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Low apoplastic water potential in trees - dehydration stress on living cells and embolism in xylem

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Academic dissertation

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ABSTRACT

Low apoplastic water potentials can affect trees by decreasing the hydraulic conductivity of xylem due to embolism and by causing dehydration stress in living cells. Low apoplastic water potentials regularly occur in trees during summer and winter. These can either be caused by loss of water due to transpiration or by freezing due to the chemical properties of ice.

In this thesis the effects of low apoplastic water potential on trees were studied by causing low water potentials with three different methods: desiccation, freezing and by adjusting the osmotic concentration of xylem sap. Tree responses in this thesis were measured with stem diameter changes, leaf gas exchange, tree temperature and xylem water potential.

Living parenchyma cells are thought to have negligible effect on xylem diameter changes but this thesis shows that the role of parenchyma can, in fact, be much more significant. Evidence for the major role of parenchyma cells in the diameter changes of frozen xylem also supported the theory of extracellular freezing. Furthermore, mesophyll cells were shown to react to freezing with a rapid depression of photosynthesis.

It was also studied how a pressure increase in the xylem conduits, resulting from low water potentials, affects tree water relations during embolism formation and ice propagation. A gas burst was detected emerging from the tree stem during freezing. A decrease in the amount of gases in the xylem conduit can benefit trees in avoiding winter embolism. It was also experimentally confirmed that the formation of embolism in trees can temporarily even help relieve water stress due to the so called ´capacitive effect´. Low apoplastic water potential affects both the xylem and living cells in trees, and the interconnectedness of these responses are also shown in this thesis.

Keywords: Water potential, dehydration, freezing, embolism, photosynthesis, diameter change, trees

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by the Roman numerals I–IV. The publications are reprinted here with the kind permission of the publishers.

I. Hölttä, T., Juurola, E., Lindfors, L., Porcar-Castell, A. (2012). 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(2): 1057-1067.
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II. Lintunen, A., Lindfors, L., Kolari, P., Juurola, E., Nikinmaa, E., Hölttä, T. (2014). Bursts of CO2 released during freezing offer a new perspective on avoidance of winter embolism in trees. Annals of Botany 114(8): 1711-1718.
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III. Lindfors, L., Hölttä, T., Lintunen, A., Porcar-Castell, A., Nikinmaa, E., Juurola, E. (2015). Dynamics of leaf gas exchange, chlorophyll fluorescence and stem diameter changes during freezing and thawing of Scots pine seedlings. Tree Physiology 35(12): 1314-1324.
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IV. Lintunen, A., Lindfors, L., Nikinmaa, E., Hölttä, T. (2016). Xylem diameter changes during osmotic stress, desiccation and freezing in *Pinus sylvestris* and *Populus tremula*. Tree Physiology 1-10.
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Author's contribution:

In study I, the author participated in designing the experiments, performed all experiments and participated in writing the paper. In study II, the author participated in designing the experiment, designed and built the custom-made gas exchange cuvette system used to measure stem respiration in the laboratory, performed all experiments and participated in writing the paper. In study III, the author participated in designing the experiment, performed most of the experiments and had the main responsibility for writing the article. In study IV, the author participated in designing the experiments, performed the osmotic stress and desiccation experiments and participated in the freezing experiments, and participated in writing the paper.

TABLE OF CONTENTS

SYMBOLS AND ABBREVIATIONS

$\Delta\psi$	Water potential difference over the xylem water pathway
ψ	Water potential
ψ_π	Osmotic potential
ψ_P	Pressure potential
ψ_g	Gravitation potential
ΦP	The operating quantum yield of photosystem II (PSII)
A	CO_2 assimilation rate
E	Transpiration rate
CO_2	Carbon dioxide
F_t	Fluorescence at time t
Fv/Fm	Maximum quantum yield of photochemistry
c_i	Intracellular CO_2 concentration
g_s	Stomatal conductance
k	Hydraulic conductance
NPQ	Non-photochemical quenching
PSII	Photosystem II
PQ	Photochemical quenching

1 INTRODUCTION

Patterns in the periods of freezing temperatures and drought causing low apoplastic water potentials in trees are expected to change due to climate change. Current dry areas are predicted to suffer more from drought and temperature is expected to rise more during winter than summer, especially in areas located at high latitudes (Stocker et al. 2014). Low apoplastic water potentials cause effects in trees such as a decrease of water conductivity in the xylem due to embolism and dehydration stress in living cells. Understanding the effects of low apoplastic water potentials on trees is now more important than ever for predicting how plants will adapt to climate change. Studying the effects of winter on trees is particularly important because the topic has received much less scientific attention than tree functioning during the growing season.

