttäolosuhteissa tehtyjä tutkimuksia.

Tämän väitöskirjan tulosten mukaan vuodenaikaiset vaihtelut maan lämpötilassa ja vesipitoisuudessa välittyvät puun vedenkuljetuskykyyn maan ja latvuston välillä. Myös lehtien ilmarakokonduktanssi, ja siten myös puun hiilensidonta, ovat kytköksissä näihin vedenkuljetuskyvyn muutoksiin kasvukauden aikana. Väitöskirjan tutkimukset tukevat vallitsevaa käsitystä nilakuljetuksen toiminnasta, ns. Münchin nilakuljetusteoriaa, missä osmoottiset pitoisuuserot muodostavat riittävän turgorpaine-eron sokereita tuottavien, tai varastoivien, ja sokereita kuluttavien solukkojen välille. Nilakuljetukselle tarvittava osmoottinen gradientti solukkojen välillä sekä painovoiman edistävä vaikutus puun juuristoa kohti tapahtuvassa nilakuljetuksessa ovat molemmat havaittavissa näissä tutkimuksissa kenttäolosuhteissa. Vettä kuljettavan ksyleemisolukon vesipotentiaali ohjailee puun nilasolukon osmolalisuutta ja turgorpainetta päivittäin, veden saatavuutta ja puun haihdutusta mukaillen. Nilakuljetusta ajavat turgorpaine-erot näyttäisivät myös määrittävän puun päivittäistä ja vuodenaikaista yhteytystuotteiden allokointia vedensaatavuuden mukaan. Kaarnakuoriaisten mukana puihin kulkeutuvat sinistäjäsienet kykenevät aiheuttamaan puun vedenkuljetuksessa häiriöitä, mitkä voivat heikentää puun elinvoimaisuutta. Äkilliset heikentymiset puun vedenkuljetuksessa voivat olla seurausta veden pintajännityksen laskusta ksyleemisolukossa intensiivisen patogeeni-infektion seurauksena.

Asiasanat: nila, ksyleemi, havupuu, vesipotentiaali, osmolalisuus, vedenjohtavuus

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ABSTRACT

Tree vascular tissues connect resource availability to tree physiological processes and growth. The xylem transports water from the soil up to the canopy of even 100-metre tall trees, whereas phloem transport connects the photosynthesis in leaves and the tree metabolic processes, including growth and tree defences against insect and pathogen attacks. Water deficit results in the closing of leaf stomata and decreasing photosynthetic production, as water and carbon dioxide are exchanged through the stomata between the leaf and ambient air. Phloem transport is driven by turgor pressure gradients generated by the interplay of phloem osmotic concentration and xylem water potential. Trees have adapted to local environmental conditions and they adjust to fast environmental changes with physiological responses. This thesis investigates tree physiological responses in vascular tissues, such as osmolality, water potential and stomatal conductance, to environmental conditions in two conifers: Scots pine and Norway spruce.

Seasonality in soil temperature and soil water content affect soil-to-leaf hydraulic conductance, and stomatal conductance is connected to these seasonal patterns in water transport. Soil environment is thus mediated to tree functionality through tree water transport. This thesis also supports Münch’s theory that it is plausible to explain phloem transport in conifers in field conditions with osmotic gradients and gravity. Xylem water potential reflects to osmotic potential and turgor pressure of the inner bark by modifying tissue solute and water content. The turgor gradients hence seem to determine daily and seasonal carbon allocation patterns according to water availability. Pathogenic infections may introduce more rapid changes in tree hydraulic conductance through a decrease in xylem sap surface tension and xylem conductivity during massive invasions of bark beetles that vector blue-stain fungi such as *Endoconidiophora polonica*. These pest attacks weaken tree vitality and may also increase tree vulnerability to hydraulic failure in the xylem.

Keywords: phloem, xylem, conifer, water potential, osmolality, hydraulic conductance

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Helsinki, July 2020

Teemu Paljakka

LIST OF ORIGINAL ARTICLES

This thesis consists of a summary of four research articles, which are reprinted after the summary section. These articles are referred to in the text by the Roman numerals I–IV. All the articles have been published in peer-reviewed journals and reprinted with the permission of publishers.

I	Lintunen A., Paljakka T., Jyske T., Peltoniemi M., Sterck F., Von Arx G., ... & Hölttä T. (2016). Osmolality and non-structural carbohydrate composition in the secondary phloem of trees across a latitudinal gradient in Europe. Frontiers in Plant Science 7: 726. https://doi.org/10.3389/fpls.2016.00726
II	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: 2160-2173. https://doi.org/10.1111/pce.13017
III	Lintunen A., Paljakka T., Salmon Y., Dewar R., Riikonen A., & Hölttä T. (2019). The influence of soil temperature and water content on belowground hydraulic conductance and leaf gas exchange in mature trees of three boreal species. Plant, Cell & Environment 43: 532-547. https://doi.org/10.1111/pce.13709
IV	Paljakka T., Rissanen K., Vanhatalo A., Salmon Y., Jyske T., Prisle N.L., Linnakoski R., Lin J.J., Laakso T., Kasanen R., Bäck J., & Hölttä T. (2020). Is decreased xylem sap surface tension associated with embolism and loss of xylem hydraulic conductivity in pathogen-infected Norway spruce saplings? Frontiers in Plant Science 11:1090. https://doi.org/10.3389/fpls.2020.01090

Author contributions:

The author conceived **Study I** with the co-authors. He also conducted the laboratory work with intern assistance and contributed to the pre-analysis, interpretation of the results, and to writing the manuscript with the co-authors. In **Study II**, the author tested the measurement techniques in trial experiments and conceived the study design with the co-authors. He conducted the sampling and on-site measurements with intern assistance and conducted the laboratory analyses himself. The author interpreted the results and wrote the article with the co-authors. In **Study III**, the author conducted the quality check, data preparation, and pre-analysis of the data. He maintained the measurements, helped with the data analysis, and contributed to writing the manuscript with the co-authors. The author conceived **Study IV** with the co-authors, and he conducted the trial studies with assistance. He established the experiment setup with the co-authors, and conducted the on-site measurements, laboratory analyses, data analysis, and writing of the manuscript with the co-authors.

TABLE OF CONTENTS

1 INTRODUCTION

Photosynthetic production and growth in trees is maintained by long-distance transport in the vascular tissues. Photosynthesis is among the key elements that provide the essence of life on this planet through the extensive forest area on the Earth's surface. The boreal region is the world's largest biome (Bonan 2008), where the distributions of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) cover large areas. These conifers extend from cool and coastal regions to mountains, and from the Mediterranean (Scots pine) to the continental climate of Siberia (Martínez-Vilalta et al. 2009; Levesque et al. 2013). Trees grow under seasonally changing environmental conditions where temperature and light globally create a gradient towards high latitudes according to radiative forcing, and the availability of water in the soil is dependent on precipitation, run-off, and evapotranspiration levels. Temperature drives tree physiological processes and seasonal phenology, e.g. by determining the length of the growing season with the quantity of light. Changing temperature and water availability are considered to globally have the largest impact on future forest productivity and health, with temperature having the strongest influence in the boreal region (Seidl et al. 2017). The surrounding environment, with its water availability level, considerably determines the efficiency and functionality of tree physiological processes. The interaction and massive exchange of energy and substances between forests and the atmosphere are possible because trees have a sophisticated system in their vascular tissues, which is efficient enough for providing resources for photosynthesis, and to the maintenance and growth of trees.

1.1 Key elements in tree physiology

1.1.1 Vascular tissues provide water and solutes for tree physiological processes

Vascular tissues transporting water (xylem) and essential organic compounds for the plant (phloem) reach almost the entire plant axial length from the roots in the soil to the leaves, where the interface of gas exchange with the ambient air is located. These vascular tissues (xylem, phloem) connect the key processes of the whole plant from water and nutrient uptake in the soil to carbon dioxide (CO_2) uptake and light interception in the leaves. The xylem and phloem exchange water and solutes in the radial direction, especially through the ray parenchyma cells (Spicer 2014). Metabolic processes in all living parts of trees require a constant input of resources, which again are provided by transport through the vascular tissues. Water is a key element in all living cells, serving as a medium for metabolic processes and in the long-distance transport of solutes. Trees also gain a large share of necessary nutrients with the uptake of water from the soil. A small proportion of tree water use goes to photosynthesis, where water molecules are oxidized to initiate photosynthetic processes that combine the solar energy with CO_2 into chemical energy in sugar products. Vast quantities of water, transported from the soil to leaves through the xylem, evaporated from the leaves during CO_2 exchange because water evaporates more rapidly from the open stomatal pores than CO_2 enters the leaves. For this reason, water becomes a limiting resource in many ecosystems, and decline or disruption in water transport will soon reflect to other important processes in a plant (Choat et al. 2012). Phloem transport is dependent on xylem water and is important in tree functionality, as it connects photosynthesis and tree carbohydrate storages to tissues in need of solutes, e.g. for the maintenance of living tissues and growth (e.g. Münch 1930; Lemoine et al. 2013; Nikinmaa et al. 2013). These locations of solute input and output in a tree are referred to as carbohydrate sources and sinks.

1.1.2 Transpiration

Plants utilize solar energy to transport water from the soil to leaves. Solar energy creates the evaporation of water from the leaves (transpiration) by heating the Earth's surface and creating a diffusive exchange of water vapour between the ambient air and leaf inner air space, where water is evaporating. Plants provide a channel, the xylem, where water can travel in very special conditions under tension driven by this evaporative pull (Dixon and Joly 1895; Tyree and Zimmermann 2002). Water consists of molecules bound together with cohesive forces. These forces originate from the polarity of water molecules, by the slightly more electronegative oxygen atom compared to the two hydrogens (Taiz and Zeiger 2015). Before water vapour diffuses into the air through stomatal pores, the liquid water evaporates. Water vapour concentrations are higher at the stomatal pores than in the ambient air resulting in movement of water vapour to air. Prior to the stomata, water has travelled through the leaf ground tissue (mesophyll) and along the xylem. The liquid surfaces on the living cells inside the stomata are held back by surface tension of the water because vaporization of water results in water deficit. Together the surface tension in the liquid surfaces of the stomata and the cohesive forces of water enable tension building in the xylem (later referred to as negative water pressure potential) and the pull of water throughout the entire axial length of the tree (Dixon and Joly 1895).

1.1.3 Characteristics of xylem tissue

Transport pathways differ in how easily water and solutes can be translocated. The water-transporting cells are mainly dead, accompanied with some living parenchyma cells functioning as resource storages. The continuum of these dead cells and the space limited by cell membranes outside the living cells is called the apoplast, whereas the interconnected continuum of living cells surrounded by cell membranes is referred to as the symplast. Tracheids are water–transporting cells in conifers and have a small diameter and high density, whereas broad-leaved trees have vessels with larger diameters and smaller densities in the xylem. Conifer tracheids connect to each other with bordered pits that reduce water flow in the xylem but also add safety to water transport (Bailey 1916; Bauch et al. 1972; Hacke et al. 2004). These bordered pits have membranes with small pores and closing valves preventing the spread of air in the xylem if these cells become non-conductive (embolized). Water and small molecules can pass through these small pores in the membranes (Hacke and Sperry 2003). Xylem cells are very stiff and can endure strong forces. After entering the tree through semi-permeable membranes in the roots, water travels without large impurities through the continuum of tracheids (or vessels in angiosperms), which support the water flow with adhesive properties in the cell walls (e.g. Tyree and Sperry 1989), inhibit impurities or large gas bubbles from entering the xylem transport stream, and have safety valves (in gymnosperms) that close when the water stream faces sudden changes. Water molecules can hold together in such conditions and withstand enormous pulling forces (Ursprung 1915).

This also enables the transport of water in a liquid form against gravity high above ground, in conditions where water would typically vaporize.

1.1.4 Characteristics of phloem tissue

Photosynthetic sugars are transported away from the leaves through the phloem, another vascular tissue located almost adjacent to xylem. Only the thin cambium, which is the active area of secondary (radial) growth, separates the secondary xylem and secondary phloem from each other. Secondary phloem consists mainly of sieve cells (in gymnosperms) that are specialized living cells, Strasburger cells accompanying the sieve cells, ray cells, and axial and ray parenchyma cells that function as storage and supporting cells (den Outer 1967). Sieve cells are accompanied by several supporting cells (Strasburger, parenchyma) with which they share the symplast (Schulz 1992). These supporting cells also have several essential cell organs that the sieve cells are missing after specializing in mass-flow transport (Sauter 1980; van Bel 2003). Sieve cells are also axially connected through pores with no membranes in between. Tissues in the phloem are more elastic, with a considerably smaller number of cells in the conducting tissue compared to the xylem (Jyske and Hölttä 2015). Long-distance transport in the phloem, driven by hydrostatic pressure (i.e. turgor pressure) differences, generates mass flow through phloem sieve cells interconnected by open pores (Knoblauch and van Bel 1998; Liesche et al. 2015). Sugars are mainly in the form of sucrose during translocation (Rennie and Turgeon 2009). The sucrose molecule is formed of glucose and fructose molecule, and it is suitable for transport, as it is a stable and soluble compound.

1.1.5 Water and solute movement according to water potential gradient

Water potential sums up the operators affecting water movement between plant cells and tissues. As water crosses the semi-permeable membranes when moving along the continuum of cells, it always moves towards a lower level of free energy of water, i.e. towards a lower water potential. Pressure (pressure potential) and osmotically active solute concentrations (osmotic potential) inside the cells are the main operators affecting the water potential. Therefore, the water potential in both tissues, xylem and phloem, may be described by

$$(-)\Psi = (-)\Psi s + (\pm)\Psi p + (-)\Psi g, \qquad (1)$$

where Ψ is water potential, Ψs is osmotic potential, Ψp is pressure potential and Ψg is gravitational potential. An increase in pressure in relation to cell surroundings also increases the water potential. On the contrary, an increase in osmotic potential in relation to its surroundings decreases the water potential of the plant cell (Taiz and Zeiger 2015). Osmotic potential is linearly proportional to osmolality, which measures the concentration of osmotically active solutes in a solution of water. The osmotic potential changes in relation to solute concentration and volume according to van’t Hoff’s Boyle’s Law

$$\Psi s = -RTc, \qquad (2)$$

where R is the gas constant (8.314 J K^{-1} mol^{-1}), T is absolute temperature (K) and c is solute concentration (mol kg^{-1}). The solute concentration (c) may be expressed with osmolality, as the amount of osmotically active solutes (n) in a mass of water (m_{water})

$$c = n / m_{water} \qquad (3).$$

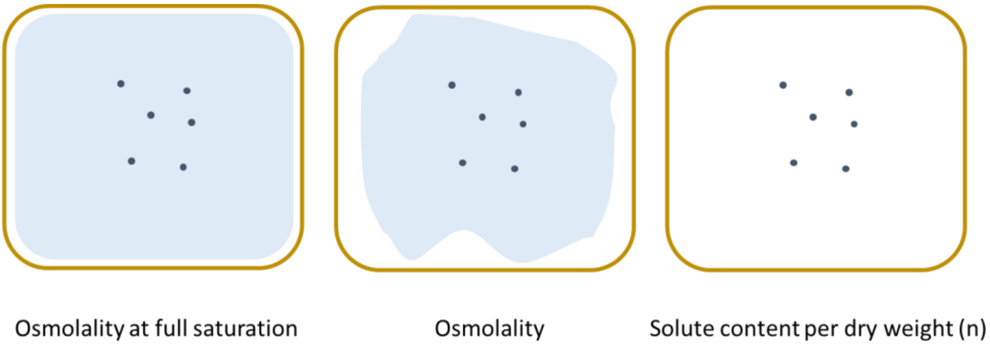


Figure 1. Illustrations of osmolality at full saturation (on the left) with a cell fully hydrated with water, osmolality (in the middle) with both water and solute levels affecting osmolality, and the solute content per dry weight (on the right) where only solutes are examined. The light blue area in the illustration refers to water whereas the points refer to osmotically active solutes (e.g. sugars, inorganic ions, amino acids, and more), and the brown border is the dry matter of the tissue (i.e. the cell structures and cell organs in addition to osmotically active solutes). Osmolality at full saturation may be used for comparison of the symplastic solute content because the water content is levelled off (e.g. Takami et al. 1981).

Changes in solution volume are inversely proportional to osmolality and osmotic potential, when solution density is known. Additionally, osmolality at full saturation describes the symplastic solute content by correcting the osmolality with the relative water content (see illustration Fig. 1).

Additionally, gravity is an important operator when studying trees because trees grow tall, and gravitation will have more impact the higher above the ground the tissues are. Gravitation increases the hydrostatic pressure by 0.01 MPa m^{-1}. The solute concentration in the xylem is small (Borghetti et al. 1991), and the water potential differences in the xylem are therefore mainly due to differences in pressure potential. The solutes and pressure both contribute considerably to water potential in living cells such as phloem and leaf mesophyll cells. Cell pressures are mainly negative in the xylem cells and always positive in the living cells. Water movement through the membranes, i.e. movement between cells and tissues, depends mainly on the water potential difference (water potential gradient) between the tissues and their surroundings (Hsiao et al. 1976). Additionally, cell membranes have proteins called aquaporins, which enhance water movement between cells (e.g. Javot and Maurel 2002). Plant cells can build up pressure because of their cell walls. For example, if a plant cell is surrounded by a dilute water solution and solutes are added in the cell, the water potential of the cell decreases in relation to its surroundings. This results in water movement from the dilute surroundings into the plant cell. As the plant cell walls are more or less elastic, the water movement will increase the pressure in the cell in relation to its surroundings. The positive pressure inside the plant cells is referred to as turgor pressure. This is the principle how living plant cells maintain their functionality even in conditions when water is less available and how growing cells can expand during the growing season, by building up high enough pressures that can expand plastic juvenile plant cells. By adding osmotically active solutes in the cells, the phloem may also attract water from the xylem to create a hydrostatic pressure gradient in the transport pathway that generates the mass flow towards lower turgor pressure in the phloem (Münch 1930). The water potentials of the xylem and phloem are expected to be close to one another (e.g. Thompson and Holbrook 2003; Hölttä et al. 2006)

$$\Psi_{\text{xylem}} = \sim\Psi_{\text{phloem}} \quad (4).$$

The relation of water potential to tissue relative water content, turgor pressure, and osmotic potential may be illustrated with the Höfler diagram (Fig. 2) (Turner 1981) or with a similar principle utilizing pressure-volume curves (Scholander 1965; Tyree and Hammel 1972). The living cells are expected to approximately follow the changes of xylem water potential, as shown in Figure 2, where the change in water potential is first related to tissue turgor pressure after the water content of the cell begins decreasing. The osmotic potential responds to the changing water potential more slowly than the turgor pressure (Noy-Meir and Ginsburg 1969; Tyree and Hammel 1972), and tissue water potential corresponds to the osmotic potential when the tissue turgor pressure is zero (indicated in Fig. 2).

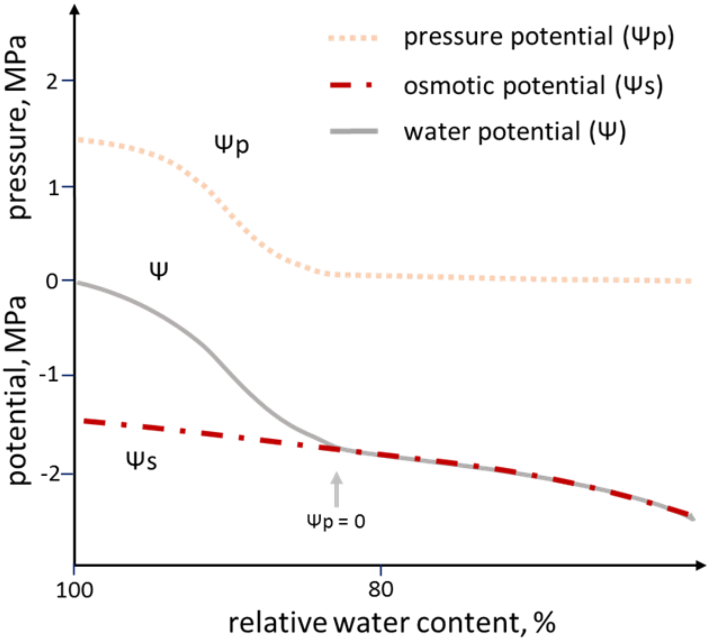


Figure 2. The components of tissue water potential (Ψ), pressure potential (Ψp) and osmotic potential (Ψs), in relation to tissue water content (illustration depicted from Turner 1981). Values in the axes are example values and not applicable to all tissues. The arrow indicates the point where tissue turgor pressure is zero and the osmotic potential component equals the tissue water potential.