Trees annually experience large fluctuations in water potential (ψ from here after) in the apoplastic space due to changing environmental conditions. Apoplastic space is defined as space outside living cells, i.e. beyond the plasma membrane of living cells including the xylem vessels. Water potential can be low during summer, when dry periods occur, and especially during winter, when water in the apoplastic space is frozen. The reasons for the low ψ in these two cases are fundamentally different. During dry periods low apoplastic ψ in trees is typically created due to low water availability in the soil and/or high transpiration rates during summer because water pressure decreases in the xylem. When water in the apoplastic spaces is frozen, a low apoplastic ψ is created by the chemical properties of ice because the ψ of ice is very low (Burke et al. 1976). A major difference in freezing-induced low apoplastic ψ compared to dry periods is that during freezing, ψ typically decreases rapidly throughout the tree in tens of minutes (Pramsohler et al. 2012), while under drought conditions this takes anywhere from hours to weeks. While freezing of water in the apoplastic spaces causes rapid dehydration stress in living cells, the dehydration is necessary for living cells as a freezing tolerance mechanism. Dehydration causes solute concentration to increase in the cell sap of living cells, leading to a decrease in freezing temperature that helps in avoiding lethal intracellular freezing. This freezing tolerance mechanism is called the extracellular freezing –strategy (Burke et al. 1976), because water leaving the cells freezes outside plasma membrane instead of freezing in the intracellular space.

Implications of freezing and the rapid dehydration of living cells on stem diameter changes and leaf gas exchange are not yet fully understood. Freezing in trees has been shown to cause detectable shrinking of the whole stem, xylem and living bark diameters. The diameter changes in living bark reflect changes in the turgor pressure of living cells (Zweifel and Häsler 2000; Ameglio et al. 2001), while in the case of xylem, the mechanism behind the diameter changes under freezing conditions has not been fully established. While xylem mainly consists of dead conduits it still holds a considerable amount of living cells that may contribute to the shrinkage of the tissue; the volume occupied by living parenchyma is for example approximately 6% for *Pinus* and 12% for *Populus* species (Spicer 2014).

Freezing temperatures are known to affect leaf gas exchange and decrease photosynthetic rates in what is called depression of photosynthesis. Many mechanisms can contribute to this depression, while their relative importance is not well understood. The mechanisms may occur within mesophyll cells where photosynthesis takes place or outside the cells. Perhaps the most well-known mechanism that occurs within the mesophyll cells is the temperature dependency of biochemical reactions in the dark reaction of photosynthesis (Huner et al. 1996). Freezing temperatures have also been shown to cause inhibition of the quantum yield

of photosynthesis (Strand and Öquist 1985) which can be related to the extracellular freezing and dehydration of mesophyll cells. Outside the mesophyll cells, ice barriers (Neuner and Pramsohler 2006) in the apoplastic space and closure of stomata (Gaumont-Guay et al. 2003) can decrease carbon dioxide (CO_2) gas exchange, which can affect photosynthetic rates. Research on the depression of photosynthesis is currently lacking investigation on the dynamics of photosynthesis at high temporal resolution along with simultaneous characterization of the dehydration of living cells that could help in determining the relative importance of the mechanisms.

Embolism is another physical phenomenon that is caused by low apoplastic ψ in the xylem conduits (e.g. Tyree and Sperry 1989). In embolism, xylem conduits are filled with air that decreases xylem water conductivity. Embolism can form either during drought or when ice in the xylem thaws. When embolism formation is related to the freezing and thawing of ice, it is called winter embolism. During drought, when trees are under water stress, embolism forms by spreading from the air-filled xylem conduits to neighbouring water-filled conduits and fills them with air.

Trees are known to decrease transpiration through the stomata to prevent the spread of embolism in the xylem (Sperry and Pockman 1993; Brodribb et al. 2003). However, how trees sense embolism and whether they are able to sense embolism directly in the xylem or whether they sense it through the low ψ that it causes in leaves is not known. During ice thawing embolism forms when gas in pockets of ice is released into the xylem sap. Instead of dissolving into the xylem sap, the gas bubbles expand and fill the xylem conduits with air (Sucoff 1969; Mayr and Sperry 2010). One major assumption usually made in connection with winter embolism formation is that all the gas dissolved in the xylem sap before freezing is trapped within the conduits and forms gas pockets within the ice, but this has not received further scientific attention.

While pressure typically changes slowly in the xylem conduits, rapid changes are also hypothesized to occur during formation of embolism and freezing, which can have great ecological significance for trees. Although embolism decreases xylem conductance, it is also hypothesized to cause a so called `capacitive effect´ that can temporarily relieve water stress (Meinzer et al. 2001). Expanding gas can pressurize and expel the remaining water out from the embolizing xylem conduits into the surrounding tissue, increasing its ψ. During freezing in trees, gas pressure may increase because of the lower specific density of ice compared with liquid water (Robson and Petty 1987). This may help trees to expel certain gases from the xylem that would otherwise cause embolism in the xylem conduits upon thawing.

2 AIM OF THE STUDY

The motivation of this thesis was to understand the implications of the low apoplastic ψ in trees that leads to dehydration of living cells and formation of embolism in the xylem vessels. Dehydration of living cells was studied for parenchyma cells in the xylem and mesophyll cells in leaves. The aims were to (1) establish the role of parenchyma cells in the diameter changes of xylem and (2) to study the depression of photosynthesis and the mechanism causing it. Embolism was studied on how trees can sense it and also the implications of the sudden changes in pressure that may occur during formation of embolism formation and freezing. The aims were to (3) experimentally test the hypothesized `capacitive effect` that can help relieve water stress in trees during drought and (4) to test if gas pressure can increase in the xylem during freezing, and if that helps decrease the amount of gasses inside xylem vessels and helps trees in respect to winter embolism. Furthermore, aim was (5) to test if trees sense formation of embolism directly in the xylem or if they detect formation of embolism indirectly with decreasing ψ in the leaves. The following hypotheses were tested in this thesis:

- Changes in apoplastic ψ influence the water content and volume of parenchyma cells that contribute significantly to xylem diameter changes (studied in paper IV).
- Freezing in trees causes depression of photosynthesis due to a decrease in the CO_2 availability for photosynthesis due to closure of the stomata and/or due to dehydration of the mesophyll cells and depression of the biochemistry of photosynthesis (studied in paper III).
- A gas burst is released from the tree stem because ice propagation during freezing pushes gas inside the stem and increases the pressure; this can help prevent winter embolism in trees (studied in paper II).
- Trees do not directly sense embolism formation in the xylem, but only through its effects on leaf water potential (studied in paper I).
- Embolism causes temporary relief of water stress due to the so called ´capacitive effect´ (studied in paper I).

3 THEORETICAL BACKGROUND

3.1 Water potential and movement of water

Water is critical for tree survival. It is needed for living cells to act as a medium for biochemical activities, for functioning of the vascular system and for CO_2 uptake into leaves for photosynthesis. The amount of water in trees is affected by environmental factors such as precipitation, sun irradiance and air temperature. While the amount of water in trees is important, water must also move to maintain functioning of the vascular system and CO_2 uptake.

Water pressure is related to both the amount of water and the movement of water. Water movement is affected by water pressure and osmotic concentration, so that it occurs towards lower pressure and higher osmotic concentration. ψ is therefore a useful parameter in describing water movement in plants. ψ is described as (Nobel 2009):

$$\psi = \psi_\pi + \psi_P + \psi_g, \quad (1)$$

where ψ_π is the osmotic potential, ψ_P is the pressure potential and ψ_g is the gravitation potential. Following e.g. Nobel (2009), the matric potential is not included as a separate term, but is included in ψ_P. Gravitational potential is not considered in the analysis due to its marginal importance in this context. Water moves towards lower ψ. The ψ_π component is important in particular when we examine the water exchange between living cells and apoplastic space. Living cells are typically able to maintain a higher ψ_P compared to that in the apoplastic space surrounding them; this is called turgor pressure. Turgor pressure is maintained by adjustingψ_π, while the absolute value of ψ_π is typically low in the apoplastic space (Nobel 2009). Water moves through semi-permeable plasma membranes that surround living cells and the rate of water movement is affected by the permeability of the membrane to water, which depends on e.g. aquaporins, i.e. water channels in the membrane (Maurel 1997).