1.1.6 Conductance of the translocation pathway

Long-distance transport is based on mass flow, meaning that solutes and water (sap) cross no membranes when translocated. Such pathways are found in the xylem and phloem cells. Mass flow in these tissues is based on hydrostatic pressure gradients and the conductance of water flow (rate of water movement per pressure difference). Conductance of these tissues is connected to mass flow by the equation

$$J = k\Delta\Psi p, \tag{5}$$

where J is the flow rate, k is the conductance of the transport pathway, and $\Delta\Psi p$ is the hydrostatic pressure difference. Sap movement in both the xylem and phloem is also related to the structure of these tissues. The conductivity of both tissues, when considering the cells as a group of transporting tubes of certain length, may be approximated with the Hagen-Poiseuille equation

$$k = n_t \pi r^4 * (8\mu L)^{-1}, \tag{6}$$

where k is conductivity, n_t is the number of tubes, r is the radius of the tubes, µ is the viscosity of the sap, and L is the length of the tubes. These equations show that the flow rates of xylem and phloem transport are influenced by the structure of the transporting tissues, the physical properties of the transported sap, and the pressure gradient.

Conductance in trees is determined by the relation of sap flow rate and the pressure difference driving it (Eq. 5) and is influenced by physiological processes, such as changes in membrane permeability of the living cells, which is enhanced by aquaporins or changes in temperature or ionic concentrations of the transported sap (Hacke 2014). Conductance differs in the stem, roots, and leaves because the tissue characteristics differ and water crosses membranes in the latter two. For example, leaf conductance is much lower than conductance in the xylem because ca. one-third of the resistance to water transport comes from water moving through leaf cells before transpiring (Sack et al. 2003), whereas the xylem cells conduct water very efficiently as they are specialized for this purpose (Tyree and Sperry 1989). Conductance in the xylem and phloem depends also on the viscosity of the transported sap. In the xylem, the sap consists mainly of water with e.g. some sugars and ions that are dissolved in the sap (Borghetti et al. 1991). However, sugar concentrations are high in the phloem, with a considerable effect on sap viscosity (Hölttä et al. 2009a). The viscosity of sap is generally also affected by temperature. Conductivity is the flow divided by length and it similarly describes the capacity for sap flow. For example, conductivity may be examined in the stem or in a cylinder where the variables affecting the water flow may be examined locally (Eq. 6). As depicted by Eq. 6, the loss of transporting cells (n_t) through embolism will affect the conductivity.

1.1.7 Embolism and hydraulic safety in water transport

Embolism occurs when the water potential inside a water-transporting cell decreases below a threshold where air-seeding occurs and conduits become filled with air (Sperry and Tyree 1988). Such a situation may occur when transpiration from the leaves is high and not enough water is available for the tree, e.g. during a hot summer day or to larger extent after prolonged drought conditions (e.g. Millburn 1966; Tyree and Sperry 1989, Cochard 1992). Water columns then break in the conduit, followed by a rapidly increasing air bubble inside the conduit. Air originates from the sap itself or from outside the embolizing cell, e.g. air may enter the tree apoplast after wounding of the outer tissues in the stem. Air entering the transpiration stream from an adjacent cell is referred to as air-seeding (Tyree and Sperry 1989). The bordered pit valves in conifers prevent the spread of the air bubble to other cells, as these valves close due to the rapid pressure change in the cell, thus narrowing down the loss of conducting cells (term "n_t" in Eq. 3). The sap itself resists air-seeding with the surface tension of the sap (Bailey 1916), as described by the Young-Laplace equation

$$\Delta\Psi p = 2\sigma \cos \alpha / r, \qquad (7)$$

where Ψp is the pressure potential in the cell, σ is surface tension, α is the contact angle of sap and cell wall, and r is the radius of the pit pore in the cell wall (Tyree and Zimmermann 2002). The surface tension of transported sap may thus affect the vulnerability of the xylem to embolism, because with lower sap surface tension a smaller change in pressure (i.e. xylem water potential) will result in embolism (Fig. 3). The contribution of surface tension to embolism is not well known, and that is one motivation for studying xylem sap surface tension in this thesis.

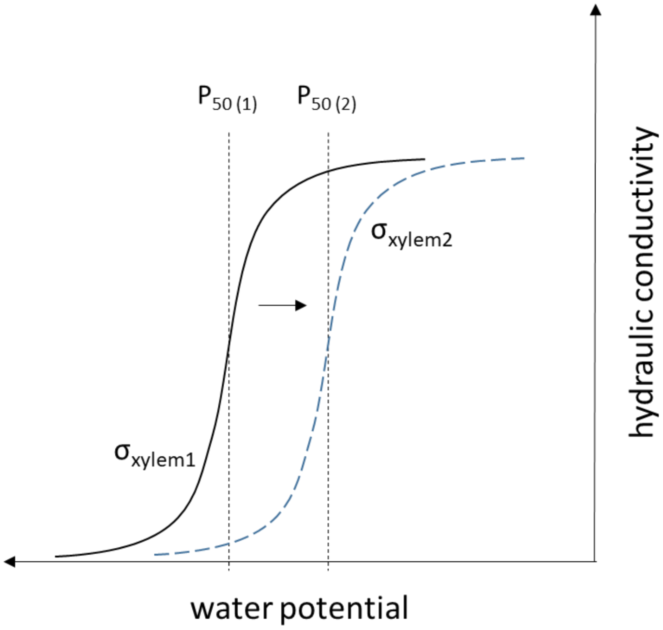


Figure 3. Tree hydraulic conductivity in relation to decreasing water potential when the xylem sap surface tension (σ) changes from a higher (σ_{xylem1}) to a lower (σ_{xylem2}) surface tension. Vulnerability to embolism is referred to as species-specific P_{50} values with the initial embolism vulnerability ($P_{50\,(1)}$) and embolism vulnerability after a decrease in xylem sap surface tension ($P_{50\,(2)}$). The reported values for $P_{50\,(1)}$ in Scots pine (*Pinus sylvestris L.*) and Norway spruce (*Picea abies L. Karst.*) are ca. 3 and 3.5 MPa, respectively (Gonzales-Munoz et al. 2018).

Without controlling transpiration, the tree would eventually end up with an exponential increase in embolism and in a loss of the conducting area (Sperry and Tyree 1988; Cochard et al. 1996). A decrease in xylem sap surface tension may enhance the loss of conducting tissue more easily (Fig. 3) (Sperry and Tyree 1988; Hölttä et al. 2011), which has also been demonstrated for conifers (Cochard et al. 2009; Hölttä et al. 2011). Trees can reduce the level of embolism by closing their leaf stomata (Stålfelt 1955; Jarvis and Jarvis 1963). The state of stomatal closure may be expressed by stomatal conductance (Cochard et al. 1996; Whitehead 1998). Should the stomata be fully open, transpiration would then be driven by the vapour pressure deficit (VPD) of the ambient air (e.g. Hodges 1967; Oren et al. 1999).

1.1.8 The phloem is a pathway between carbon sources and sinks

Trees produce carbohydrates that are constantly translocated away from the leaves or accumulated as starch to be translocated afterwards (Ainsworth and Bush 2010; Sala et al. 2011; Carbone et al. 2013) because trees need to maintain a large system of organs with daily and seasonal dynamics related to growth, defence, and constant maintenance respiration. Above-zero temperatures increase photosynthesis, metabolism, and tree growth rates until respiration from cell metabolism uses an increasingly larger share of tree carbon reserves (Amthor 2000; Körner 2015). Water transport in the xylem is closely coupled with the transport of solutes in the phloem (Münch 1930; Christy and Ferrier 1973; van Bel 2003; Hölttä et al. 2006; Pfautsch et al. 2015a). Phloem water potential needs to respond to changes in xylem water potential so that turgor pressure may be maintained in the cells (Kaufmann and Kramer 1967; Christy and Ferrier 1973; Cernusak et al. 2003; Hölttä et al. 2006). Additionally, the phloem osmotic potential is therefore expected to respond to vertical water potential gradients also found in the xylem (Woodruff 2004, Domec et al. 2008). Phloem osmotic potential should be lower than the corresponding xylem water potential for attracting water from the xylem for generating a turgor gradient between the sugar sources and sinks (Münch 1930; Kauffman and Kramer 1967; Hölttä et al. 2006). Phloem loading, i.e. transported sugars entering the transporting cells in the phloem, function with the passive movement of sugars from the mesophyll cells to the phloem in the needles in conifers such as Scots pine (Liesche et al. 2011). This passive movement is generated when the sugar, mainly sucrose, concentration is higher in the leaf mesophyll than in the leaf phloem. As the driving force for phloem transport is an osmotically generated turgor gradient, cause e.g. by sucrose, the sinks consuming the sugars should determine the direction of the phloem transport (Minchin et al. 1993). The phloem also functions as an information pathway of source and sink strength, i.e. the production and consumption of sugars, developmental phases of growth tissues, or invading pest attacks (e.g. Körner 2015). This information travels as pressure changes (Thompson and Holbrook 2004; Hölttä et al. 2006; Mencuccini and Hölttä 2010; Sellier and Youcef Mammeri 2019) and as chemical signals such as in the form of hormones and proteins (van Bel 2003) between tree compartments (leaves, branches, stem, roots). The phloem is located adjacent to the xylem practically along the whole tree length, with only the cambial cells in between, and is connected to the xylem with ray parenchyma cells (e.g. Pfautsch et al. 2015a, 2015b). These parenchyma cells support the exchange of water and solutes between the xylem and phloem. Ray parenchyma cells are connected to the conducting tissues of the xylem and phloem, and they remain functional longer than the conducting tissue (Spicer 2014). Changes in water potential are thus transmitted rapidly through the xylem between the canopy and roots, as well as simultaneously to the phloem and carbon sinks (Perämäki et al. 2001; Sevanto et al. 2002; Hölttä et al. 2010). Tree compartments are therefore interactively connected by the vascular tissues providing sugars for the most-consuming tissues at the sinks (Kirschbaum 2011; Ainsworth and Bush 2011; Körner 2015; Hölttä et al. 2017). The phloem transport rate and the input of solutes to sinks is thus related to the hydraulic conductance of the xylem (Hölttä et al. 2010), in addition to photosynthesis in the leaves and activity at the sinks.

1.1.9 Carbon allocation in Norway spruce and Scots pine

Changes in temperature and light drive the seasonality to which trees are adapted to with their phenology. Trees adjust to this seasonal rhythm somewhat according to the accumulated temperature sum (Suni et al. 2003, Sutinen et al. 2012). Processes e.g. growth, reproduction, and preparation for winter dormancy generate seasonal patterns of carbon sinks that are determined by species characteristics and the regional adaptation of trees along with soil temperature, water content (Sutinen et al. 2014), and local climate. Photosynthesis and the activity of metabolic processes increase with temperature, as photosynthesis is limited by temperature in the spring, and the enzymatic processes in metabolism are also driven by temperature (Berry and Björkman 1980). Therefore, temperature also drives the activity and consumption of sugars at the sinks (Ainsworth and Bush 2011; Lemoine et al. 2013; Körner 2015). Carbon allocation in the timing of flowering, bud burst, onset of shoot and stem growth, and in the allocation to storages are somewhat synchronized in Scots pine and Norway spruce (Antonova and Stasova 2006; Sutinen et al. 2012; Swidrak et al. 2014). Norway spruce and Scots pine allocate a considerable proportion of recently sequestered carbon to storages and belowground to fine root growth and for the mycorrhizal fungus in the roots (Mildner et al. 2014; Henriksson et al. 2015; von Arx et al. 2017). These storages are utilized especially further away from the canopy (Mildner et al. 2014). Larger carbon use requirements may also occur after mechanical strain (Körner 2003), forest disturbances, e.g. storms and fire, or because of biotic operators due to strain caused by insects and tree diseases (Lemoine et al. 2013). However, tree resources seem to be limited by the use of carbon storages and water availability together and not solely by the carbon reserves (Körner 2003; Sala et al. 2012).

1.1.10 Soil water availability and tree water uptake

Trees grow under the availability of resources that are considerably determined by the characteristics of the growing site. After precipitation and snowmelt have determined the input of water to soil in a catchment, soil characteristics thereafter determine the water and nutrient availability for trees (Duursma et al. 2008; Ilvesniemi et al. 2010). Soil coarseness influences the water holding capacity and oxygen levels in the soil. Coarse soils have a smaller water holding capacity and the excess water runs through the soil, whereas fine soil particles have a larger surface area for holding water and thus their water holding capacity is higher (Duursma et al. 2008). The soil water potential describes the tendency of water movement in the soil, but it also describes how easily trees can uptake water. For water movement to occur through the fine roots and all the way to the root xylem, the water potential in the tree roots must be lower than in the soil. Soil water potentials are often expected to be close to zero in boreal conditions, but this depends on soil characteristics. Soil water potential is more sensitive to changes in soil water content in fine soils with clay particles (typical growing sites of Norway spruce), whereas they are much more decoupled in coarse sandy soils (typical growing sites of Scots pine) and soil water potential declines rapidly only in a very low soil water content. Roots are sensitive to embolism (Hacke 2014) and thus low soil water potentials may require physiological adjustments in the roots to recover the water uptake (Hagedorn et al. 2016). However, such conditions seldom occur in Finnish soils, where springtime soil water availability is often substantial after snowmelt. The soil water content usually decreases towards the late summer, when temperatures and deficits of water vapour in the air (VPD) are the highest. The hydrostatic forces and osmotic potential gradients aid in maintaining water uptake in the plant roots with modifications in root membrane permeability (Henzler et al 1999; Javot and Maurel 2002). Water needs to pass a series of living cells before reaching the xylem, but water flow is also enhanced by the aquaporins in the root cell membranes (Oliviusson et al. 2001; Javot and Maurel 2002).

1.2 Tree responses to environmental conditions

1.2.1 Responses of vascular tissues to water availability

Drastic changes in environmental conditions have consequences on tree productivity and survival in all habitats because tree species are locally adapted to prevailing growth conditions (Allen et al. 2010; Choat et al. 2012; Bouche et al. 2014). Water deficit is among the most critical aspects in the distribution patterns of tree species, as it is considered the ultimate cause for tree mortality (Adams et al. 2018). Water availability in the soil connects to leaf photosynthesis and gas exchange through stomatal control (e.g. Saliendra et al. 1995; Kellomäki and Wang 1995; Irvine et al. 1998; Duursma et al. 2008). However, water deficits may cause decline in tree hydraulic conductance (Tyree and Sperry 1989), and also affect the phloem transport because less water is available to maintain phloem turgor, and thus the turgor gradient driving phloem transport becomes weaker between the carbon sources and sinks (e.g. Hölttä et al. 2010; Nikinmaa et al. 2014; Salmon et al. 2019). Occasional dry periods are not critical for trees, although the tree productivity or growth may temporally decline (Nardini et al. 2018), because trees are able to recover from these mild events (Brodibb and Cochard 2009). Prolonged water stress is especially stressful for trees, as it causes an accumulating decline in tree water transport capacity that may be challenging for tree recovery (Brodribb et al 2010; Urli et al. 2013). Trees aim to maintain their performance in various growing sites by controlling their stomatal openings and by adjusting the allocation in tree compartments (Martínez-Vilalta et al. 2009; Rosas et al. 2019) in addition to adaptation (Maherali et al. 2004). Trees not only adjust to growing site conditions through structural characteristics but also through long-term physiological adjustments to prevailing conditions, e.g. through osmotic adjustment, that may enhance resistivity against leaf wilting or in the maintenance of water uptake in the roots (Grime and Mackey 2002; Bartlett et al. 2014). The conductance in both vascular tissues responsible for long-distance transport, i.e. the xylem and phloem, depends largely on tissue properties, e.g. the number of conduits, conduit size, and diameter (e.g. Hacke et al. 2006; Liesche et al. 2015), and thus tree growth also determines the limits for water and solute transport. Water availability affects the xylem conduit diameter because cell expansion during growth is sensitive to turgor pressure in the growing tissue (Cosgrove 1986; Antonova et al. 1995). Environmental conditions influence the characteristics of the xylem and phloem in terms of the number of cells and cell width, which together determine the annual radial increment of wood and hydraulic conductivity and the thickness of cell walls, which determines the carbon used for the cell walls providing mechanical strength and hydraulic safety, also in boreal conifers (Antonova and Stasova 1993; Kilpeläinen et al. 2003; Jyske et al. 2010; Eilmann et al. 2011).

Water deficits during growth result in smaller conduits (Antonova et al. 1995; Jyske et al. 2010) or even in growth cessation (Eilmann et al. 2011). Considerably more xylem than phloem is produced annually (Jyske and Hölttä 2015), with more constant annual increments in the phloem (Antonova and Stasova 2006; Swidrak et al. 2014). Xylem production is more variable to temperatures and water availability than phloem production is (Antonova and Stasova 2006; Swidrak et al. 2014), although water availability also influences the amount of produced phloem in Norway spruce (Gričar et al. 2014). Newly formed phloem is conductive for only 1–2 years (Sauter 1980; Gričar and Cufar 2008; Swidrak et al. 2014), whereas xylem cells are functional for several years, with far more conductive cells in the xylem than the phloem (Jyske and Hölttä 2015). Phloem transport rates are slow in conifers, likely because of high resistance in the sieve cells (Liesche et al. 2015). The xylem is considerably larger in volume compared to the phloem when considering that phloem requires a relatively small amount of water from the xylem to transport solutes (Pfautsch et al. 2015b). However, the elastic inner bark tissue, which includes tissues between the vascular cambium and cork cambium (e.g. parenchyma, ray and sieve cells), also function as a water storage providing security to xylem water transport (Zweifel 2000; Hölttä et al. 2006; Pfautsch et al. 2015b).

1.2.2 Adaptation of Scots pine and Norway spruce to local environmental conditions

Conifers are gymnosperms that have acclimated to freezing temperatures as well as to dry growing sites with a small number of structural hydraulic traits (Hacke et al. 2015) and wide distribution, as they are the tree-line species at both high altitudes and in the northern boreal region. Conifers have optimized the structure of the water transporting xylem with small conduits and bordered pits in between (Hacke et al. 2004). Such a xylem structure is also suitable for cold environments (Sperry 2011; Mayr et al. 2014), but not as efficient in water transport as the xylem in angiosperm tree species (mostly broadleaved tree species). Conifers can add hydraulic safety with their bordered pit structure, which functions as valves when disruptions occur in water transport (Maherali et al. 2004; Bouche et al. 2014). Conifers seem to adapt their anatomical properties (Bouhe et al. 2014; Lopez et al. 2016) and acclimate growth and carbon allocation to meet local site conditions (Maherali et al. 2004; Martínez-Vilalta et al. 2009; Gričar et al. 2015; Gonzales-Munoz 2018). They also adjust their water use with stomatal closure more sensitively than broadleaved trees (Brodribb and Cochard 2009; Klein 2014). Scots pines in particular close their stomata more easily to avoid water loss through evapotranspiration and maintain their water potential above a certain limit (Irvine et al. 1998; Barlett et al. 2016), having similar water potential thresholds across climates (Martínez-Vilalta et al. 2009). Scots pines adjust to the environment through modifications in canopy area, growth and water transport efficiency rather than by modifying the drought resistance of their water-transporting tissue (Martínez-Vilalta 2009; Eilmann et al. 2011; Poyatos et al. 2013; Salmon et al. 2015). The P50 value (water potential threshold where 50% of the conductivity in the water-conducting tissue is lost) is less negative in Scots pine and Norway spruce compared to many other species (Bartlett et al. 2016; Gonzales-Munoz 2018), meaning that stomatal control prevents hydraulic failure by preventing the water potential from decreasing too much in both of these conifer species (Jarvis and Jarvis 1963; Bengtson 1980; Irvine et al. 1998). The structural characteristics of the xylem and phloem in Norway spruce may differ between growing sites, as the species is able to adapt well to local conditions (Gričar et al. 2015). Norway spruce recovers rapidly after winter hardening (Christersson 1972) and drought (Jyske et al. 2010). However, hot and dry summers are not optimal for Norway spruce (Solberg 2004; Gričar et al. 2015), and its growth is sensitive to changes in water availability and temperature (Levesque et al. 2013; Gričar et al. 2015).