3.1.1 Transpiration-driven xylem transport

Transpiration from leaves that drives xylem transport in trees decreases the ψ in the xylem conduits and in the apoplastic space. Xylem consists mostly of dead conduits and is used to transport water and nutrients from the roots to the leaves. Water moves in the xylem towards lower water pressure (ψ_P) along the ψ_P gradient. Maintaining this gradient in the xylem requires water to be released into the atmosphere from the leaves and taken up from the soil by the roots. Water evaporates into gas phase from water-air surfaces covering air cavities within the leaves, and from there it diffuses out from the leaves into the atmosphere through the stomatal aperture.

Most of the gas exchange between the leaves and atmosphere occurs though the stomata. The stomatal aperture is surrounded by a pair of guard cells that enable plants to change the size of their stomatal pore to decrease or increase gas exchange, including the diffusion of water vapour and CO_2. Their movement through the stomata occurs along a concentration gradient towards a lower concentration. During the day, concentration gradients are typically such that the flux of water vapour heads out from the leaves and the flux of CO_2 into the leaves. Irradiance and temperature are important factors affecting gas exchange through the

stomata. Trees have a gas exchange optimization problem in relation to the stomata; while plants are able to use stomata to control the water flow and pressure gradient in the xylem, the stomata concurrently also affect the photosynthesis rate (Hari et al. 1986; Nikinmaa et al. 2013). Trees stops the water pressure decreasing too low in the xylem because it can causes embolism. Trees can avoid low water pressures by closing stomata but then they cannot photosynthesize without inflow of CO_2 into the leaves. Photosynthesis requires CO_2 for enzymatic reactions to synthetize sugars.

3.1.2 Freezing and ice in the apoplastic space

When freezing occurs in trees, it spreads rapidly throughout the apoplastic space and fills it with ice. Ice has a low water potential that depends on its temperature. The water potential of ice decreases by 1.2 MPa per a 1-°C decrease in temperature (Rajashekar and Burke 1982). Ice therefore exerts a strong pull on water that freezes on contact. To protect living cells from freezing, many tree species employ an extracellular freezing strategy (Burke et al. 1976), in which apoplastic water freezes while lethal intracellular freezing is avoided due to an increase in osmotic concentration of the cell sap. Due to the low water potential, apoplastic freezing initially creates a steep water potential gradient between the apoplastic ice and cell sap. Because water always moves towards a lower water potential through a semipermeable membrane, living cells experience a net loss of water to the apoplastic ice, leading to dehydration and shrinkage (Figure 1). Osmotic concentration increases within the cells during this dehydration and the cell volume decreases.

Trees must cold-acclimate during the autumn to achieve the ability to survive freezing and the extreme dehydration of living cells. Cold acclimation involves e.g. an increase in solute concentration in living cells and an increase in the fluidity of the plasma membrane (see Sakai and Larcher 1987). The increasing solute concentration decreases the equilibrium freezing temperature and changes to mechanical properties of the plasma membrane are important in maintaining cellular integrity. If the cold acclimation level of living cells is insufficient, thelow apoplastic water potential may lead to loss of turgor pressure and plasmolysis due to desiccation stress (Ashworth et al. 1993; Walters et al. 2002) and cell membrane rupture due to apoplastic ice crystals (Steponkus 1984; Ristic and Ashworth 1993; Charrier et al. 2013). In the worst case the living cells undergo lethal intracellular freezing (e.g. Mazur 1969; Wolfe and Bryant 2001).