1.2.3 Physiological adjustments to stressful conditions

Sink activity is limited by temperature during parts of the growing season in the boreal region, which affects the consumption of sugars in the sink and thus also their translocation rate in the phloem (Körner 2003). Temperature also controls cambial activity, which determines the majority of the sink activity during radial growth (Gruber et al. 2010). Growth is among the first tree physiological processes to decline due to stressful conditions and photosynthesis follows thereafter (McDowell 2011). Stressful conditions may enhance the impact of other abiotic stressors on trees, e.g. combined water deficit and high temperatures result in increasing leaf temperatures (Urban et al. 2017; Birami et al. 2018) and in higher transpiration rates as VPD increases (Oren et al. 1999). Water stress in trees may further proceed to decline in vitality due to insects and pathogens (biotic stress) when temperatures are suitable for insect generations (Roualt et al. 2004) and storages of water and energy no longer buffer against the stressful conditions (Allen et al. 2010; McDowell et al. 2011; Jactel et al. 2012). Tree physiological responses in water transport under pathogenic invasion are not well known, although there is a consensus that water stress and pest attacks are connected (Jactel et al. 2012; Netherer et al. 2019) and that several pathogens fairly directly hamper tree water use (Oliva et al. 2014). The European bark beetle (*Ips typographus*) is the economically most important pest of Norway spruce trees in Europe. The species also vectors several species of blue-stain fungi (Francheschi et al. 2005), including the most virulent of these fungi, *Endoconidiophora polonica* (Krokene and Solheim 1998), which this thesis also examines in terms of tree water transport in Norway spruce saplings. This fungus causes rapid declines in tree water transport, eventually leading to tree mortality (Horndvedt 1983; Kirisits and Offenthaler 2002). One proposed mechanism for the rapid decline in water transport is that the pathogen increases tree vulnerability to embolism through a decrease in xylem sap surface tension (Sperry and Tyree 1988; Christiansen and Fjone 1993; Kuroda 2005). Phloem transport is expected to be hampered under water stress (Sevanto 2018; Salmon et al. 2019), and thus, the sugar transport for the use of induced tree defence and stress adjustments is likely weaker under water stress (Nagy et al. 2000; Francheschi 2005; Sala et al. 2010), hampering tree resistance against abiotic and biotic stress (Savage et al. 2015; Sevanto 2018). Although, in stressful conditions trees may allocate to defence instead of growth (Baier et al. 2002) to enhance resistance against biotic stress.

1.2.4 Research on phloem transport

Research on vascular transport in trees has been extensive for over a century. The theory behind phloem transport was also proposed nearly a century ago (Münch 1930). Surprisingly, demonstrating this theory empirically has proven difficult (Knoblauch and Oparka 2012; Carvalho et al. 2018; Liesche and Schulz 2018), although it is widely accepted in the research community (Holbrook and Knoblauch 2018; Liesche and Schulz 2018). Phloem tissue is difficult to study because the cells are under positive pressure and the native conditions in these cells are easily disturbed (van Bel 2003). Conifer phloem osmotic potential gradients in particular have been studied much less after an intensive period of studies in the 1920s and 1930s (Rosner et al. 2001). Phloem research still requires convenient methods and studies in field conditions (Salmon et al. 2019). The theoretical assumptions in conifer phloem transport have therefore remained unverified in field conditions until presently. The existing theory behind phloem transport, i.e. Münchs theory, had not been comprehensively demonstrated for conifers in field conditions (Münch 1930; Steppe et al. 2015; Savage 2015) when Study II was conducted, although plenty of research had been performed on angiosperms (e.g. Hammel 1968; Kaufmann and Kramer 1967; Sovonick-Dunford 1981). After Münch's time, only a couple studies have examined the vertical osmotic or turgor gradients in conifers (e.g. Rosner et al. 2001; Woodruff 2004; Mencuccini et al. 2013; Woodruff 2014). Afterwards, certain publications have measured the phloem turgor gradient in angiosperms (e.g. Knoblauch et al. 2016), and later on also in Scots pine (Lazzarin et al. 2017; Liesche and Schulz 2018). Critics have questioned whether the turgor gradient is sufficient to drive phloem transport in tall trees (Thompson 2006); conifers in particular have more resistance in their phloem pathways (Turgeon 2010). The requirements for realistic mass flow may be fulfilled in theory (Tyree et al. 1974; Hölttä et al. 2013). One motivation for our Study II was to test Münch's theory in conifers in field conditions. The osmotic potential of the phloem or other living plant tissues have been measured for decades (Kaufmann and Kramer 1967; Tyree and Hammel 1972; Turner and Jarvis 1975; Westgate and Boyer 1985; Kikuta and Richter 1992; Rosner et al. 2001; Callister et al. 2006; Devaux et al. 2009), but so far no standard and convenient method has been developed for measuring osmotic potential from living tissues (Bartlett et al. 2012), as many previous methods are laborious. Fascinatingly, several fundamental physiological traits, such as water and solute transport, are still only partially understood (Savage et al. 2015; Jensen 2018). The blood circulation of humans and animals is very well understood compared to the long-distance transport in plants, although similar physical principles apply to both (Arieff et al. 1972; Gisselsson et al. 1998). The plant cell wall can withstand exceptional forces and enables the build-up of water potential gradients that occur in plant bodies and this is a major difference between animal and plant cells. Deficiency in the knowledge of these fundamental tree traits also results in uncertainty when predicting forest productivity and species distributions in a changing environment (Sevanto 2018). The majority of models have very simplified variables explaining the connection of tree productivity and tree health to the environment, e.g. providing inaccurate estimations of tree productivity in extreme conditions, such as during drought periods, and they may be unable to predict tree growth properly due to gaps in understanding tree water use (Baudena et al. 2014). Especially, variability in key physiological traits: stomatal conductance, xylem water transport, and translocation of solutes in the phloem and their sensitivity to the environment, have been scarcely studied in connection to each other in field conditions because the phloem is challenging to study. The ambient CO_2 concentration has increased rapidly in the recent century, which is predicted to have a positive effect on tree productivity provided that other resources, such as nitrogen, are sufficiently available and growth can utilize the enhanced photosynthetic production (Kirschbaum 2011). By understanding how tree functional traits are connected to each other in whole-tree-level physiology and the ways in which the environment is connected to tree physiology, we may better understand how tree productivity, growth, and tree health respond under a changing environment, e.g. with changes in temperature, water availability and CO2 concentration.

1.3 Aims of the study

The present thesis investigates tree physiological responses to the environment in connection to vascular transport and tree gas exchange. The research is conducted on mature trees and saplings of two conifer species, Scots pine and Norway spruce, in field and laboratory conditions. Study I investigated the latitudinal changes of branch osmolality in dry and moist sites together with branch non-structural carbohydrates in two conifers and two broadleaved species. The aim was to understand how inner bark osmolality, describing phloem osmolality, varies across the climate and across growing sites in relation to water and sugar concentrations. Study I hypothesized that osmolality changes are mainly due to changes in tissue solute content and that branch tissue osmolality and solute content increases towards more drought- and cold- prone areas in northern and southern Europe. This study thus provides insight to what extent trees adjust their physiology across the growing sites within species and between the studied species. The aim of Study II was to answer to what extent the xylem and phloem water potentials are connected in their daily dynamics, whether Münch's theory of phloem transport is valid in mature conifer trees in field conditions, and how the phloem osmotic potential and water content respond to changing xylem water potentials to generate turgor gradients that drive the mass flow in the phloem. Study II hypothesized that needle and inner bark osmolality responds to xylem water potential, and that a sufficient osmotic gradient exists to drive the phloem transport in the bark of studied trees. Osmolality changes were investigated in the needles, branches, upper stem (lower part of the living canopy), and stem base to understand the osmotic gradients at the whole-tree level in mature trees and saplings of Scots pine and Norway spruce. Study III investigated which environmental variables affect the belowground hydraulic conductance and how stomatal conductance is connected to belowground hydraulic conductance in field conditions at a seasonal scale in the boreal region. Study III hypothesized that belowground hydraulic conductance link soil conditions and leaf gas exchange. The study was conducted in forest and urban sites with Scots pine and two broadleaved species. Study IV investigated xylem hydraulic conductivity and whole-tree water relations during biotic stress induced by pathogenic fungus. Xylem sap was studied as a dynamic solution that may cause sudden changes in the vascular system and in whole-tree physiology. The aims of Study IV were to investigate the mechanisms of how pathogens influence tree water transport and whether the change in xylem sap surface tension may provide an explanation to hydraulic disruption in Norway spruce saplings, as hypothesized in previous studies. Thus, the study hypothesis was that pathogenic fungus lowers the xylem sap surface tension and thus increase tree vulnerability to embolism, as depicted in Fig. 3.

2 MATERIAL AND METHODS

2.1 Measurement sites and plant material

Plant material for the branch inner bark osmolality in Study I was collected from a latitudinal gradient across Europe. The Scots pine and Norway spruce samples were collected from SMEAR 1 (Station for Measuring Forest Ecosystem-Atmosphere Relations) in Värriö (North Finland) and SMEAR 2 in Hyytiälä (South Finland), from the Netherlands, the Czech Republic, Switzerland, and Italy in late summer 2014. Sites were classified as dry and moist based on soil type and vegetation characteristics, as described in more detail in Study I. Branch samples 5 cm in length were cut between distances of 60–70 cm from the branch tips from trees five metres or more in height. Samples were preserved in liquid nitrogen or dry ice immediately after collection.

Study II sample collection and on-site measurements in mature trees and saplings of Scots pine and Norway spruce were conducted as follows. Mature Scots pine sample collection and on-site measurements were conducted at SMEAR 2 station in Hyytiälä, Finland (61° 51'N, 24° 17'E, 181 m above sea level). The mean annual rainfall was ca. 700 mm and mean temperature 3.5 °C in 1980–2010 (Simola et al. 2012). The station is equipped with measurements in the soil, and at the ground and tree levels. The aim of the station is to measure interactions between the ecosystem and the atmosphere (Hari et al. 2013). The studied forest is a Scots pine-dominated managed stand established in 1962 by sowing. Similar forest ecosystems are estimated to cover ca. 8-% of the Earth's surface (Hari et al. 2013). The site is classified as sub-xeric heath forest (*Vaccinium* type) (Cajander 1926). Needles, and the inner bark of the branches, upper stem, and stem base were collected from two dominant Scots pine trees equipped with continuous tree measurements or from trees located next to these trees. The sampled trees were ca. 17 metres in height and 16 cm in diameter at breast height in 2011, and on average 18 metres in height and 18.5 cm in breast height diameter in 2014 and 2015. The needle samples were hand-picked (five pairs per sample) and the branch inner bark was sampled by first cutting the branch piece and then peeling the sample along the surface of the xylem for the osmolality measurement. Needle samples were collected from branches exposed to light. The stem bark samples for osmolality measurement were detached from the stem by cutting a ca. 1 x 2-cm bark sample with a scalpel (Fig. 4). The easily removable dead bark was removed from the bark samples before preserving them in a dry shipper with liquid nitrogen. Additionally, samples for the water content measurements and sugar measurements were collected simultaneously along with the osmolality samples in July and September. Samples for water content measurements were preserved in a cool box, whereas the sugar samples were preserved in a dry shipper. Sample collection from mature Scots pines for Study II was conducted at heights of 13 to 17 m (needles and branches), 11–13 m height (upper stem), and 1.3 m (stem base). Sampling occured from morning to evening between 07:00 and 19:30, as described in more detail in Study II. Scots pine saplings were 40 cm in height and were grown in a similar forest site (see above) as the mature Scots pine trees near SMEAR 2 in Hyytiälä, Finland. Saplings were potted using the growing site soil material and studied in a greenhouse. This same Scots pine sapling material was also used in Aaltonen et al. (2016). Needles and the inner bark of the stems and roots were collected in August 2013 following the same procedure as with mature

Figure 4. Inner bark sampled from the upper stem (left) and sampled branches (right) in Study II.

Scots pines. Mature Norway spruce trees were studied in southern Finland in Haapastensyrjä (60 4'N, 24 3'E) during the growing season of 2012. They were ca. 30-year-old dominant trees, 23 m in height, and were growing on fertile agricultural land (Jyske et al. 2015). Samples were collected from the canopy, upper branches, upper stem, and stem base from similar heights as in mature Scots pines. Norway spruce saplings were grafted from cuttings and were ca. 60 cm in height above the grafting. These trees were also studied in a greenhouse in Haapastensyrjä in 2012. The sample collection from mature trees and saplings followed similar procedures as with Scots pine.

Study III forest site measurements were also conducted at SMEAR 2, Hyytiälä, Finland. The continuous tree stem measurements in Scots pines were from trees adjacent or near to trees in Study II with similar average heights and an average diameter at breast height of ca. 20 cm. The study years were 2013, 2015, and 2016. The angiosperms were studied at urban experimental sites in Helsinki, Finland (Riikonen et al. 2011), with average tree heights of 11 m and 6 m, and average diameter at breast heights of ca. 15 cm and 13 cm in *Alnus glutinosa* x pyramidalis and *Tilia vulgaris*, respectively. The urban sites were studied in 2010, 2012, and 2013.

Study IV plant material incorporated 44 Norway spruce saplings that consisted of two clones (clone nrs 64 and 1510) (Fig. 5). Saplings were dug and collected from Haapastensyrjä nursery (see location details above) in late spring 2015. Saplings were then moved to the greenhouse and allocated into three treatment groups, with 20 saplings in both the infected and wounded control treatments and 4 saplings in the intact control treatment. The study was conducted in a greenhouse in Helsinki during the summer of 2016. One group of saplings was inoculated in three stem positions with blue-stain fungus *Endoconidiophora polonica*. Trees termed "Wounded control" were mock-inoculated with 2% Malt Extract Agar, and the control trees were left intact. Inoculations were made with a sterile 6-mm cork borer exposed on the xylem surface. Inoculation sites were covered with the cut bark flap and sealed with parafilm.

Figure 5. Sapling material (Norway spruce) in Study IV.

2.2 Measurements

Xylem water potential

Water potential was measured from the needles (Study II) or needle tips (Study IV) after the intact needles were kept in sealed aluminium foil bags for ca. 20 min to equilibrate with the xylem water potential. This way the measured water potential represented the stem water potential and not the leaf water potential (Scholander et al. 1965; Turner 1988). Additionally, the leaf water potential, i.e. without equilibrating with the xylem water potential, was used in the Scots pine saplings in Study II. Water potential was measured by sealing the sampled needle in the pressure chamber. The leaf petiole was cut with a scalpel prior to the measurement. Pressure was increased until water appeared in the cut surface.

Osmolality (Studies I and II)

One method used in this thesis measures the water and osmotically active substances in the inner bark, i.e. the secondary phloem, which consists of tissues from the cambium to the cork cambium. The method for measuring *in situ* osmolality (referred solely as “osmolality” in the text) in this thesis resembles certain previously reported methods for measuring needle and inner bark osmolality (Turner and Jarvis 1975; Tyree 1976; Sobrado and Turner 1983; Callister et al. 2006; Devaux et al. 2009), where needle and inner bark samples are deep frozen and thawed before mechanical extraction (Tyree 1976; Sobrado and Turner 1983; Callister et al. 2006). Needle and inner bark sap was extracted after deep freezing of the samples with liquid nitrogen or dry ice. Sap extraction was conducted with a centrifuge (Heraeus Fresco 17 Centrifuge, Thermo Scientific, Massachusetts, USA) with a relative centrifugal force of 14 000 g for 10 min (Devaux et al. 2009; Paljakka et al. 2019). Prior to

Figure 6. Extracted sap from the needles (light yellow colour on the left), branch (orange in the middle) and stem base (light orange on the right) of a mature Scots pine.

mechanical sap extraction, the needle and inner bark samples were thawn in the cryo tubes in which they were previously preserved in the deep freezer (-80 °C). Thereafter, a freshly cut slice was removed from the sample end of the inner bark samples with a scalpel, and the needles were cut in half, with five pairs of Scots pine needles per sample. The samples were then placed in the centrifuge with the fresh cut facing the silica-based membrane in the collection tubes (GeneJET Plasmid Miniprep Kit, Thermo Scientific, Massachusetts, USA). Next, the sap was moved to the osmolality measurement tubes using pipettes (Fig. 6) prior to the osmolality measurement with a freezing point osmometer (Osmomat 030 cryoscopic osmometer, Gonotec, Berlin, DE). Osmolality values are in molar concentrations, i.e. moles of solutes per mass of water expressed in units mol kg^{-1}. The method description is explained in detail in Paljakka et al. (2019).

Water content (Study II)

The fresh weight of the needles and inner bark samples was measured immediately after sample collection from a separate sample collected along with the osmolality samples. Water content samples were not frozen prior to the measurements. Samples were dried at 80 °C for 72 h before measuring the dry weight. The water content of the needles and inner bark were calculated as the difference of fresh sample weight and the oven dried sample weight per sample dry weight

$$WC = (FW-DW)/DW, \quad (8)$$

where WC is the water content, FW the fresh weight and DW the dry weight of the samples.

Relative water content (inner bark and needles in Studies I and II; stem in Study IV)

Relative water content was calculated from the needle and inner bark samples, as well as from the xylem samples in Study IV. Prior to the calculation, the saturated weight of the samples was measured after soaking the samples in sealed tubes in milli-Q water for 48 h in a cold room at 5 °C after the measurement of sample fresh weight. The fresh and dry weights were measured as described above. Thereafter, the excess water was carefully wiped with a moist tissue and the sample weighed. Relative water content was thereafter calculated as (Weatherley 1950)

$$RWC = (FW-DW) / (SW - DW), \qquad (9)$$

where RWC is the relative water content and SW the saturated water content.

Solute content (Study I)

Solute content (per dry mass) of the osmolality samples (n) was examined with the water content samples by multiplying the osmolality with the water content

$$n = \text{osmolality} * WC \qquad (10).$$

Osmolality at full saturation (Study II)

The symplastic solute content between various tissues in the tree was examined with osmolality at full saturation by multiplying the osmolality with sample RWC (Takami et al. 1981).

$$\text{osmolality}_{\text{fullsaturation}} = \text{osmolality} * RWC \qquad (11).$$

Xylem diameter change (Studies II and III)

Xylem diameter changes were used to estimate the stem water potential change (Irvine and Grace 1997; Perämäki et al. 2001; Sevanto et al. 2002; Chan et al. 2018). Xylem diameter change was measured using point dendrometer sensors (LVDT) (SolartronAX/5.0/S, Solartron Inc., West Sussex, UK). Sensor accuracy is less than a micrometre. The daily variations in xylem diameter change are in the magnitude of tens of micrometres.

Turgor pressure (Study II)

Turgor pressure was estimated in the branches with Equations 1 and 2, where the water potential equilibrium is expected between the xylem and phloem (e.g. Kaufmann and Kramer 1967). Therefore, turgor pressure was estimated with the xylem water potential and the inner bark osmotic potential of the branches by simplifying these equations

$$\Psi p_{phloem} = (-)\Psi p_{xylem} - (-)\Psi s_{phloem}, \qquad (12)$$

where Ψp_{phloem} is the turgor pressure of the inner bark, Ψp_{xylem} the xylem water potential and Ψs_{phloem} the osmotic potential of the inner bark (note the negative sign in front of the osmotic potential).

Sap flow (Study III)

Water flow in the stem and tree-level transpiration may be estimated with sap flow measurement (Granier 1987). Sap flow was measured with custom-made heated and reference temperature sensors at breast height. The temperature difference between the heated and reference sensors estimate the water flow in the stem. Zero sap flow was estimated with a 7-day moving average from the night-time maximum temperature differences.

Tree-level transpiration (Study IV)

Transpiration of the saplings in Study IV was measured by gravimetrically weighing the sapling weight difference between a few hours of transpiration during the warmest time of the day.

Belowground hydraulic conductance (Study III)

Daily maximum transpiration estimated by sap flow (sap_{max}) was divided with the daily amplitude in xylem diameter change, as depicted by Eq. 5, to estimate the belowground hydraulic conductance of the trees (Martínez-Vilalta et al. 2007). Ten per cent daily minimum and maximum means were first calculated from the sap flow and xylem diameter data. The maximum xylem diameter was thus expected to represent night-time soil water potential. Belowground conductance (k_{bg}) was estimated by simplifying Eq. 5, with the difference in daily minimum (d_{min}) and night-time maximum (d_{max}) xylem diameters, i.e. the driving force for the sap flow in the stem, and the daily maximum sap flow sap_{max} as follows

$$k_{bg} = sap_{max} / (d_{max} - d_{min}) \quad (13).$$

Whole-tree conductance (Study IV)

Tree-level hydraulic conductance in Study IV was calculated following the same principle as in Study III, but tree-level transpiration was measured gravimetrically and the xylem water potential was measured as described above in “Xylem water potential”. The soil water potential was estimated from a pre-dawn water potential measurement in a trial study.

Stomatal conductance

The canopy conductance, which estimates the stomatal conductance at the canopy level, was calculated as the daily maximum transpiration divided by the maximum VPD, which is the driving force of transpiration in ambient air. The VPD was calculated with temperature and relative humidity data at the SMEAR 2 station (Junninen et al. 2009) using an empirical equation. Thereafter the canopy conductance (Gs) was calculated by

$$Gs = sap_{max} / VPD_{max} \quad (14).$$

Canopy conductance in Study IV was calculated with tree-level transpiration divided by the mean VPD at the time of measurement. VPD was calculated from the greenhouse environmental data.

Hydraulic conductivity (Study IV)

Hydraulic conductivity is the hydraulic conductance per a given length and cross-sectional area (e.g. within a stem segment) (Eq. 6). Hydraulic conductivity was measured as the water flow rate after pushing water through sampled stem segments at approximately a 0.1-bar pressure (Sperry et al. 1988). This pressure was created with a 1-metre column of water solution through a tube with a diameter between 4 – 9 mm according to the diameter of the stem piece. The solution consisted of HCl, KCl, and milli-Q water, where pH was ~2.2 and solution osmolality was 0.03 mol kg-1. Samples for the hydraulic conductivity measurement were cut stem segments (ca. 10 cm in length), which were sealed in plastic zipper bags after collection. Prior to measurement, one centimetre of the stem bark was peeled from the ends of the stem segments and sealed with silicon tape to ensure that water flow originated only through the xylem. Stem segment ends were cut under water and plastic tubes were attached to the stem ends. Water flow rate was measured from the water meniscus with a calliper for 20 min as the volume of water flow through the stem segment to the plastic tube at the end of the segment. The volumetric water flow rate through the stem piece was calculated by multiplying with the cross-sectional tube area.

Xylem sap surface tension (Study IV)

The first water drops coming through the xylem of the stem segments in the hydraulic conductivity measurement were collected in plastic tubes. These samples were used for xylem sap surface tension measurement. The first drop was discarded to avoid contamination of the sample. The surface tension measurements were conducted with a capillary rise method with a similar setup as in Vanhanen et al. (2008). Glass capillary tubes were thoroughly cleaned with sulphuric acid and distilled water, and tubes were dried before each measurement. The contact on angle of the capillary tubes was expected to be close to zero, as it is for clean glass (Cras et al. 1999). The capillary tubes were then placed in a holder in the collected samples in plastic tubes at room temperature (20 °C) for 1 h. The capillary rise was measured with a hand-held caliper after tubes had been in the sample at room temperature (20 °C) for 1 h. Distilled water was used to test the radius of the capillary tubes, as the water surface tension is well known. Surface tension was then calculated as

$$\sigma = 1/2\, \rho g r\, (h + r/3), \qquad (15)$$

where σ is surface tension (N m-1), ρ is water density (kg m-3), g is the gravitational acceleration (9.81 m s-2), r is the radius of the capillary tube (m), and h is the height of capillary rise inside the tube (m).

2.3 Statistical testing

Two level mixed effect model, with species and region included, were used with spatial analyses of osmolality, water content and solute and sugar contents in Study I. Linear regression was used for statistical testing in Study II. Normalized values of belowground conductance were used in a mixed-effect model between the years in different species in Study III, with the effect of soil temperature and soil water content included. The seasons in Study were defined as the soil temperature being < 8 °C in spring and autumn. Student’s t-test and linear regression were used for statistical testing in Study IV. All statistical tests were conducted in R (R Foundation for Statistical Computing, Vienna, Austria) apart from the mixed-effect model analyses in Study II which were conducted in SAS (SAS, version 9.4, SAS Institute Inc., Cary, NC).

3 RESULTS

3.1 Osmolality in relation to solutes, water content and xylem water potential

Studies I and II investigated needle and inner bark osmolality and to what extent the water content or solutes contribute to osmolality. The xylem water potential was measured in Study II with the inner bark osmolality to understand how water potential is transmitted to the living tissues of the phloem and needles (Fig. 7a and b). The branch inner bark osmolality was largely driven by the tissue water content in both species in Study I because the mean solute content was rather steady between the study sites (see the original Fig. 3 in Study I paper). The branch inner bark osmolality increased towards the northern and southern measurement sites.

The relation of osmolality and osmolality at full saturation (Figs. 7c and d) is shown here, although it was not shown in Study I, to compare the symplastic solute contribution to osmolality between Studies I and II. The relation of osmolality and osmolality at full saturation and their range were similar in Scots pine and Norway spruce across the sites (Fig. 7c). The tissue turgid weight and osmolality also correlated in the present data (not shown), thus indicating an osmotic adjustment through cell volume changes to water availability, as also discussed in Study I. Osmolality and osmolality at full saturation were also correlated in all the tree compartments examined in Study II (Fig. 7d). The osmolality at full saturation in the needles was much larger in June compared to the other measurement periods (Fig. 7d). Changes in osmotically active solutes explained ca. 30-% of the changes in osmolality in the branches and 50-% in the stems of the Scots pines in Study II. Osmolality at full saturation gradually decreased with increasing water contents towards the stem base, resulting in gradually decreasing osmolality from the needles towards the stem base (see the original Figure 5 in Study II paper). Solute content per dry weight remained rather steady between the tree compartments, which indicates a similar relationship in both Studies I and II with the tissue osmolality, water content, and solute levels in relation to changing water potential. An osmolality gradient was found decreasing towards stem base in both osmolality and in osmolality at full saturation, although the latter was more distinct, and in relative water content in Study II. The inner bark sugar osmolality (osmolality calculated from sugar concentrations) also decreased towards the stem base in Study II. These axial osmotic adjustments in sugar concentrations resulted from larger changes in monosaccharides than sucrose (see the original Fig. 8a in Study II paper).

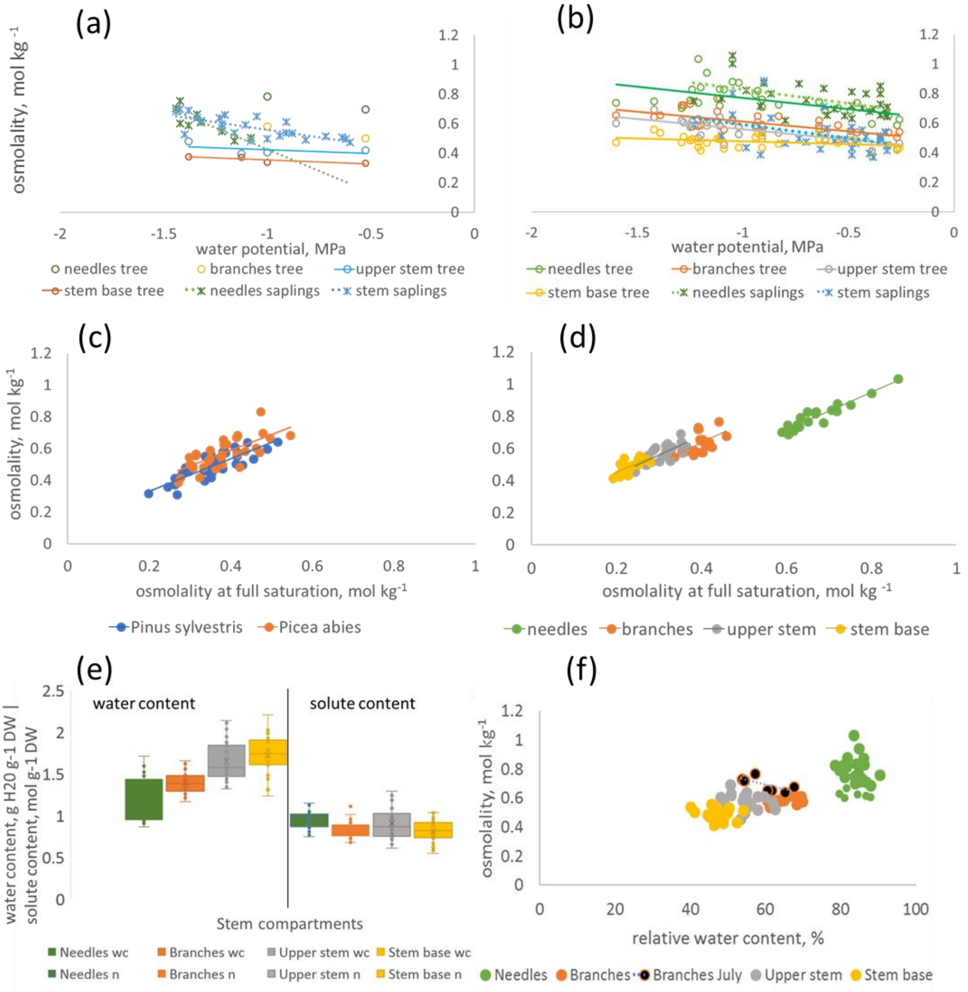


Figure 7. Inner bark and needle osmolality in relation to xylem water potential in Norway spruce (a) and in Scots pine (b), and to osmolality at full saturation (c,d), the solute content (n) and water content (wc) in the different tree compartments (e), and the relation of osmolality and relative water content (f).

Xylem water potential correlated with needle and inner bark osmolality in all the studied tree compartments in mature trees and in saplings of both species (Fig. 7e and f). Both mature trees and saplings had similar relations between xylem water potentials and osmolality. The highest correlations with xylem water potential were found in the inner bark osmolality of branches and upper stems in mature Scots pines, and with needles and stem inner bark in the saplings of both species. Relations of needle and inner bark osmolality to water potential were similar in Scots pine and Norway spruce, although the small number of observations prevented comprehensive statistical analyses in mature Norway spruce. The water contents of the upper stem and stem base were the highest with the lowest relative water contents (Fig. 7e and f). Water content measurements were not conducted with Norway spruce in Study II.

3.2 Inner bark osmotic potential and turgor pressure in the branches

The components of branch inner bark water potential in the measurement periods of mature Scots pine are shown in Figure 8a (note the negative sign in front of the osmotic potential). Osmotic potential increased (was less negative) with increasing turgor pressure in the branches when the water potentials experienced by the trees were not too low such as in June and September (see the original Fig. 5a in Study II paper). However, the branch osmotic potential decreased with increasing turgor pressure in July contrary to other measurement periods (Fig. 8a and b). This seems to result mainly from the sufficient decrease in the tissue water content, as the osmotic potential and branch relative water content correlated during this period (Fig. 7e). The turgor pressure in the branch was zero when the branch inner bark water content changed by ca. 10% in July. At this time in July, tree seemed to control the stomata as it is shown in the Figure 8b, where the components of branch inner bark water

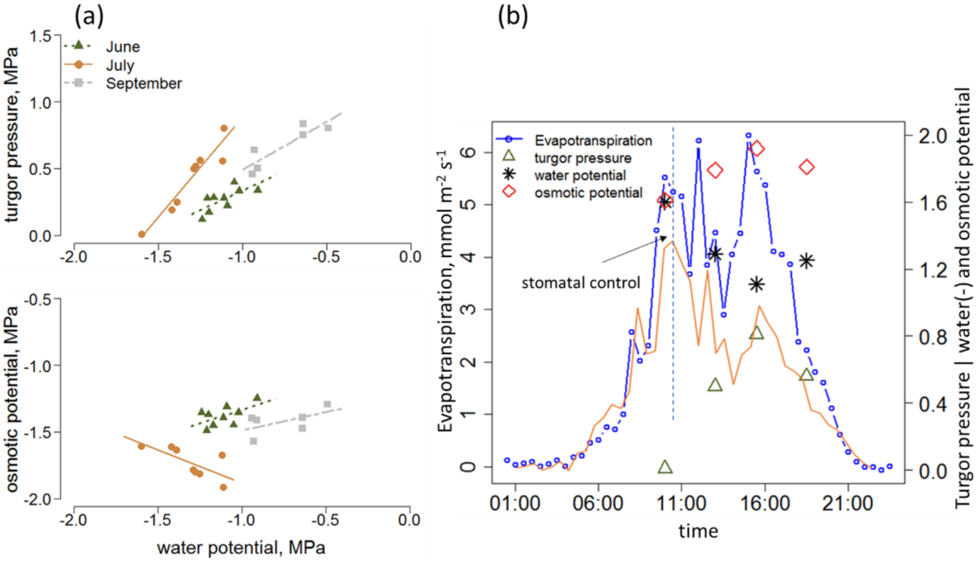


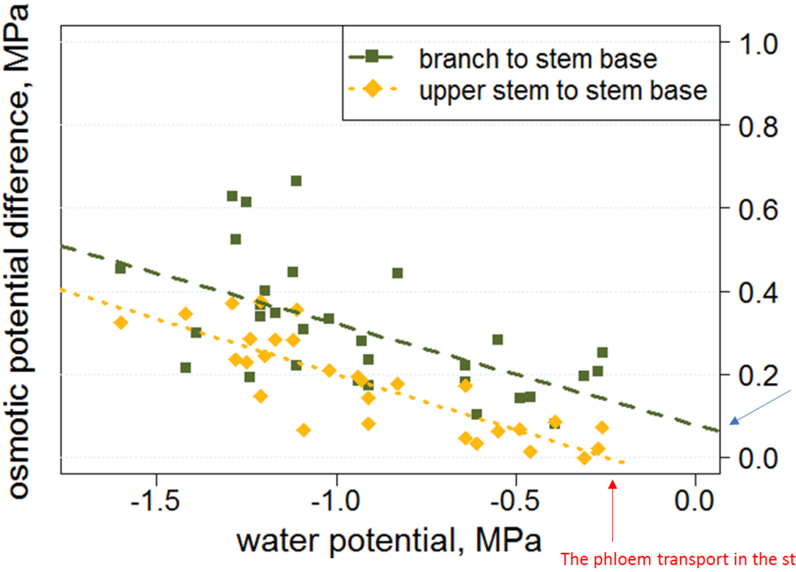
Figure 8. Components of water potential, i.e. turgor pressure and osmotic potential, in branches in relation to xylem water potential in the measurement periods in Scots pine (a). The negative sign in front of the osmotic potential describes the effect of osmotic potential to tissue water potential. The tissue water potential components together with xylem water potential, ecosystem-level evapotranspiration (measured with eddy covariance) (Vesala et al. 2005), and surface conductance (orange line) indicating stomatal control (b) during this clear and warm summer day in the measurement site at the SMEAR 2 station in Hyytiälä in July 2014.

potential, i.e. the osmotic potential and turgor pressure, are compared to surface conductance (stomatal conductance in the ecosystem level) calculated from the eddy covariance measured evapotranspiration. Although, making firm conclusions based on observations from only one day is challenging. Turgor loss point in the branches was ca. -1.6 MPa at all the measurement periods in Study II.

There is an unfortunate error in Study II (Study II Figure 6), where one measurement point from July is represented as an observation from September. Additionally, one point from September is missing from the figure but is shown above in Figure 8. In practice, this does not change the slope of the regression, and the turgor loss point is the same in September in both figures (see Fig 8. above and Fig. 6 in Study II).

3.3 Axial osmotic potential gradients in relation to xylem water potential

One key finding of Study II in the context of phloem transport was that a sufficient osmotic gradient remained from the branches to the stem base, although the xylem water potential was close to zero (see the blue arrow in Fig. 9). Study II also showed that the osmotic gradient in the stem was supported by gravity when the xylem water potential corresponded to pre-dawn conditions (see the red arrow in Fig. 9). The gravitational potential in the canopy of a 20-metre tall tree is ca. 0.2 MPa.



Osmotic gradient from branch to stem base when xylem water potential is zero.

The phloem transport in the stem is aided by gravity in pre-dawn conditions, i.e. during night time.

Figure 9. Osmotic potential gradient between the branches and stem base and between the upper stem and stem base in relation to xylem water potential. The blue points refer to the osmolality gradient between the branches and stem base and the brown points refer to the osmotic potential gradient between the upper stem and stem base.

3.4 Axial tissue elasticity and direction of turgor pressure gradients

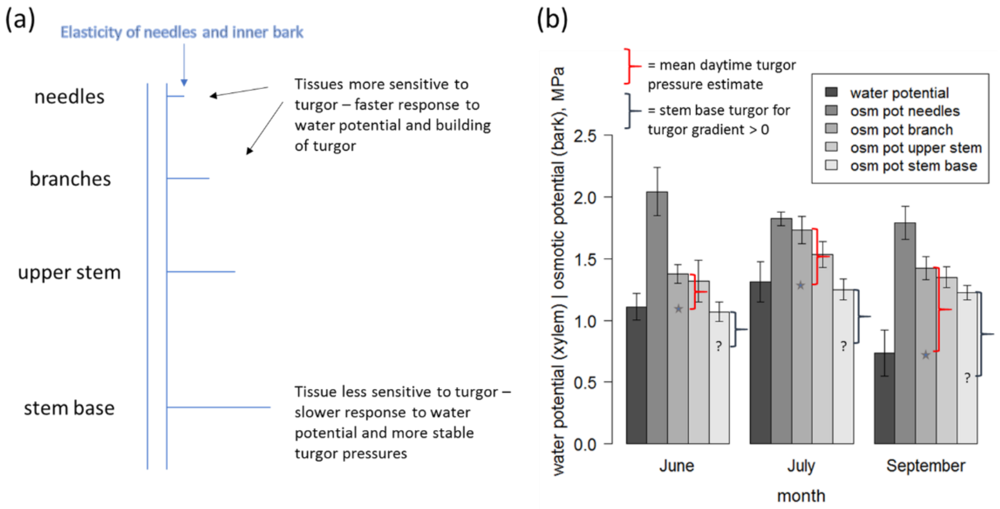


Figure 10. Tissue elasticity between tree compartments derived from their water content and relative water content (a), xylem water potential measured from the needles, and osmotic potential of all studied positions in mature Scots pines. The xylem water potential is negative, but expressed as an absolute value in this figure to help point out the turgor estimates in different positions. Xylem water potential is less negative towards the stem base. Parentheses indicate turgor pressure estimates in the branch and baseline turgor pressure in the stem for a zero turgor gradient. The impact of gravity is not considered in the stem base turgor estimate in the figure.

The tissue elasticity of needles and inner bark of branches and stem is estimated in relation to these tree compartments (Fig. 10a) based on the water content and relative water content measurements in Fig. 7e and 7f. These estimations indicate that inner bark tissue elasticity was higher in the stem base than in the canopy. Certain preconditions can be estimated for the turgor gradients, based on the branch turgor estimate in Study II. The mean water potential in the stem base needs to be lower than -0.8 MPa in June and July and lower than -0.6 MPa in September for an equal daytime turgor pressure in the branches and stem base (Fig. 10b). After extracting the gravitational potential from these values, the mean stem base water potential should be lower than -0.6 MPa in June and July, and -0.4 MPa in September for an equal daytime turgor pressure in the branches and stem base.

3.5 Whole-tree conductance and stomatal conductance during the growing season

The tree belowground hydraulic conductance increased with increasing soil temperature in spring and autumn in Scots pine. Additionally, the stomatal conductance followed this increase, meaning that the leaf gas exchange was related to belowground hydraulic conductance. During the summer the hydraulic conductance was influenced by the soil water content.

3.6 Tree water transport under pathogenic invasion

Stem xylem conductivity decreased rapidly because of the pathogenic infection caused by *E. polonica* in Study IV. Decreased xylem sap surface tension was associated with the declining conductivity of the xylem (Fig. 11a) and a decline in stem relative water content (Fig. 11b). Xylem sap surface tension differed from pure water in all the studied trees in Study IV. The hampering of water transport resulted in local loss in the xylem conductivity, as tree-level conductance or transpiration did not clearly differ between the treatments. Infection resulted in visible symptoms, such as wilted branches, and, in the stem, as reacting xylem or spreading of fungus hyphae in the desiccated xylem.

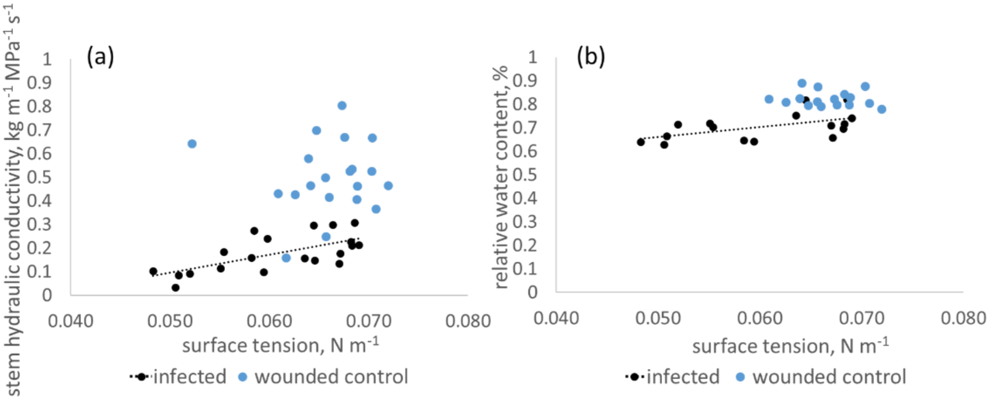


Figure 11. Xylem sap surface tension in relation to stem hydraulic conductivity (a) and stem relative water content (b) in *E. polonica* infected and control Norway spruce saplings in Study IV.