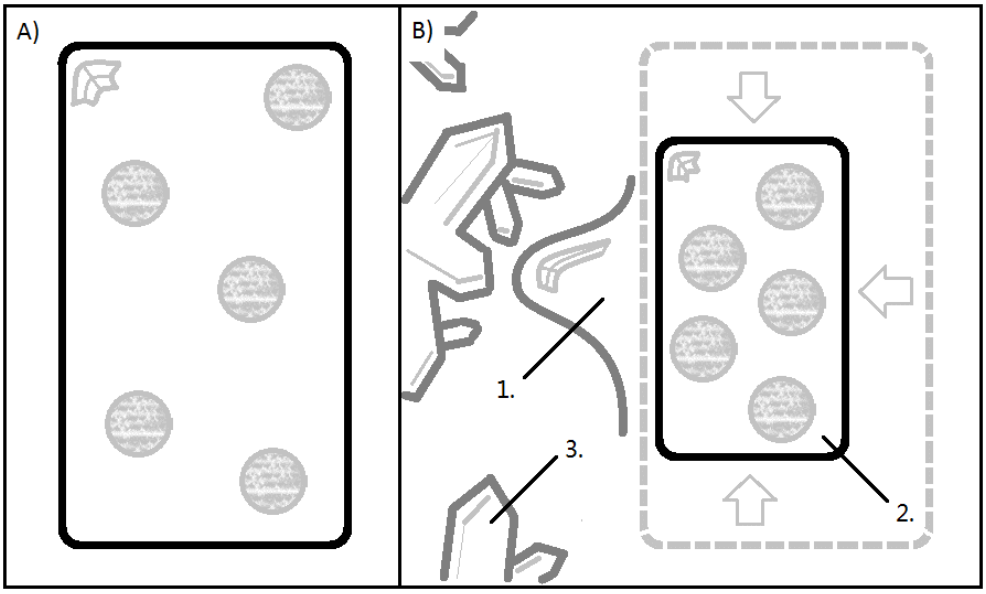

Figure 1. Schematic presentation of extracellular freezing. (A) A living cell before freezing begins. (B) A living cell dehydrates during freezing. Water moves (B1) out from the living cells (B2) along a water potential gradient upon propagation of ice (B3) into the apoplastic space closet to the cell. In parallel, the living cell shrinks and its osmotic concentration increases because the ratio between water and solute concentration changes in the cell sap. The dashed grey line indicates the size of the living cells before dehydration has begun. The gray round shapes indicate the amount of solutes and white area amount of water inside the living cell (B2). As the living cell dehydrates the amount of water decreases while the relative proportion of solutes increases. This is visualized as an increase in area that the gray shapes occupy inside the living cell.

Temperature measurements in trees have been widely applied to detect the timing and temperature at which ice nucleation occurs. Ice nucleation in trees can be detected based on the exothermic reaction (Weiser 1970; Burke et al. 1976; Pramsohler et al. 2012), in which water releases energy as heat due to its phase change from liquid to solid state. Most tree species are able to avoid nucleation to some extent by supercooling (Wisniewski et al. 2014) to a few degrees below 0 °C (Burke et al. 1976). In the exothermic reaction, thermal energy is released due to the phase change of water into ice that can be seen as an increase in temperature. In contrast, the thawing moment can be identified based on the absorption of energy by water in the endothermic reaction (Silk et al. 1986). An example of ice nucleation detection utilizing temperature measurement is shown in Figure 2.

3.2 Relationship between pressure, cell volume and stem diameter

Pressure affects the volume within structures, such as plasma membranes, cell walls and walls of xylem vessels, by causing the structures to expand when pressure increases and to shrink when pressure decreases around them (Nobel 2009). Shrinking and expanding due to pressure changes can also be detected on the whole stem-scale as measurable stem diameter changes. A linear relationship between these two, based on Hooke's law, has been applied to both the xylem (Irvine and Grace 1997) and the living bark (Mencuccini et al. 2013), although some non-linearity likely occurs in the pressure vs. volume relation (De Schepper and Steppe 2010), complicating the interpretation of the diameter change measurements. Diameter changes can be categorized as reversible when they follow changes in pressure in both directions, or irreversible when they do not follow the pressure changes due to e.g. cambial growth or damage to living cells and loss of turgor pressure due to e.g. freezing.