4 DISCUSSION

4.1 Drivers of needle and inner bark osmolality across sites, and in axial osmotic gradients

Across the latitudes the branch inner bark osmolality is more driven by water than solutes. Thus, inner bark tissue water contents contribute to tissue osmolality more than was expected in Study I. Branch inner bark osmolality increases towards northern and southern latitudes indicating to physiological acclimation to cold and dry environments. For example, higher osmolality is required to retain water in the inner bark at lower xylem water potentials, which may also be the situation in drier climates. Osmolality was also studied axially in relation to water and solute contents in Study II. The osmolality in the needles is driven by solutes over water. The osmolality in other tree compartments, especially in the branches, is driven more by tissue water content. Stem osmolality is considerably driven by both water and solutes, which is also supported by the dendrometer and sugar concentration measurements (see also Figures 3 and 8b in the original Study II paper). A decrease in the water content increases osmolality because there is less water in relation to solutes in the tissue, as depicted in Eq. 3 (e.g. Tyree et al. 1978). Observations of stem water contents and the high correlation of stem inner bark osmolality and xylem dendrometers, which detect the volumetric changes in the stem base, support that tissue water contents change considerably and contribute to stem base inner bark osmolality with the solutes (Sevanto et al. 2011). Early summer is the most intensive time for shoot growth and the reason for the higher solute content and the larger contribution of solutes to osmolality in the needles may also relate to this seasonality in tree growth (Antonova and Stasova 2006; Woodruff and Meinzer 2011).

Osmolality at full saturation indicates that solutes contribute more to branch inner bark osmolality than do the solute content estimates across the latitudes. A comparison of solute content alone is difficult because the tissue characteristics differ between tree compartments and studied sites (Rosner et al. 2001; Antonova and Stasova 2006; Gričar and Cufar 2008), and these differences, e.g. the proportion of cell walls of the tissue volume, affect the solute content through dry weight. Osmolality at full saturation describes the osmotic potential when the water potential is zero, meaning that under these conditions the tissue turgor pressure corresponds to the osmotic potential. Thus, osmolality at full saturation is also affected by the elastic properties of the tissue cell walls. Osmotic adjustment may also occur through changes in cell volume, which can be seen in the relation of tissue turgid weight and osmotic potential (Hsiao 1973; Sobrado and Turner 1983; Turner et al. 1987). The elastic properties of the inner bark influence the turgid weight when sample tissues are hydrated, which affect the osmolality at full saturation. Nevertheless, osmolality at full saturation may be more suitable for comparing the osmotic adjustment than the solute content (Rosner et al. 2001), as it is related to water potential (i.e. water potential is zero). However, both these measures, as well as the *in situ* osmolality, contain uncertainties that are discussed more in the “Method assessment” section.

Axial gradients in osmolality, osmolality at full saturation and relative water content are distinct, with all these variables gradually decreasing towards the stem base. Water contents increase from needles towards the stem base. Gradient of osmolality at full saturation is more distinct than osmolality gradient. Rosner et al. (2001) reported that osmolality at full saturation decreased gradually towards the stem base but osmolality did not show a clear gradient vertically. Vertical gradients in water potential exist in trees because gravity and the pathway resistance contribute to the xylem water potential (Hellkvist et al. 1974; Marshal and Monserud 2003; Woodruff et al. 2004). Such gradients also reflect to the phloem, as the living cells need to maintain turgor pressure (Woodruff et al. 2004). Vertical adjustment in osmolality relates to vertical gradients in water potential, in wood sugar concentrations (Sala and Hoch 2009; Woodruff and Meinzer 2011), in phloem tissue sugar osmolality (Woodruff 2014), and in phloem sap sugar concentrations in the sieve cells (Cernusak et al. 2003). Gradually decreasing sugar osmolality is observed with considerably larger variation in the monosaccharides in Study II, which is also observed in the phloem tissues of Douglas-fir (Woodruff 2014). Mainly sucrose is transported in the phloem of conifers (Pate 1976; Rennie and Turgeon 2009). The role of sucrose in the inner bark sugar osmolality likely increases if the analyses concentrate more on the conducting phloem instead of the whole inner bark (Rosner et al. 2001).

Xylem water potential is transmitted to all tree compartments, especially to needles, branches and upper stem as was hypothesized in Study II. Although, needle osmolality seems to be somewhat decoupled from the xylem and needle water potentials in the mature trees and saplings in Scots pine compared to Norway spruce saplings. A large share of resistance to water transport is to be expected from the leaf xylem to the mesophyll cells (e.g. Hacke 2014). Water potential appears to be well transmitted to the upper stem. Based on the water content measurements, stems have more capacity to store water than the canopy. Changes in water potential are thus rapidly transmitted to osmolality through the water content (Noy-Meir and Ginsburg 1969). Water content measurements were not conducted with Norway spruce in Study II, which may have different patterns in water storing capacity between the canopy and stem bark (Zweifel et al. 2001) compared to Scots pine. Water potential measured from the needles is also not as clearly transmitted to the stem base as to compartments in the canopy (Hellkvist 1974; Perämäki et al. 2005; Woodruff 2004). Additionally, there is a time lag in how rapidly water potential changes are transmitted to the stem base (Sevanto et al. 2002). The dendrometer measurements can efficiently detect the relation of xylem water potential and inner bark osmolality in the stem base because they detect the change in tissue turgor and volume (Irvine and Grace 1997).

4.2 Components of inner bark water potential in the branches

With measurements of xylem water potential and branch inner bark osmotic potential, measured close to the needles at the canopy, the branch turgor pressure can be roughly estimated. This allows investigation of branch inner bark osmotic potential, turgor pressure and water potential together. The relation of osmotic potential to turgor pressure and xylem water potential have seldom been analysed in field conditions in past studies. Branch inner bark osmotic potential increases with increasing turgor pressure in June and September, but opposite behaviour is observed in July. In July, the branch osmotic potential likely decreases because of the decreasing branch relative water content. Osmotic potential correlates with the relative water content only when the decrease in the branch relative water content is substantial, such as in the branches in July, after a sufficient drop of turgor pressure (Hsiao

1973). The inner bark tissue water potential in the branches and stem is hence mainly driven by the change in turgor pressure in Study II, which covers the relations of water content and osmolality until turgor is zero (Turner 1981) and solute contents play only a minor role (Tyree and Hammel 1972). When tissue turgor is zero, also referred to as the turgor loss point, the osmotic potential is expected to follow the xylem water potential (Turner 1981). Turgor loss point in branch inner bark is at xylem water potential ca. 1.6 MPa in all measurement periods. The relationship between water potential and tissue water content, osmotic potential and turgor pressure may be understood with pressure-volume curves (e.g. Weatherley and Slatyer 1957; Tyree and Hammel 1972) (see also Fig. 2), where the water potential is first passed on as a change in turgor pressure with small initial changes in relative water content and osmotic potential. Water movement through the cell membrane is much faster than the membrane permeability to solutes (Zimmermann 1978). Cell wall elasticity determines the sensitivity of turgor pressure and osmotic potential to changes in water potential (Noy-Meir and Ginsburg 1969). Therefore, osmolality changes more rapidly in relation to turgor pressure in elastic cells, whereas turgor pressure is more sensitive to water potential (Noy-Meir and Ginsburg 1969) in rigid cells such as those in the canopy (Gross and Koch 1991). Thus, the gradually decreasing relative water content indirectly indicates a gradually decreasing turgor pressure towards the stem base (Rosner et al. 2001). The higher water content along with the smaller relative water content indicate greater cell wall elasticity such as in the stem compared to the branches and needles in Study II.

One possible explanation for the contrary behaviour of branch osmotic potential and turgor pressure in July could be that the tree closes its stomata because the xylem water potential is low (as shown in Fig. 8b). Closing the stomata prevents losing water in transpiration and thus enables the increase in the xylem water potential and turgor pressure of the branches. Scots pines close their stomata rapidly at water potentials of approximately -1.5 MPa and may open and close their stomata multiple times during the day (Jarvis and Jarvis 1963; Hodges 1967; Irvine et al. 1998). Scots pine is reported to maintain the minimum water potentials steadily above -1.5 MPa in the xylem (Irvine et al. 1998; Martínez-Vilalta et al. 2009). The observed turgor loss point value of ca. -1.6 MPa in the branches is reasonable in Study II considering the stomatal behaviour reported for Scots pine. Eventually, the tree needs to open its stomata for photosynthesis and to gain nutrients with water transport (Woodruff and Meinzer 2011). Similarly, Scots pine is sensitive to prolonged drought as it maintains the minimum water potentials at high levels by stomatal closure (Martínez-Vilalta et al. 2009; Sterck et al. 2012), but this also reduces photosynthetic production. Turgor loss point is usually estimated from the leaves and is considered a good indicator for the drought tolerance of a species (Bartlett et al. 2014). Moreover, it does not appear to have been estimated previously for Scots pine (Bartlett et al. 2016). Trees can also acclimate to their environments by osmotic adjustment, thereby also adjusting the turgor loss point to water stress (Hsiao 1976; Bartlett et al. 2014). The turgor pressure estimate shown above includes the errors of the water potential measurement as along with those from the osmolality sap extraction method, and it is therefore a rough estimate of turgor pressure (Turner 1988). Turgor pressure was not estimated for the branches in Norway spruce. Norway spruce is reportedly less sensitive compared to Scots pine to stomata closure when water potentials decrease (Jarvis and Jarvis 1963; Gonzales-Munoz 2018).

4.3 Osmotic potential gradients and Münch’s theory of phloem transport

A sufficient osmotic gradient from branches to stem base was found in Study II, which supports the Münch hypothesis and these osmotic gradients enable the turgor gradient-driven phloem transport (Zimmerman 1957; Hammel 1968). Study II indicates also that during times of high xylem water potential and small transpiration rates, e.g. at night or during rainy conditions (Hammel 1968), the driving force for mass flow in the stem is already provided by gravity. At other times, the osmotic gradient provides the additional driving force needed for mass flow. The role of gravity in the phloem transport has been previously reported in modelling studies (Hölttä et al. 2009a). The osmotic potential may be even higher in the conducting phloem (Rosner et al. 2001) than when studying the osmotic potential of the whole secondary phloem such as in this thesis. Additionally, the sap extracted from the whole inner bark may be more dilute than the sap measured directly from the sieve cells because the apoplastic water mixes with the sap from the symplast (Tyree 1976; Meinzer et al. 1986; Kikuta and Richter 1992). Therefore, the resulting turgor pressures or even the turgor pressure gradients may be higher in the sieve cells than in the whole inner bark. A gradual decrease in the inner bark relative water content towards the stem base observed in Study II also supports the existence of turgor gradients from the canopy to stem base (Tyree and Hammel 1972; Rosner et al. 2001). Turgor pressure is very challenging to measure directly from the phloem (Turgeon and Wolf 2009) and indirect turgor pressure estimates are therefore used. Study II supports the view that Münch’s theory, where osmotic gradients create a hydrostatic pressure gradient to drive mass flow in the phloem, is a plausible theory to explain phloem transport in conifers in field conditions.

4.4 Turgor gradient between sources and sinks

The axially smaller conductivity resulting from an axially decreasing water potential (Woodruff 2014) is considered to hinder the formation of a turgor gradient for driving the phloem transport in conifers (Woodruff et al. 2008; Turgeon 2010), although structural chracteristics allow for a sufficient turgor gradient (Hölttä et al. 2013). However, larger elasticity towards the stem base observed in Study II, and discussed above, already supports the formation of a turgor gradient from the canopy towards the stem base through the differing sensitivities of turgor pressure towards changes in water potential in the canopy and stem tissues (as shown in Fig. 10a). Stem water potential should be known for making proper turgor estimates in the stem, but it is not well known in conifers. Water potential is expected to be considerably higher in the stem base than in the canopy (Hellkvist 1974; Woodruff 2004; Perämäki et al. 2005; Hölttä et al. 2009b). Regardless of these uncertainties, the phloem transport seems to progress up towards the canopy during the daytime, as previously suggested in a modelling study by Nikinmaa et al. (2014), unless the xylem water potential is lower than these values at the stem base. Trees should be able to maintain their solute transport towards the sinks at the tree base and belowground (Lemoine et al. 2013). However, this already seems challenging in conventional environmental conditions during the daytime. It is thus no surprise that the phloem transport velocity decreases during water stress (Dannoura et al. 2011 and 2019). The transport velocity in the phloem is ca. 0.1 m h^{-1} and 0.2 m h^{-1} in Scots pine and Norway spruce, respectively (Liesche et al. 2015), meaning it may take up to 12 days for sugars in the canopy to reach belowground (Mildner et al. 2014).

Therefore, it is reasonable that trees maintain large carbohydrate storages (Sala et al. 2011) that are used in the sinks further away from the canopy (Mildner et al. 2014). If daytime turgor gradients are challenging to maintain towards the stem base, radial growth rates are reasonably at their highest in the evening and night-time (Antonova et al. 1995). Radial growth is also sensitive to low water potentials, as turgor pressure is needed in cell expansion (Cosgrove 1986). The critical thresholds for growth may be encountered at stem water potentials of ca. -0.5 to -1 MPa (Körner 2015). Stem base turgor pressures have also been shown to connect to photosynthesis (Mencuccini et al. 2013) and thus, through turgor pressure changes, to solute transport between the phloem loading sites at the sources (Liesche et al. 2011) and sinks (Hölttä et al. 2006, Mencuccini et al. 2013). Water potential at the stem base likely operates in a smaller range compared to the canopy (Perämäki et al. 2005), and the cell wall elasticity smoothens the turgor changes at the stem base. Together these issues indicate that the turgor gradient is more determined in the canopy. Additionally, the sink activity affects the osmotic potential and thus the turgor pressure, which feeds back to photosynthesis (Kirschbaum 2011; Nikinmaa et al. 2013; Hölttä et al. 2017). For example, roots consume the sugars transported there, which lowers the osmotic potential and the turgor pressure at the stem base when sink strength is high. Maintaining the turgor gradient towards the roots seems to be easier in September because xylem water potential needs to only be lower than -0.4 MPa at the stem base during the daytime. This is in line with the known patterns that trees allocate photosynthetic products towards the roots in autumn (Hansen and Beck 1994).

4.5 Osmolality sap extraction method assessment

The present method for osmolality sap extraction with the deep freeze-thaw treatment is investigated in Studies I and II by comparing it to several other physiological variables, including the water potential, water content, and sugar content. Mechanically extracted sap account on average for ca. 25% of the inner bark and ca. 45% of the needle tissue water content after sap collection with a centrifuge (not shown). Callister et al. (2006) also reported ca. 50% water sap extraction of leaf water content in broadleaved trees with a larger centrifugal force. The amount of extracted sap also correlate with osmolality in the inner bark (not shown). However, comparisons to other osmotic potential measurements have demonstrated that the amount of extracted sap does not cause large deviation from the compared methods (Kikuta and Richter 1992; Callister et al. 2006). Extracted sap amounts may be related to symplastic and apoplastic water fractions (Callister et al. 2006). The error caused by apoplastic water to symplastic osmotic potentials is estimated to dilute the osmolality samples by 10% to 20% in the conventional range of the water potentials (Tyree 1976; Wenkert 1980; Callister et al. 2006), as the apoplastic water is dilute (Turner 1981). Thus, the apoplastic water will affect the osmolality measurement. Apoplastic water is difficult to assess and corrections of it may result in larger errors in osmotic potentials (Wenkert 1980). An increase in the proportion of cell walls to cell volume increases the apoplastic water quantity in the cell walls (Markhart 1981). The apoplastic water quantity is related to the symplastic water potential because apoplastic and symplastic water potentials are expected to equilibrate (Thompson and Holbrook 2003). Tissue water content measurement also includes the water content of both the apoplast and symplast (Tyree 1976), and the estimates for solute content and osmolality at full saturation therefore also include uncertainty because of the water content measurements that are used for estimating these variables (Rosner and Kikuta 2002). This dilution of osmolality at full saturation depends on the studied species (Turner et al. 1987). Previously used methods have most often been used for studying leaves. Devaux et al. (2009) compared the centrifugation method in inner bark samples with phloem sap exudation methods and reported that mechanical sap without the freeze-thaw treatment extraction underestimates the compound content in the sap. One advantage is that no additional solvents are needed, which are commonly used in phloem sap exudation methods. The freezing treatment or osmotic changes due to enzymatic activity during thawing will not affect the results, not even after an hour (Bartlett et al. 2012). Evaporation from the small samples needs to be considered when determining the osmotic potential (Rosner and Kikuta 2002; Bartlett et al. 2012), which is in line with the test measurements in this thesis. Freeze-thaw treatment mixes the symplastic and apoplastic solutions, and the largest error for estimating solute concentrations in this method may originate from apoplastic water dilution (Tyree 1976; Turner et al. 1981). The method used in this study (Paljakka et al. 2019) is robust, easy to use, and it is not as sensitive to evaporation that accurately targets the conducting phloem.

4.6 Progress of whole-tree conductance and stomatal conductance along with seasonal phenology

Study III showed that the seasonal progress in soil variables also determine the preconditions of water transport in Scots pine and in two broadleaved species. In Scots pine, a tree's belowground hydraulic conductance increases with increasing soil temperature having more influence on tree water transport in the spring and autumn. During summer, soil water availability influences the hydraulic conductance, and thus the tree water transport, over soil temperature in Southern Finland. Soil temperatures affect the root water uptake through the temperature dependency of viscosity and the water flow regulation through aquaporins (Oliviusson et al. 2001) as well as through changes in membrane permeability (Henzler et al. 1999; Javot and Maurel 2002). The relation of soil water content and belowground hydraulic conductance is to be expected in the summer time because less water in the soil will lower root conductance and hamper tree water uptake (Duursma et al. 2008; Rodriguez-Gamir et al. 2019). Trees may promote belowground hydraulic conductance if water availability hampers water uptake by adding carbohydrate levels in the roots (Hagedorn et al. 2016; Rodriguez-Gamir et al. 2019). The findings in Study III suggest that increasing soil temperatures in spring and autumn may also result in higher annual tree photosynthetic production and carbon uptake in addition to the positive effect of temperature to photosynthesis (Berry and Björkman 1980; Stinziano et al. 2015). The longer growing season would be advantageous for carbon uptake in the spring, as light limits the growing season in the autumn (Way 2011) but an autumnal temperature increase would nevertheless benefit Norway spruce at least (Stinziano et al. 2015). Increased spring and autumn temperatures have already changed the timing of tree phenological events in past decades (Chung et al. 2013). Soil temperatures are also related to bud burst and shoot growth in Norway spruce (Sutinen et al. 2014). The onset of radial growth is determined by early spring temperatures, meaning that vascular tissues in Scots pine and Norway spruce are tuned already during the early growing season (Antonova and Stasova 2006; Swidrak et al. 2014). In addition to hydraulic conductance, tree recovery includes the awakening of the photosynthetic capacity in both these species (Bergh and Linder 1999; Kostiainen et al. 2009; Linkosalo et al. 2014) and several physiological adjustments (Turner and Jarvis 1975). Norway spruce recovers more rapidly than Scots pine after winter, which may enhance its photosynthetic production along with exposing it to frost damages (Christersson 1972; Linkosalo et al. 2014). Predicting the effects of an earlier spring or later autumn on tree productivity is difficult because varying temperatures affect the progress of tree phenology and winter acclimation if they result in cold stress or freezing injuries when the growing season is ongoing (Chung et al. 2013). Decreasing snow cover may cause lower soil temperatures and delay soil thawing and thus the springtime recovery of tree water transport and growth in both Norway spruce and Scots pine (Repo et al. 2004; Sutinen et al. 2014). A longer growing season with higher spring temperatures may also enable more insect generations, causing stress to trees, and possibly also influence the distribution of insects (Roualt et al. 2004).

4.7 Tree vulnerability to embolism increases under pathogenic invasion

The increase in spring temperatures may increase the number of insect generations that encounter favourable flying days (temperatures above 16 °C) (Netherer et al. 2015). Additionally, trees in spring time are more intensively attacked by bark beetles because trees are likely more attractive during spring recovery (Netherer et al. 2015). Attacks of bark beetles also involve ophiostomatoid fungi, such as *E. polonica*, which was studied in Study IV. The conclusions in Study IV imply that tree vulnerability to embolism may increase in conifers because of pathogenic infections such as *E. polonica*. The decrease in the stem hydraulic conductivity, along with a decreased stem relative water content (Rosner et al. 2019), indicate towards embolism in the stem caused by the decreased xylem sap surface tension in the *E. polonica* inoculated trees. The resulting decrease in xylem sap surface tension is, in other words, proportional to increasing tree vulnerability to water stress, as shown in Fig. 3 (Sperry and Tyree 1988; Cochard et al. 2009; Hölttä et al. 2011). The local decrease in stem hydraulic conductivity in infected trees is not apparent in the tree transpiration in Study IV, likely because the stem is well buffered (Hölttä et al. 2009b), and locations for hydraulic disruptions are more likely to occur closer to the canopy with higher resistance to water transport (Zwieniecki et al. 2002). Studies of embolism vulnerability assume that xylem sap is not dynamic, and the effect of surface tension has often been neglected (Domec 2011). Losso et al. (2018) observed also that xylem sap surface tension differed from that of pure water in Norway spruce trees growing in field conditions, with similar range of xylem sap surface tension values to Study IV. However, the cause for the lowered xylem sap surface tension was not detected in Study IV. *E. polonica* has been observed progressing through the living cells in the xylem and located in the bordered pits of Norway spruces (Francheschi et al. 1998). Bordered pits are in a key position to prevent the spread of air-seeding from embolized cells elsewhere in the xylem (Hacke et al. 2004; Sperry 2011). Hampered water transport eventually leads to decline in tree vitality, as indicated also by the visible symptoms observed in Study IV (Fig. 12b). Pathogens, such as *E. polonica*, may thus introduce a more rapid change in hydraulic conductance when the infection is sufficiently intense (Horntvedt et al. 1983; Kirisits and Offenthaler 2002) (Fig. 12a), compared to the slow seasonal change observed in Study III. For example, massive bark beetle invasions can cause such intensity while introducing the fungus at the wood surface (Christiansen 1985). Large disturbances have been encountered after bark beetle invasions

(Solberg 2004; Williams et al. 2012). Such disturbances are expected to increase in the future climate (Allen et al. 2010; Seidl et al. 2017). Tree resistance along with the resin ducts are the first line of defence (Rissanen et al. 2015), and versatile constitutive defence compounds aid in maintaining conifer functionality (Krokene 2015). After a biotic attack, conifers may enhance their resistance with induced terpene and phenolic defence compounds (Krokene et al. 1999; Baier et al. 2002; Nagy et al. 2004). These defence enhancements are costly for the tree, and after depletion of easily accessible carbon reserves the tree needs to maintain its photosynthetic production and vascular transport (McDowell 2011; Garcia-Forner 2016). Tree declines caused by insects and pathogens are often related to water stress and low tree water potentials (Jactel et al. 2012; Netherer et al. 2015; Netherer et al. 2019).

Figure 12. Bark beetle-bored holes in the stem of a desiccated Norway spruce under natural conditions (a). Symptoms of infection in the branches of *E. polonica*-infected Norway spruce saplings studied in Study IV (b).

5 CONCLUDING REMARKS

This thesis provides further evidence with field measurements for the prevailing theories concerning tree long-distance transport in the xylem and phloem, and on how they are interconnected with each other and to tree gas exchange. Research on simultaneous physiological measurements of the xylem and phloem has been scarce in conifers in field conditions. This thesis supports the tightly connected relations between the vascular tissues and leaf gas exchange in mature trees and saplings in the studied species, and these relations seem to be universal plant traits based on the recently published literature. The studies in this thesis have also produced new knowledge of how trees are connected to their surrounding environments and how interconnected the tree compartments are during environmental changes. The main findings in this thesis were that inner bark osmotic potentials were driven by both water content and solutes in the branches of Scots pine and Norway spruce and in all tree compartments in Scots pine, with water having the strongest influence on osmotic potential in relation to changes in water potential. The osmotic adjustment due to tissue volumetric changes was partly investigated and may cause some of the change observed in tissue solute contents. This study was the first to measure the branch inner bark osmotic potential on a continental scale. The prevailing phloem transport theory, i.e. the Münch hypothesis, was supported by this thesis in mature conifers with field measurements. Xylem water potential was connected to osmolality in all tree compartments, and the interplay between the xylem and phloem determined the turgor gradient driving the long-distance transport in the phloem. This thesis was able to outline that the theory is plausible in the field conditions for Scots pine, with similar indications for Norway spruce. Field measurements are generally important because they eventually show to what extent the phenomena observed in laboratory conditions actually contribute in nature. This thesis supports the idea that solute translocation in the phloem is tightly connected to water transport in the xylem, which has been demonstrated in many modelling and laboratory studies. Sufficient turgor gradients exist in the phloem, and they can drive the mass flow in the phloem of these conifer species with the aid of gravity. Daytime turgor gradients may be difficult to maintain in the canopy towards the tree base, which may complicate the allocation of photosynthetic products during the daytime or during times of water deficits. Such conditions would also affect the supply for the sinks and thus also growth. Furthermore, the allocation of recent photosynthates to growth and to the stem base may be easiest at night and allocation towards the stem base is also easier in autumn. This may be one possible reason for the daily and seasonal allocation patterns observed in previous studies. The conclusions concerning the osmotic potential and turgor pressure in this thesis are also affected by the study method. The method for measuring inner bark osmolality used in this study proved to measure osmotic potential conveniently with robust measurement routines. It also proved to be sensitive to tissue-level physiological changes. The novelty of this method relies on the practicality of sap extraction from the inner bark samples, allowing for large numbers of direct measurements of the inner bark sap. This method resembled methods used in the past that have incorporated more laborious measurement routines for similar purposes. No standard method currently exists for measuring the osmolality of the inner bark. The belowground hydraulic conductance was connected to soil temperature and soil water content in the forest with Scots pine. Similar patterns were also found at the urban sites with two broadleaved species. The studied broadleaved tree species in Study III also add to the importance of these findings being common traits among tree species, although this thesis concentrates on conifers. These results were gained with continuous stem measurements, i.e. sap flow and dendrometer change, which are convenient for long-term measurements. The connection of leaf gas exchange and belowground hydraulic conductance reported in this study may also provide the means for assessing certain presumptions of tree leaf gas exchange based on the growing site properties. Thus, the soil temperature and soil water content are involved in the phenological and seasonal patterns of tree gas exchange during the growing season, at least during spring recovery. Finally, pathogenic infections, such as those caused by *E. polonica* that was studied in this thesis, may introduce more rapid changes in tree water transport. A decrease in xylem sap surface tension may render trees more vulnerable to water deficits, which may add another variable to the investigations of tree vulnerability to embolism formation with the dynamic xylem sap properties. Research on tree embolism vulnerability has so far relied mainly on the structural characteristics of the water-transporting tissue that set the preconditions to tree water stress vulnerability with hardly any attention to xylem sap properties. The dynamic nature of xylem sap requires more investigation to comprehensively understand its significance to tree water transport.

Together with all these studies, this thesis advocates that important tree physiological processes, including leaf gas exchange and water and solute translocation, are interconnected and mediated with soil water availability. Changes in water availability impact the physiological processes at the whole-tree-level. Decreasing xylem water potential transmits to the phloem as decreasing turgor pressure and osmotic adjustments if the water deficit is severe. This will hamper phloem transport, as it is driven by turgor pressure gradients. With less available water for the tree, the demands of growing tissue and other sink tissues may not be fulfilled, which may result in declines in tree growth and vitality. With additional demands from other abiotic or biotic stresses, such as insect or pathogen strains, the maintenance of tree functionality may become challenging. Interactions between trees and insects or pathogens require further studies, as these interactions are often indirect and not well known. For example, the occurrence of bark beetles in large numbers depends much on the environmental conditions that trees experience. Temperature has a major influence on seasonal phenology and metabolic activity. Hydraulic traits determine the frame where vascular transport can maintain the whole-tree functioning of a given species, which defines the distribution where these species can survive and flourish. Other physiological aspects, e.g. hormones and proteins, contribute to the developmental phases of sinks and in signalling, but these issues are not discussed in this thesis. The future scenarios of rainfall and temperature patterns predict that forests will experience more water stress in all habitats where the water balance is expected to change because trees are adapted to local conditions. Prolonged changes in environmental conditions may promote adaptation. However, this is a much longer process than changes occurring in the environment. Humankinds need to understand its surroundings create urgent demands for understanding trees. Humans have a large impact on the vegetation and climate of the Earth, and thus, it is also important to understand what means are available for forests to mitigate their changing environments.

6 REFERENCES

Aaltonen, H., Lindén, A., Heinonsalo, J., Biasi, C. & Pumpanen, J. (2017). Effects of prolonged drought stress on Scots pine seedling carbon allocation. Tree physiology 37: 418-427. https://doi.org/10.1093/treephys/tpw119.

Adams, H. D., Zeppel, M. J., Anderegg, W. R., Hartmann, H., Landhäusser, S. M., Tissue, D. T. et al. (2017). A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. Nature ecology & evolution 1: 1285-1291.

Ainsworth, E. A. & Bush, D. R. (2011). Carbohydrate export from the leaf: A highly regulated process and target to enhance photosynthesis and productivity. Plant Physiology 155: 64-69. https://doi.org/10.1104/pp.110.167684.

Allen, C. D., Macalady, A. K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M. et al. (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management 259: 660-684. https://doi.org/10.1016/j.foreco.2009.09.001.

Amthor, J. S. (2000). The McCree–de Wit–Penning de Vries–Thornley respiration paradigms: 30 years later. Annals of botany 86: 1-20. https://doi.org/10.1006/anbo.2000.1175.

Antonova, G. F. & Stasova, V. V. (1993). Effects of environmental factors on wood formation in Scots pine stems. Trees 7: 214-219. https://doi.org/10.1007/BF00202076.

Antonova, G. F., Cherkashin, V. P., Stasova, V. V. & Varaksina, T. N. (1995). Daily dynamics in xylem cell radial growth of Scots pine (*Pinus sylvestris* L.). Trees 10: 24-30. https://doi.org/10.1007/BF00197776.

Antonova, G. & Stasova, V. (2006). Seasonal development of phloem in Scots pine stems. Russian Journal of Developmental Biology 37: 306-320. https://doi.org/10.1134/S1062360406050043.

Arieff, A. I., Kleeman, C. R., Keushkerian, A. & Bagdoyan, H. (1972). Brain tissue osmolality: Method of determination and variations in hyper-and hypo-osmolar states. The Journal of laboratory and clinical medicine 79: 334-343.

Baier, P., Führer, E., Kirisits, T. & Rosner, S. (2002). Defence reactions of Norway spruce against bark beetles and the associated fungus *Ceratocystis polonica* in secondary pure and mixed species stands. Forest Ecology and Management 159: 73-86. https://doi.org/10.1016/S0378-1127(01)00711-3.

Bailey, I. W. (1916). The structure of the bordered pits of conifers and its bearing upon the tension hypothesis of the ascent of sap in plants. Botanical Gazette 62: 133-142. https://doi.org/10.1086/331877.

Bartlett, M. K., Zhang, Y., Kreidler, N., Sun, S., Ardy, R., Cao, K. & Sack, L. (2014). Global analysis of plasticity in turgor loss point, a key drought tolerance trait. Ecology Letters 17: 1580-1590. https://doi.org/10.1111/ele.12374.

Bartlett, M. K., Scoffoni, C., Ardy, R., Zhang, Y., Sun, S., Cao, K. & Sack, L. (2012). Rapid determination of comparative drought tolerance traits: Using an osmometer to predict turgor loss point. Methods in Ecology and Evolution 3: 880-888. https://doi.org/10.1111/j.2041-210X.2012.00230.x.

Bartlett, M. K., Klein, T., Jansen, S., Choat, B. & Sack, L. (2016). The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought. Proceedings of the National Academy of Sciences of the United States of America 113: 13098-13103. https://doi.org/10.1073/pnas.1604088113.

Bauch, J., Liese, W. & Schultze, R. (1972). The morphological variability of the bordered pit membranes in gymnosperms. Wood Science and Technology 6: 165-184. https://doi.org/10.1007/BF00351575.

Baudena, M., Dekker, S. C., van Bodegom, P. M., Cuesta, B., Higgins, S. I., Lehsten, V. et al. (2014). Forests, savannas and grasslands: Bridging the knowledge gap between ecology and dynamic global vegetation models. Biogeosciences Discussions 11: 9471-9510. https://doi.org/10.5194/bgd-11-9471-2014.

Bengtson, C. (1980). Effects of water stress on Scots pine. Ecological Bulletins 205-213.

Berry, J. & Bjorkman, O. (1980). Photosynthetic response and adaptation to temperature in higher plants. Annual Review of plant physiology 31: 491-543. https://doi.org/10.1146/annurev.pp.31.060180.002423.

Birami, B., Gattmann, M., Heyer, A. G., Grote, R., Arneth, A. & Ruehr, N. K. (2018). Heat waves alter carbon allocation and increase mortality of Aleppo pine under dry conditions. Frontiers in Forests and Global Change 18. https://doi.org/10.3389/ffgc.2018.00008.

Bonan, G. B. (2008). Forests and climate change: forcings, feedbacks, and the climate benefits of forests. science, 320, 1444-1449. https://doi.org/10.1126/science.1155121.

Borghetti, M., Edwards, W., Grace, J., Jarvis, P. & Raschi, A. (1991). The refilling of embolized xylem in *Pinus sylvestris* L. Plant, Cell & Environment 14: 357-369. https://doi.org/10.1111/j.1365-3040.1991.tb00944.x.

Bouche, P. S., Larter, M., Domec, J., Burlett, R., Gasson, P., Jansen, S. & Delzon, S. (2014). A broad survey of hydraulic and mechanical safety in the xylem of conifers. Journal of experimental botany 65: 4419-4431. https://doi.org/10.1093/jxb/eru218.

Brodribb, T. J., Bowman, D. J., Nichols, S., Delzon, S. & Burlett, R. (2010). Xylem function and growth rate interact to determine recovery rates after exposure to extreme water deficit. New Phytologist 188: 533-542. https://doi.org/10.1111/j.1469-8137.2010.03393.x.

Brodribb, T. J. & Cochard, H. (2009). Hydraulic failure defines the recovery and point of death in water-stressed conifers. Plant Physiology 149: 575-584. https://doi.org/10.1104/pp.108.129783.

Cajander, A. K. (1926). The theory of forest types. Acta For Fenn 291-108. https://doi.org/10.14214/aff.7193.

Callister, A. N., Arndt, S. K. & Adams, M. A. (2006). Comparison of four methods for measuring osmotic potential of tree leaves. Physiologia Plantarum 127: 383-392. https://doi.org/10.1111/j.1399-3054.2006.00652.x.

Carbone, M. S., Czimczik, C. I., McDuffee, K. E. & Trumbore, S. E. (2007). Allocation and residence time of photosynthetic products in a boreal forest using a low-level 14C pulse-chase labeling technique. Global Change Biology 13: 466-477. https://doi.org/10.1111/j.1365-2486.2006.01300.x.

Carvalho, M. R., Losada, J. M. & Niklas, K. J. (2018). Phloem networks in leaves. Current opinion in plant biology 4329-35. https://doi.org/10.1016/j.pbi.2017.12.007.

Cernusak, L. A., Arthur, D. J., Pate, J. S. & Farquhar, G. D. (2003). Water relations link carbon and oxygen isotope discrimination to phloem sap sugar concentration in eucalyptus globulus. Plant Physiology 131: 1544-1554. https://doi.org/10.1104/pp.102.016303.

Chan, T., Berninger, F., Kolari, P., Nikinmaa, E. & Hölttä, T. (2018). Linking stem growth respiration to the seasonal course of stem growth and GPP of Scots pine. Tree physiology 38(9): 1356-1370. https://doi.org/10.1093/treephys/tpy040.

Choat, B., Jansen, S., Brodribb, T. J., Cochard, H., Delzon, S., Bhaskar, R. et al. (2012). Global convergence in the vulnerability of forests to drought. Nature 491: 752. https://doi.org/10.1038/nature11688.

Christersson, L. (1972). The transpiration rate of unhardened, hardened, and dehardened seedlings of spruce and pine. Physiologia Plantarum 26: 258-263. https://doi.org/10.1111/j.1399-3054.1972.tb08554.x.

Christiansen, E. (1985). *Ceratocysts polonica* inoculated in Norway spruce: Blue-staining in relation to inoculum density, resinosis and tree growth. Forest Pathology 15: 160-167. https://doi.org/10.1111/j.1439-0329.1985.tb00880.x.

Christiansen, E. & Fjone, G. (1993). Pruning enhances the susceptibility of *Picea abies* to infection by the bark beetle-transmitted blue-stain fungus, ophiostoma polonicum. Scandinavian Journal of Forest Research 8: 235-245. https://doi.org/10.1080/02827589309382773.

Christy, A. L. & Ferrier, J. M. (1973). A mathematical treatment of Munch's pressure-flow hypothesis of phloem translocation. Plant Physiology 52: 531-538. https://doi.org/10.1104/pp.52.6.531.

Chung, H., Muraoka, H., Nakamura, M., Han, S., Muller, O. & Son, Y. (2013). Experimental warming studies on tree species and forest ecosystems: A literature review. Journal of Plant Research 126: 447-460. https://doi.org/10.1007/s10265-013-0565-3.

Cochard, H. (1992). Vulnerability of several conifers to air embolism. Tree physiology 11: 73-83. https://doi.org/10.1093/treephys/11.1.73.

Cochard, H., Breda, N. & Granier, A. (1996). Whole tree hydraulic conductance and water loss regulation in Quercus during drought: evidence for stomatal control of embolism? 53: 197-206. https://doi.org/10.1051/forest:19960203.

Cochard, H., Holtta, T., Herbette, S., Delzon, S. & Mencuccini, M. (2009). New insights into the mechanisms of water-stress-induced cavitation in conifers. Plant Physiology 151: 949-954. https://doi.org/10.1104/pp.109.138305.

Cosgrove, D. (1986). Biophysical control of plant cell growth. Annual Review of Plant Physiology 37: 377-405. https://doi.org/10.1146/annurev.pp.37.060186.002113.

Cras, J., Rowe-Taitt, C., Nivens, D. & Ligler, F. (1999). Comparison of chemical cleaning methods of glass in preparation for silanization. Biosensors and bioelectronics 14: 683-688. https://doi.org/10.1016/S0956-5663(99)00043-3.

Dannoura, M., Epron, D., Desalme, D., Massonnet, C., Tsuji, S., Plain, C. et al. (2019). The impact of prolonged drought on phloem anatomy and phloem transport in young beech trees. Tree physiology 39: 201-210. https://doi.org/10.1093/treephys/tpy070.

Dannoura, M., Maillard, P., Fresneau, C., Plain, C., Berveiller, D., Gerant, D. et al. (2011). In situ assessment of the velocity of carbon transfer by tracing 13C in trunk CO2 efflux after pulse labelling: Variations among tree species and seasons. New Phytologist 190: 181-192. https://doi.org/10.1111/j.1469-8137.2010.03599.x.

den Outer, R. W. (1967). Histological investigations of the secondary phloem of gymnosperms (Doctoral dissertation, Veenman).

Devaux, M., Ghashghaie, J., Bert, D., Lambrot, C., Gessler, A., Bathellier, C. et al. (2009). Carbon stable isotope ratio of phloem sugars in mature pine trees throughout the growing season: Comparison of two extraction methods. Rapid Communications in Mass Spectrometry 23: 2511-2518. 10.1002/rcm.4092. https://doi.org/10.1002/rcm.4092.

Dixon, H. H. & Joly, J. (1895). XII. on the ascent of sap. Philosophical Transactions of the Royal Society of London.(B.) (186): 563-576. https://doi.org/10.1098/rstb.1895.0012.

Domec, J. (2011). Let's not forget the critical role of surface tension in xylem water relations. Tree physiology 31: 359-360. https://doi.org/10.1093/treephys/tpr039.

Domec, J. C., Lachenbruch, B., Meinzer, F. C., Woodruff, D. R., Warren, J. M. & McCulloh, K. A. (2008). Maximum height in a conifer is associated with conflicting requirements for xylem design. Proceedings of the National Academy of Sciences of the United States of America 105: 12069-12074. https://doi.org/10.1073/pnas.0710418105.

Duursma, R. A., Kolari, P., Peramaki, M., Nikinmaa, E., Hari, P., Delzon, S. et al. (2008). Predicting the decline in daily maximum transpiration rate of two pine stands during drought based on constant minimum leaf water potential and plant hydraulic conductance. Tree physiology 28: 265-276. https://doi.org/10.1093/treephys/28.2.265.

Franceschi, V. R., Krokene, P., Christiansen, E. & Krekling, T. (2005). Anatomical and chemical defenses of conifer bark against bark beetles and other pests. New Phytologist 167: 353-376. https://doi.org/10.1111/j.1469-8137.2005.01436.x.

Franceschi, V., Krekling, T., Berryman, A. & Christiansen, E. (1998). Specialized phloem parenchyma cells in Norway spruce (*pinaceae*) bark are an important site of defense reactions. American Journal of Botany 85: 601-615. https://doi.org/10.2307/2446529.

Garcia-Forner, N., Sala, A., Biel, C., Savé, R. & Martínez-Vilalta, J. (2016). Individual traits as determinants of time to death under extreme drought in *Pinus sylvestris* L. Tree physiology 36: 1196-1209. https://doi.org/10.1093/treephys/tpw040.

Gisselsson, L., Smith, M. & Siesjö, B. K. (1998). Influence of hypoglycemic coma on brain water and osmolality. Experimental brain research 120: 461-469. https://doi.org/10.1007/s002210050419.

Gonzalez-Munoz, N., Sterck, F., Torres-Ruiz, J. M., Petit, G., Cochard, H., von Arx, G. et al. (2018). Quantifying in situ phenotypic variability in the hydraulic properties of four tree species across their distribution range in Europe. PloS one 13: e0196075. https://doi.org/10.1371/journal.pone.0196075.

Granier, A. (1987). Evaluation of transpiration in a douglas-fir stand by means of sap flow measurements. Tree physiology 3: 309-320. https://doi.org/10.1093/treephys/3.4.309.

Gricar, J., Prislan, P., De Luis, M., Gryc, V., Hacurová, J., Vavrčík, H. & Čufar, K. (2015). Plasticity in variation of xylem and phloem cell characteristics of Norway spruce under different local conditions. Frontiers in plant science 6730. https://doi.org/10.3389/fpls.2015.00730.

Gričar, J. & Čufar, K. (2008). Seasonal dynamics of phloem and xylem formation in silver fir and Norway spruce as affected by drought. Russian Journal of Plant Physiology 55: 538-543. https://doi.org/10.1134/S102144370804016X.

Gričar, J., Prislan, P., Gryc, V., Vavrčík, H., De Luis, M. & Čufar, K. (2014). Plastic and locally adapted phenology in cambial seasonality and production of xylem and phloem cells in *Picea abies* from temperate environments. Tree physiology 34(8): 869-881. https://doi.org/10.1093/treephys/tpu026.

Grime, J. P. & Mackey, J. (2002). The role of plasticity in resource capture by plants. Evolutionary Ecology 16: 299-307. https://doi.org/10.1023/A:1019640813676.

Gross, K. & Koch, W. (1991). Water relations of *Picea abies*. I. comparison of water relations parameters of spruce shoots examined at the end of the vegetation period and in winter. Physiologia Plantarum 83: 290-295. https://doi.org/10.1034/j.1399-3054.1991.830213.x.

Gruber, A., Strobl, S., Veit, B. & Oberhuber, W. (2010). Impact of drought on the temporal dynamics of wood formation in *Pinus sylvestris*. Tree physiology 30: 490-501. https://doi.org/10.1093/treephys/tpq003.

Hacke, U. & Sperry, J. (2003). Limits to xylem refilling under negative pressure in *Laurus nobilis* and acer negundo. Plant, Cell & Environment 26: 303-311. https://doi.org/10.1046/j.1365-3040.2003.00962.x.

Hacke, U. G. (2014). Variable plant hydraulic conductance. Tree physiology 34: 105-108. https://doi.org/10.1093/treephys/tpu007.

Hacke, U. G., Sperry, J. S. & Pittermann, J. (2004). Analysis of circular bordered pit function II. gymnosperm tracheids with torus-margo pit membranes. American Journal of Botany 91: 386-400. https://doi.org/10.3732/ajb.91.3.386.

Hacke, U. G., Sperry, J. S., Wheeler, J. K. & Castro, L. (2006). Scaling of angiosperm xylem structure with safety and efficiency. Tree physiology 26: 689-701. https://doi.org/10.1093/treephys/26.6.689.

Hacke, U. G., Lachenbruch, B., Pittermann, J., Mayr, S., Domec, J. & Schulte, P. J. (2015). The hydraulic architecture of conifers. Functional and ecological xylem anatomy. Springer, p. 39-75. https://doi.org/10.1007/978-3-319-15783-2_2.

Hagedorn, F., Joseph, J., Peter, M., Luster, J., Pritsch, K., Geppert, U. et al. (2016). Recovery of trees from drought depends on belowground sink control. Nature plants 2: 1-5. https://doi.org/10.1038/nplants.2016.111.

Hammel, H. T. (1968). Measurement of turgor pressure and its gradient in the phloem of oak. Plant Physiology 43: 1042-1048. https://doi.org/10.1104/pp.43.7.1042.

Hansen, J. & Beck, E. (1994). Seasonal changes in the utilization and turnover of assimilation products in 8-year-old Scots pine (*Pinus sylvestris* L.) trees. Trees 8: 172-182. https://doi.org/10.1007/BF00196844.

Hari, P., Nikinmaa, E., Pohja, T., Siivola, E., Bäck, J., Vesala, T. & Kulmala, M. (2013). Station for measuring ecosystem-atmosphere relations: SMEAR. Physical and physiological forest ecology. Springer, p. 471-487. https://doi.org/10.1007/978-94-007-5603-8_9.

Hellkvist, J., Richards, G. & Jarvis, P. (1974). Vertical gradients of water potential and tissue water relations in Sitka spruce trees measured with the pressure chamber. Journal of Applied Ecology 637-667. https://doi.org/10.2307/2402215.

Henriksson, N., Tarvainen, L., Lim, H., Tor-Ngern, P., Palmroth, S., Oren, R. et al. (2015). Stem compression reversibly reduces phloem transport in *Pinus sylvestris* trees. Tree physiology 35: 1075-1085. https://doi.org/10.1093/treephys/tpv078.

Henzler, T., Waterhouse, R. N., Smyth, A. J., Carvajal, M., Cooke, D. T., Schäffner, A. R. et al. (1999). Diurnal variations in hydraulic conductivity and root pressure can be correlated with the expression of putative aquaporins in the roots of *Lotus japonicus*. Planta 210: 50-60. https://doi.org/10.1007/s004250050653.

Hodges, J. D. (1967). Patterns of photosynthesis under natural environmental conditions. Ecology 48: 234-242. https://doi.org/10.2307/1933105.

Holbrook, N. M. & Knoblauch, M. (2018). Editorial overview: Physiology and metabolism: Phloem: A supracellular highway for the transport of sugars, signals, and pathogens. Current opinion in plant biology 43iii-vii. https://doi.org/10.1016/j.pbi.2018.05.013.

Hölttä, T., Mencuccini, M. & Nikinmaa, E. (2009). Linking phloem function to structure: Analysis with a coupled xylem-phloem transport model. Journal of theoretical biology 259: 325-337. https://doi.org/10.1016/j.jtbi.2009.03.039.

Hölttä, T., Vesala, T., Sevanto, S., Perämäki, M. & Nikinmaa, E. (2006). Modeling xylem and phloem water flows in trees according to cohesion theory and Münch hypothesis. Trees 20: 67-78. https://doi.org/10.1007/s00468-005-0014-6.

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

Hölttä, T., Juurola, E., Lindfors, L. & Porcar-Castell, A. (2011). Cavitation induced by a surfactant leads to a transient release of water stress and subsequent ‘run away’embolism in scots pine (*Pinus sylvestris*) seedlings. Journal of experimental botany 63: 1057-1067. https://doi.org/10.1093/jxb/err349.

Hölttä, T., Cochard, H., Nikinmaa, E. & Mencuccini, M. (2009). Capacitive effect of cavitation in xylem conduits: Results from a dynamic model. Plant, Cell & Environment 32: 10-21. https://doi.org/10.1111/j.1365-3040.2008.01894.x.

Hölttä, T., Lintunen, A., Chan, T., Mäkelä, A. & Nikinmaa, E. (2017). A steady-state stomatal model of balanced leaf gas exchange, hydraulics and maximal source–sink flux. Tree physiology 37: 851-868. https://doi.org/10.1093/treephys/tpx011.

Hölttä, T., Mäkinen, H., Nöjd, P., Mäkelä, A. & Nikinmaa, E. (2010). A physiological model of softwood cambial growth. Tree physiology 30: 1235-1252. https://doi.org/10.1093/treephys/tpq068.

Horntvedt, R., Christiansen, E., Solheim, H. & Wang, S. (1983). Artificial inoculation with *Ips typographus*-associated blue stain fungi can kill healthy Norway spruce trees. Meddelelser fra Skogforsk .

Hsiao, T. C., Acevedo, E., Fereres, E. & Henderson, D. (1976). Water stress, growth and osmotic adjustment. Philosophical Transactions of the Royal Society of London.B, Biological Sciences 273: 479-500. https://doi.org/10.1098/rstb.1976.0026.

Hsiao, T. C. (1973). Plant responses to water stress. Annual review of plant physiology 24: 519-570. https://doi.org/10.1146/annurev.pp.24.060173.002511.

Ilvesniemi, H., Pumpanen, J., Duursma, R., Hari, P., Keronen, P., Kolari, P. et al. (2010). Water balance of a boreal Scots pine forest. Boreal Environment Research 15.

Irvine, J. & Grace, J. (1997). Continuous measurements of water tensions in the xylem of trees based on the elastic properties of wood. Planta 202: 455-461. https://doi.org/10.1007/s004250050149.

Irvine, J., Perks, M. P., Magnani, F. & Grace, J. (1998). The response of *Pinus sylvestris* to drought: Stomatal control of transpiration and hydraulic conductance. Tree physiology 18: 393-402. https://doi.org/10.1093/treephys/18.6.393.

Jactel, H., Petit, J., Desprez-Loustau, M., Delzon, S., Piou, D., Battisti, A. & Koricheva, J. (2012). Drought effects on damage by forest insects and pathogens: A meta-analysis. Global Change Biology 18: 267-276. https://doi.org/10.1111/j.1365-2486.2011.02512.x.

Jarvis, P. & Jarvis, M. S. (1963). The water relations of tree seedlings.: IV. some aspects of the tissue water relations and drought resistance. Physiologia Plantarum 16: 501-516. https://doi.org/10.1111/j.1399-3054.1963.tb08327.x.

Javot, H. & Maurel, C. (2002). The role of aquaporins in root water uptake. Annals of Botany 90: 301-313. https://doi.org/10.1093/aob/mcf199.

Jensen, K. H. (2018). Phloem physics: Mechanisms, constraints, and perspectives. Current opinion in plant biology 4396-100. https://doi.org/10.1016/j.pbi.2018.03.005.

Junninen, H., Lauri, A., Keronen, P., Aalto, P., Hiltunen, V., Hari, P. & Kulmala, M. (2009). Smart-SMEAR: On-line data exploration and visualization tool for SMEAR stations. Boreal Environ.Res 14: 447-457.

Jyske, T. & Hölttä, T. (2015). Comparison of phloem and xylem hydraulic architecture in *Picea abies* stems. New Phytologist 205: 102-115. https://doi.org/10.1111/nph.12973.

Jyske, T., Hölttä, T., Mäkinen, H., Nöjd, P., Lumme, I. & Spiecker, H. (2010). The effect of artificially induced drought on radial increment and wood properties of Norway spruce. Tree physiology 30: 103-115. https://doi.org/10.1093/treephys/tpp099.

Jyske, T. M., Suuronen, J., Pranovich, A. V., Laakso, T., Watanabe, U., Kuroda, K. & Abe, H. (2015). Seasonal variation in formation, structure, and chemical properties of phloem in *Picea abies* as studied by novel microtechniques. Planta 242: 613-629. https://doi.org/10.1007/s00425-015-2347-8.

Kaufmann, M. R. & Kramer, P. J. (1967). Phloem water relations and translocation. Plant Physiology 42: 191-194. https://doi.org/10.1104/pp.42.2.191.

Kellomäki, S. & Wang, K. (1996). Photosynthetic responses to needle water potentials in Scots pine after a four-year exposure to elevated CO2 and temperature. Tree physiology 16: 765-772. https://doi.org/10.1093/treephys/16.9.765.

Kikuta, S. B. & Richter, H. (1992). Leaf discs or press saps? A comparison of techniques for the determination of osmotic potentials in freeze-thawed leaf material. Journal of experimental botany 43: 1039-1044. https://doi.org/10.1093/jxb/43.8.1039.

Kilpeläinen, A., Peltola, H., Ryyppö, A., Sauvala, K., Laitinen, K. & Kellomäki, S. (2003). Wood properties of scots pines (*Pinus sylvestris*) grown at elevated temperature and carbon dioxide concentration. Tree physiology 23: 889-897. https://doi.org/10.1093/treephys/23.13.889.

Kirisits, T. & Offenthaler, I. (2002). Xylem sap flow of Norway spruce after inoculation with the blue-stain fungus *Ceratocystis polonica*. Plant Pathology 51: 359-364. https://doi.org/10.1046/j.1365-3059.2002.00722.x.

Kirschbaum, M. U. (2011). Does enhanced photosynthesis enhance growth? lessons learned from CO2 enrichment studies. Plant Physiology 155: 117-124. https://doi.org/10.1104/pp.110.166819.

Klein, T. (2014). The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours. Functional Ecology 28: 1313-1320. https://doi.org/10.1111/1365-2435.12289.

Knoblauch, M. & Oparka, K. (2012). The structure of the phloem–still more questions than answers. The Plant Journal 70: 147-156. https://doi.org/10.1111/j.1365-313X.2012.04931.x.

Knoblauch, M. & van Bel, A. J. (1998). Sieve tubes in action. The Plant Cell 10: 35-50. https://doi.org/10.1105/tpc.10.1.35.

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. https://doi.org/10.7554/eLife.15341.025.

Körner, C. (2015). Paradigm shift in plant growth control. Current opinion in plant biology 25107-114. https://doi.org/10.1016/j.pbi.2015.05.003.

Körner, C. (2003). Carbon limitation in trees. Journal of Ecology 91: 4-17. https://doi.org/10.1046/j.1365-2745.2003.00742.x.

Kostiainen, K., Kaakinen, S., Saranpää, P., Sigurdsson, B. D., Lundqvist, S., Linder, S. & Vapaavuori, E. (2009). Stem wood properties of mature Norway spruce after 3 years of continuous exposure to elevated [CO2] and temperature. Global Change Biology 15: 368-379. https://doi.org/10.1111/j.1365-2486.2008.01755.x.

Krokene, P. (2015). Conifer defense and resistance to bark beetles. Bark beetles. Elsevier, p. 177-207. https://doi.org/10.1016/B978-0-12-417156-5.00005-8.

Krokene, P. & Solheim, H. (1998). Pathogenicity of four blue-stain fungi associated with aggressive and nonaggressive bark beetles. Phytopathology 88: 39-44. https://doi.org/10.1094/PHYTO.1998.88.1.39.

Krokene, P., Christiansen, E., Solheim, H., Franceschi, V. R. & Berryman, A. A. (1999). Induced resistance to pathogenic fungi in Norway spruce. Plant Physiology 121: 565-570. https://doi.org/10.1104/pp.121.2.565.

Kuroda, K. (2005). Xylem dysfunction in Yezo spruce (*Picea jezoensis*) after inoculation with the blue-stain fungus *Ceratocystis polonica*. Forest Pathology 35: 346-358. https://doi.org/10.1111/j.1439-0329.2005.00424.x.

Lazzarin, M., Zweifel, R., Anten, N. & Sterck, F. J. (2019). Does phloem osmolality affect diurnal diameter changes of twigs but not of stems in Scots pine? Tree physiology 39: 275-283. https://doi.org/10.1093/treephys/tpy121.

Lemoine, R., La Camera, S., Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N. et al. (2013). Source-to-sink transport of sugar and regulation by environmental factors. Frontiers in plant science 4272. https://doi.org/10.3389/fpls.2013.00272.

Lévesque, M., Saurer, M., Siegwolf, R., Eilmann, B., Brang, P., Bugmann, H. & Rigling, A. (2013). Drought response of five conifer species under contrasting water availability suggests high vulnerability of Norway spruce and European larch. Global Change Biology 19: 3184-3199. https://doi.org/10.1111/gcb.12268.

Liesche, J. & Schulz, A. (2018). Phloem transport in gymnosperms: A question of pressure and resistance. Current opinion in plant biology 4336-42. https://doi.org/10.1016/j.pbi.2017.12.006.

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: 376-386. https://doi.org/10.1093/treephys/tpv020.

Liesche, J., Martens, H. & Schulz, A. (2011). Symplasmic transport and phloem loading in gymnosperm leaves. Protoplasma 248: 181-190. https://doi.org/10.1007/s00709-010-0239-0.

Linkosalo, T., Heikkinen, J., Pulkkinen, P. & Mäkipää, R. (2014). Fluorescence measurements show stronger cold inhibition of photosynthetic light reactions in Scots pine compared to Norway spruce as well as during spring compared to autumn. Frontiers in plant science 5264. https://doi.org/10.3389/fpls.2014.00264.

López, R., Cano, F. J., Choat, B., Cochard, H. & Gil, L. (2016). Plasticity in vulnerability to cavitation of *Pinus canariensis* occurs only at the driest end of an aridity gradient. Frontiers in plant science 7769. https://doi.org/10.3389/fpls.2016.00769.

Losso, A., Beikircher, B., Damon, B., Kikuta, S., Schmid, P. & Mayr, S. (2017). Xylem sap surface tension may be crucial for hydraulic safety. Plant Physiology 175: 1135-1143. https://doi.org/10.1104/pp.17.01053.

Maherali, H., Pockman, W. T. & Jackson, R. B. (2004). Adaptive variation in the vulnerability of woody plants to xylem cavitation. Ecology 85: 2184-2199. https://doi.org/10.1890/02-0538.

Markhart III, A. H., Sionit, N. & Siedow, J. N. (1981). Cell wall water dilution: An explanation of apparent negative turgor potentials. Canadian Journal of Botany 59: 1722-1725. https://doi.org/10.1139/b81-231.

Marshall, J. D. & Monserud, R. A. (2003). Foliage height influences specific leaf area of three conifer species. Canadian Journal of Forest Research 33: 164-170. https://doi.org/10.1139/x02-158.

Martínez-Vilalta, J., Korakaki, E., Vanderklein, D. & Mencuccini, M. (2007). Below-ground hydraulic conductance is a function of environmental conditions and tree size in Scots pine. Functional Ecology, 21: 1072-1083. https://doi.org/10.1111/j.1365-2435.2007.01332.x.

Martínez-Vilalta, J., Cochard, H., Mencuccini, M., Sterck, F., Herrero, A., Korhonen, J. et al. (2009). Hydraulic adjustment of Scots pine across Europe. New Phytologist 184: 353-364.

Matthews, B., Netherer, S., Katzensteiner, K., Pennerstorfer, J., Blackwell, E., Henschke, P. et al. (2018). Transpiration deficits increase host susceptibility to bark beetle attack: Experimental observations and practical outcomes for *Ips typographus* hazard assessment. Agricultural and Forest Meteorology 26369-89. https://doi.org/10.1016/j.agrformet.2018.08.004.

Mayr, S., Schmid, P., Laur, J., Rosner, S., Charra-Vaskou, K., Damon, B. & Hacke, U. G. (2014). Uptake of water via branches helps timberline conifers refill embolized xylem in late winter. Plant Physiology 164: 1731-1740. https://doi.org/10.1104/pp.114.236646.

McDowell, N. G. (2011). Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. Plant Physiology 155: 1051-1059. https://doi.org/10.1104/pp.110.170704.

Meinzer, F., Rundel, P., Sharifi, M. & Nilsen, E. (1986). Turgor and osmotic relations of the desert shrub *Larrea tridentata*. Plant, Cell & Environment 9: 467-475. https://doi.org/10.1111/j.1365-3040.1986.tb01762.x.

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: 189-203. https://doi.org/10.1111/j.1469-8137.2009.03050.x.

Mencuccini, M., Hölttä, T., Sevanto, S. & Nikinmaa, E. (2013). Concurrent measurements of change in the bark and xylem diameters of trees reveal a phloem-generated turgor signal. New phytologist, 198: 1143-1154. https://doi.org/10.1111/nph.12224.

Milburn, J. A. (1966). The conduction of sap: I. water conduction and cavitation in water stressed leaves. Planta 34-42. https://doi.org/10.1007/s00442-014-2935-5.

Mildner, M., Bader, M. K., Leuzinger, S., Siegwolf, R. T. & Körner, C. (2014). Long-term 13 C labeling provides evidence for temporal and spatial carbon allocation patterns in mature *Picea abies*. Oecologia 175: 747-762.

Minchin, P., Thorpe, M. & Farrar, J. (1993). A simple mechanistic model of phloem transport which explains sink priority. Journal of experimental botany 44: 947-955. https://doi.org/10.1093/jxb/44.5.947.

Münch, E. 1930. Stoffbewegungen in der pflanze.

Nagy, N. E., Franceschi, V. R., Solheim, H., Krekling, T. & Christiansen, E. (2000). Wound-induced traumatic resin duct development in stems of Norway spruce (*pinaceae*): Anatomy and cytochemical traits. American Journal of Botany 87: 302-313. https://doi.org/10.2307/2656626.

Nagy, N. E., Fossdal, C. G., Krokene, P., Krekling, T., Lönneborg, A. & Solheim, H. (2004). Induced responses to pathogen infection in Norway spruce phloem: Changes in polyphenolic parenchyma cells, chalcone synthase transcript levels and peroxidase activity. Tree physiology 24: 505-515. https://doi.org/10.1093/treephys/24.5.505.

Nardini, A., Savi, T., Trifilò, P. & Gullo, M. L. (2018). Progress in botany.

Netherer, S., Panassiti, B., Pennerstorfer, J. & Matthews, B. (2019). Acute drought is an important driver of bark beetle infestation in austrian Norway spruce stands. Frontiers in Forests and Global Change 239. https://doi.org/10.3389/ffgc.2019.00039.

Netherer, S., Matthews, B., Katzensteiner, K., Blackwell, E., Henschke, P., Hietz, P. et al. (2015). Do water-limiting conditions predispose Norway spruce to bark beetle attack? New Phytologist 205: 1128-1141. https://doi.org/10.1111/nph.13166.

Nikinmaa, E., Sievänen, R. & Hölttä, T. (2014). Dynamics of leaf gas exchange, xylem and phloem transport, water potential and carbohydrate concentration in a realistic 3-D model tree crown. Annals of botany 114: 653-666. https://doi.org/10.1093/aob/mcu068.

Nikinmaa, E., Hölttä, T., Hari, P., Kolari, P., Mäkelä, A., Sevanto, S. & Vesala, T. (2013). Assimilate transport in phloem sets conditions for leaf gas exchange. Plant, Cell & Environment 36: 655-669. https://doi.org/10.1111/pce.12004.

Noy-Meir, I. & Ginzburg, B. (1969). An analysis of the water potential isotherm in plant tissue. Australian Journal of Biological Sciences 22: 35-52. https://doi.org/10.1071/BI9690035.

Oliva, J., Stenlid, J. & Martínez-Vilalta, J. (2014). The effect of fungal pathogens on the water and carbon economy of trees: Implications for drought-induced mortality. New Phytologist 203: 1028-1035. https://doi.org/10.1111/nph.12857.

Oliviusson, P., Salaj, J. & Hakman, I. (2001). Expression pattern of transcripts encoding water channel-like proteins in Norway spruce (*Picea abies*). Plant Molecular Biology 46: 289-299. https://doi.org/10.1023/A:1010611605142.

Oren, R., Sperry, J., Katul, G., Pataki, D., Ewers, B., Phillips, N. & Schäfer, K. (1999). Survey and synthesis of intra-and interspecific variation in stomatal sensitivity to vapour pressure deficit. Plant, Cell & Environment 22: 1515-1526. https://doi.org/10.1046/j.1365-3040.1999.00513.x.

Paljakka, T., Lintunen, A., Salmon, Y. & Hölttä, T. (2019). Measurement of inner bark and leaf osmolality. In: Phloem. Humana, New York, NY. p. 135-142. https://doi.org/10.1007/978-1-4939-9562-2_11.

Pate, J. (1976). Nutrients and metabolites of fluids recovered from xylem and phloem: Significance in relation to long-distance transport in plants. Transport and transfer processes in plants 253-281. https://doi.org/10.1016/B978-0-12-734850-6.50029-0.

Peñuelas, J., & Staudt, M. (2010). BVOCs and global change. Trends in plant science, 15: 133-144. https://doi.org/10.1016/j.tplants.2009.12.005.

Perämäki, M., Vesala, T. & Nikinmaa, E. (2005). Modeling the dynamics of pressure propagation and diameter variation in tree sapwood. Tree physiology 25: 1091-1099. https://doi.org/10.1093/treephys/25.9.1091.

Pfautsch, S., Hölttä, T. & Mencuccini, M. (2015a). Hydraulic functioning of tree stems—fusing ray anatomy, radial transfer and capacitance. Tree physiology 35: 706-722. https://doi.org/10.1093/treephys/tpv058.

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

Pirinen, P., Simola, H., Aalto, J., Kaukoranta, J., Karlsson, P. & Ruuhela, R. (2012). Tilastoja Suomen ilmastosta 1981-2010. Ilmatieteen laitos.

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: 388-401. https://doi.org/10.1111/nph.12278.

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: 14162-14167. https://doi.org/10.1073/pnas.0902279106.

Repo, T., Kalliokoski, T., Domisch, T., Lehto, T., Mannerkoski, H., Sutinen, S. & Finér, L. (2005). Effects of timing of soil frost thawing on Scots pine. Tree physiology 25: 1053-1062. https://doi.org/10.1093/treephys/25.8.1053.

Riikonen, A., Lindén, L., Pulkkinen, M. & Nikinmaa, E. (2011). Post-transplant crown allometry and shoot growth of two species of street trees. Urban forestry & Urban greening 10: 87-94. https://doi.org/10.1016/j.ufug.2010.09.001.

Rissanen, K., Hölttä, T., Vanhatalo, A., Aalto, J., Nikinmaa, E., Rita, H. & Bäck, J. (2016). Diurnal patterns in Scots pine stem oleoresin pressure in a boreal forest. Plant, Cell & Environment 39: 527-538. https://doi.org/10.1111/pce.12637.

Rodríguez-Gamir, J., Xue, J., Clearwater, M. J., Meason, D. F., Clinton, P. W. & Domec, J. (2019). Aquaporin regulation in roots controls plant hydraulic conductance, stomatal conductance, and leaf water potential in *Pinus radiata* under water stress. Plant, Cell & Environment 42: 717-729. https://doi.org/10.1111/pce.13460.

Rosas, T., Mencuccini, M., Barba, J., Cochard, H., Saura-Mas, S. & Martínez-Vilalta, J. (2019). Adjustments and coordination of hydraulic, leaf and stem traits along a water availability gradient. New Phytologist 223: 632-646. https://doi.org/10.1111/nph.15684.

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: 472-482. https://doi.org/10.1007/s00468-001-0131-9.

Rosner, S., & Kikuta, S. B. (2002). Determination of the osmotic potential in secondary phloem of Norway spruce (Picea abies (L.) Karst.) using tissue discs-A technical report. PHYTON-HORN-, 42: 79-98.

Rosner, S., Heinze, B., Savi, T. & Dalla-Salda, G. (2019). Prediction of hydraulic conductivity loss from relative water loss: New insights into water storage of tree stems and branches. Physiologia Plantarum 165: 843-854. https://doi.org/10.1111/ppl.12790.

Rouault, G., Candau, J., Lieutier, F., Nageleisen, L., Martin, J. & Warzée, N. (2006). Effects of drought and heat on forest insect populations in relation to the 2003 drought in Western Europe. Annals of Forest Science 63: 613-624. https://doi.org/10.1051/forest:2006044.

Sack, L., Cowan, P., Jaikumar, N. & Holbrook, N. (2003). The ‘hydrology’of leaves: Co-ordination of structure and function in temperate woody species. Plant, Cell & Environment 26: 1343-1356. https://doi.org/10.1046/j.0016-8025.2003.01058.x.

Sala, A. & Hoch, G. (2009). Height-related growth declines in ponderosa pine are not due to carbon limitation. Plant, Cell & Environment 32: 22-30. https://doi.org/10.1111/j.1365-3040.2008.01896.x.

Sala, A., Woodruff, D. R. & Meinzer, F. C. (2012). Carbon dynamics in trees: Feast or famine? Tree physiology 32: 764-775. https://doi.org/10.1093/treephys/tpr143.

Sala, A., Piper, F. & Hoch, G. (2010). Physiological mechanisms of drought-induced tree mortality are far from being resolved. New Phytologist 186: 274-281. https://doi.org/10.1111/j.1469-8137.2009.03167.x.

Saliendra, N. Z., Sperry, J. S. & Comstock, J. P. (1995). Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in betula occidentalis. Planta 196: 357-366. https://doi.org/10.1007/BF00201396.

Salmon, Y., Dietrich, L., Sevanto, S., Hölttä, T., Dannoura, M. & Epron, D. (2019). Drought impacts on tree phloem: From cell-level responses to ecological significance. Tree physiology 39: 173-191. https://doi.org/10.1093/treephys/tpy153.

Salmon, Y., Torres-Ruiz, J. M., Poyatos, R., Martínez-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: 2575-2588.

Sauter, J. J. (1980). The Strasburger cells—equivalents of compagnion cells. *Berichte der Deutschen Botanischen Gesellschaft*, *93*: 29-42.

Savage, J. A., Clearwater, M. J., Haines, D. F., Klein, T., Mencuccini, M., Sevanto, S. et al. (2016). Allocation, stress tolerance and carbon transport in plants: How does phloem physiology affect plant ecology? Plant, Cell & Environment 39: 709-725. https://doi.org/10.1111/pce.12602.

Scholander, P. F., Bradstreet, E. D., Hemmingsen, E. A. & Hammel, H. T. (1965). Sap pressure in vascular plants: Negative hydrostatic pressure can be measured in plants. Science (New York, N.Y.) 148: 339-346. https://doi.org/10.1126/science.148.3668.339.

Schulz, A. (1992). Living sieve cells of conifers as visualized by confocal, laser-scanning fluorescence microscopy. Protoplasma, 166: 153-164. https://doi.org/10.1007/BF01322778.

Seidl, R., Thom, D., Kautz, M., Martin-Benito, D., Peltoniemi, M., Vacchiano, G. et al. (2017). Forest disturbances under climate change. Nature climate change 7: 395. https://doi.org/10.1038/nclimate3303.

Sellier, D. & Mammeri, Y. (2019). Diurnal dynamics of phloem loading: Theoretical consequences for transport efficiency and flow characteristics. Tree physiology 39: 300-311. https://doi.org/10.1093/treephys/tpz001.

Sevanto, S. (2018). Drought impacts on phloem transport. Current opinion in plant biology 4376-81. https://doi.org/10.1016/j.pbi.2018.01.002.

Sevanto, S., Vesala, T., Perämäki, M. & Nikinmaa, E. (2002). Time lags for xylem and stem diameter variations in a Scots pine tree. Plant, Cell & Environment 25: 1071-1077. https://doi.org/10.1046/j.1365-3040.2002.00884.x.

Sevanto, S., Hölttä, T. & Holbrook, N. M. (2011). Effects of the hydraulic coupling between xylem and phloem on diurnal phloem diameter variation. Plant, Cell & Environment 34: 690-703. https://doi.org/10.1111/j.1365-3040.2011.02275.x.

Sobrado, M. & Turner, N. C. (1983). A comparison of the water relations characteristics of *Helianthus annuus* and *Helianthus petiolaris* when subjected to water deficits. Oecologia 58: 309-313. https://doi.org/10.1007/BF00385228.

Solberg, S. (2004). Summer drought: A driver for crown condition and mortality of Norway spruce in Norway. Forest Pathology 34: 93-104. https://doi.org/10.1111/j.1439-0329.2004.00351.x.

Sovonick-Dunford, S., Lee, D. R. & Zimmermann, M. H. (1981). Direct and indirect measurements of phloem turgor pressure in white ash. Plant Physiology 68: 121-126. https://doi.org/10.1104/pp.68.1.121.

Sperry, J. S. (2011). Hydraulics of vascular water transport. Mechanical integration of plant cells and plants. Springer, p. 303-327. https://doi.org/10.1007/978-3-642-19091-9_12.

Sperry, J., Donnelly, J. & Tyree, M. (1988). A method for measuring hydraulic conductivity and embolism in xylem. Plant, Cell & Environment 11: 35-40. https://doi.org/10.1111/j.1365-3040.1988.tb01774.x.

Sperry, J. S. & Tyree, M. T. (1988). Mechanism of water stress-induced xylem embolism. Plant Physiology 88: 581-587. https://doi.org/10.1104/pp.88.3.581.

Stålfelt, M. (1955). The stomata as a hydrophotic regulator of the water deficit of the plant. Physiologia Plantarum 8: 572-593. https://doi.org/10.1111/j.1399-3054.1955.tb07753.x.

Steppe, K., Sterck, F. & Deslauriers, A. (2015). Diel growth dynamics in tree stems: Linking anatomy and ecophysiology. Trends in plant science 20: 335-343. https://doi.org/10.1016/j.tplants.2015.03.015.

Sterck, F. J., Martínez-Vilalta, J., Mencuccini, M., Cochard, H., Gerrits, P., Zweifel, R. et al. (2012). Understanding trait interactions and their impacts on growth in Scots pine branches across Europe. Functional Ecology 26: 541-549. https://doi.org/10.1111/j.1365-2435.2012.01963.x.

Suni, T., Berninger, F., Markkanen, T., Keronen, P., Rannik, Ü. & Vesala, T. (2003). Interannual variability and timing of growing-season CO2 exchange in a boreal forest. Journal of Geophysical Research: Atmospheres 108(D9). https://doi.org/10.1029/2002JD002381.

Sutinen, S., Roitto, M. & Repo, T. (2015). Vegetative buds, needles and shoot growth of Norway spruce are affected by experimentally delayed soil thawing in the field. Forest Ecology and Management 336: 217-223. https://doi.org/10.1016/j.foreco.2014.10.029.

Sutinen, S., Partanen, J., Viherä-Aarnio, A. & Häkkinen, R. (2012). Development and growth of primordial shoots in Norway spruce buds before visible bud burst in relation to time and temperature in the field. Tree physiology 32: 987-997. https://doi.org/10.1093/treephys/tps063.

Swidrak, I., Gruber, A. & Oberhuber, W. (2014). Xylem and phloem phenology in co-occurring conifers exposed to drought. Trees 28: 1161-1171. https://doi.org/10.1007/s00468-014-1026-x.

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

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: 399-407. https://doi.org/10.1111/j.1365-3040.1981.tb02118.x.

Thompson, M. V. (2006). Phloem: The long and the short of it. Trends in plant science 11: 26-32. https://doi.org/10.1016/j.tplants.2005.11.009.

Thompson, M. V. & Holbrook, N. M. (2004). Scaling phloem transport: information transmission. Plant, Cell and Environment 24: 509-519. https://doi.org/10.1111/j.1365-3040.2003.01148.x.

Thompson, M. V. & Holbrook, N. M. (2003). Scaling phloem transport: water potential equilibrium and osmoregulatory flow. Plant, Cell and Environment 26: 1561-1577. https://doi.org/10.1046/j.1365-3040.2003.01080.x.

Turgeon, R. & Wolf, S. (2009). Phloem transport: Cellular pathways and molecular trafficking. Annual review of plant biology 60: 207-221. https://doi.org/10.1146/annurev.arplant.043008.092045.

Turgeon, R. (2010). The role of phloem loading reconsidered. Plant Physiol 152: 1817-1823. https://doi.org/10.1104/pp.110.153023.

Turner, N. & Jarvis, P. (1975). Photosynthesis in Sitka spruce (*Picea sitchensis* (bong.) carr. IV. response to soil temperature. Journal of Applied Ecology 561-576. https://doi.org/10.2307/2402174.

Turner, N. C. (1981). Techniques and experimental approaches for the measurement of plant water status. Plant and Soil 58: 339-366. https://doi.org/10.1007/BF02180062.

Turner, N. C., Stern, W. R. & Evans, P. (1987). Water relations and osmotic adjustment of leaves and roots of lupins in response to water deficits 1. Crop Science 27: 977-983. https://doi.org/10.2135/cropsci1987.0011183X002700050032x.

Turner, N. (1988). Measurement of plant water status by the pressure chamber technique. Irrigation Science 9: 289-308. https://doi.org/10.1007/BF00296704.

Tyree, M. T. & Sperry, J. S. (1989). Vulnerability of xylem to cavitation and embolism. Annual review of plant biology 40: 19-36. https://doi.org/10.1146/annurev.pp.40.060189.000315.

Tyree, M. (1976). Negative turgor pressure in plant cells: Fact or fallacy? Canadian Journal of Botany 54: 2738-2746. https://doi.org/10.1139/b76-294.

Tyree, M. & Zimmermann, M. (2002). Xylem structure and the ascent of sap springer. Springer-Verlag, Berlin, Germany. https://doi.org/10.1007/978-3-662-04931-0.

Tyree, M. & Hammel, H. (1972). The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. Journal of experimental botany 23: 267-282. https://doi.org/10.1093/jxb/23.1.267.

Tyree, M. T., Christy, A. L. & Ferrier, J. M. (1974). A simpler iterative steady state solution of Münch pressure-flow systems applied to long and short translocation paths. Plant Physiology 54: 589-600. https://doi.org/10.1104/pp.54.4.589.

Urban, J., Ingwers, M. W., McGuire, M. A. & Teskey, R. O. (2017). Increase in leaf temperature opens stomata and decouples net photosynthesis from stomatal conductance in *Pinus taeda* and *Populus deltoides* x nigra. Journal of experimental botany 68: 1757-1767. https://doi.org/10.1093/jxb/erx052.

Urli, M., Porté, A. J., Cochard, H., Guengant, Y., Burlett, R. & Delzon, S. (2013). Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. Tree physiology 33: 672-683. https://doi.org/10.1093/treephys/tpt030.

Ursprung, A. (1915). Über die Kohäsion des Wassers im Farnannulus.

Van Bel, A.J.E. (2003). The phloem, a miracle of ingenuity. Plant, Cell & Environment 26: 125-149. https://doi.org/10.1046/j.1365-3040.2003.00963.x.

Vanhanen, J., Hyvärinen, A., Anttila, T., Viisanen, Y. & Lihavainen, H. (2008). Ternary solution of sodium chloride, succinic acid and water? surface tension and its influence on cloud droplet activation. Atmospheric Chemistry and Physics 8: 4595-4604. https://doi.org/10.5194/acp-8-4595-2008.

Vesala, T., Suni, T., Rannik, Ü., Keronen, P., Markkanen, T., Sevanto, S. et al. (2005). Effect of thinning on surface fluxes in a boreal forest. Global Biogeochemical Cycles 19(2). https://doi.org/10.1029/2004GB002316.

von Arx, G., Arzac, A., Fonti, P., Frank, D., Zweifel, R., Rigling, A. et al. (2017). Responses of sapwood ray parenchyma and non-structural carbohydrates of *Pinus sylvestris* to drought and long-term irrigation. Functional Ecology 31: 1371-1382. https://doi.org/10.1111/1365-2435.12860.

Way, D. A. (2011). Tree phenology responses to warming: Spring forward, fall back? Tree physiology 31: 469-471. https://doi.org/10.1093/treephys/tpr044.

Weatherley, P. & Slatyer, R. (1957). Relationship between relative turgidity and diffusion pressure deficit in leaves. Nature 179: 1085-1086. https://doi.org/10.1038/1791085a0.

Weatherley, P. (1950). Studies in the water relations of the cotton plant: I. the field measurement of water deficits in leaves. New Phytologist 49: 81-97. https://doi.org/10.1111/j.1469-8137.1950.tb05146.x.

Wenkert, W. (1980). Measurement of tissue osmotic pressure. Plant Physiology 65: 614-617. https://doi.org/10.1104/pp.65.4.614.

Westgate, M. & Boyer, J. (1985). Osmotic adjustment and the inhibition of leaf, root, stem and silk growth at low water potentials in maize. Planta 164: 540-549. https://doi.org/10.1007/BF00395973.

Whitehead, D. (1998). Regulation of stomatal conductance and transpiration in forest canopies. Tree physiology 18: 633-644. https://doi.org/10.1093/treephys/18.8-9.633.

Williams, A. P., Allen, C. D., Macalady, A. K., Griffin, D., Woodhouse, C. A., Meko, D. M. et al. (2013). Temperature as a potent driver of regional forest drought stress and tree mortality. Nature climate change 3: 292-297. https://doi.org/10.1038/nclimate1693.

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

Woodruff, D. R. & Meinzer, F. C. (2011). Water stress, shoot growth and storage of non-structural carbohydrates along a tree height gradient in a tall conifer. Plant, Cell & Environment 34: 1920-1930. https://doi.org/10.1111/j.1365-3040.2011.02388.x.

Woodruff, D. R., Meinzer, F. & Lachenbruch, B. (2008). Height-related trends in leaf xylem anatomy and shoot hydraulic characteristics in a tall conifer: Safety versus efficiency in water transport. New Phytologist 180: 90-99. https://doi.org/10.1111/j.1469-8137.2008.02551.x.

Woodruff, D., Bond, B. & Meinzer, F. (2004). Does turgor limit growth in tall trees? Plant, Cell & Environment 27: 229-236. https://doi.org/10.1111/j.1365-3040.2003.01141.x.

Zimmermann, U. (1978). Physics of turgor-and osmoregulation. Annual Review of Plant Physiology 29: 121-148. https://doi.org/10.1146/annurev.pp.29.060178.001005.

Zimmermann, M. H. (1957). Translocation of organic substances in trees. I. the nature of the sugars in the sieve tube exudate of trees. Plant Physiology 32: 288-291. https://doi.org/10.1104/pp.32.4.288.

Zweifel, R. & Häsler, R. (2000). Frost-induced reversible shrinkage of bark of mature subalpine conifers. Agricultural and Forest Meteorology 102: 213-222. https://doi.org/10.1016/S0168-1923(00)00135-0.

Zweifel, R., Item, H. & Häsler, R. (2001). Link between diurnal stem radius changes and tree water relations. Tree physiology 21: 869-877. https://doi.org/10.1093/treephys/21.12-13.869.

Zwieniecki, M. A., Melcher, P., Boyce, C. K., Sack, L. & Holbrook, N. (2002). Hydraulic architecture of leaf venation in *Laurus nobilis* L. Plant, Cell & Environment 25: 1445-1450. https://doi.org/10.1046/j.1365-3040.2002.00922.x.