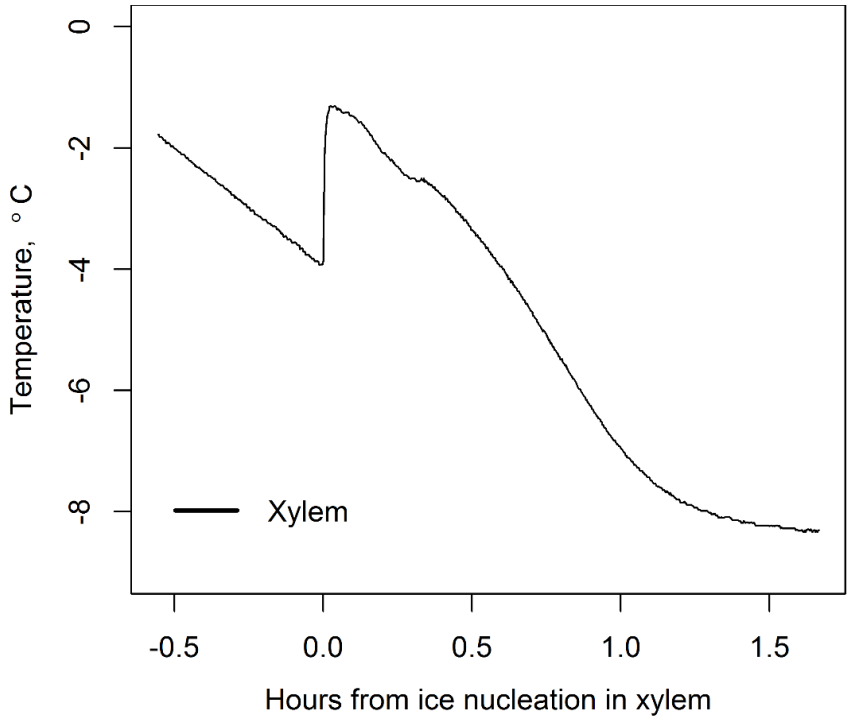

Figure 2. An example of measured temperature dynamics in the xylem of the stem during freezing. Air temperature decreased in a linear fashion in an environmental chamber. The temperature suddenly increased by ~2 °C (time 0.0 in the Figure) because the phase change of water from liquid to solid form releases energy in the form of heat in a phenomenon called an exothermic reaction.

Reversible diameter change measurements of the xylem (Irvine and Grace 1997) and living bark, consisting of the phloem and vascular cambium (Mencuccini et al. 2013), have been extensively used to study changes in the water content of these tissues during summer conditions (Sevanto et al. 2011; De Swaef et al. 2015; Pfautsch et al. 2015). Changes in the negative hydrostatic pressure of the xylem and in the positive turgor pressure of the living bark may also be derived from diameter change measurements provided that the pressure–volume relations of the tissues are known (De Schepper and Steppe 2010).

3.3 Factors limiting photosynthesis in freezing temperatures

It is well known that tree water status greatly influences leaf transpiration and photosynthesis during the growing season (Nobel 2009). Photosynthetic rate is largely reduced during sub-freezing temperatures (Troeng and Linder 1982).Mesophyll cells in needles of evergreen trees experience similar freezing-associated water stress as the living cells of the stem, which is seen as mesophyll cell shrinkage (Roden et al. 2009). The stem and needles are hydraulically connected through the xylem and phloem, and therefore an ice front and water potential can propagate rapidly within the plant (Pramsohler et al. 2012) unless barriers exist, e.g. inside buds, which could prevent the spreading of the ice front (Jones et al. 2000).

Several factors related to water stress may contribute to the depression of photosynthesis during freezing in addition to the direct effect of temperature. The direct temperature effect refers to the temperature dependency of biochemical reactions in the dark reaction of photosynthesis (Huner et al. 1996) that leads to a reduction in photosynthetic rate, independent of any effect that water freezing may have on trees. Factors related to water stress caused by the freezing of apoplastic water may also contribute to the depression of photosynthesis in addition to the direct effect of temperature. Depression may result from decreased stomatal conductance with lowering water potential. The depression may also occur due to non-stomatal reasons such as biochemical limitations in the Calvin–Benson cycle (Strand and Öquist 1985), a decrease in the diffusion rate of CO_2 into mesophyll cells due to formation of ice into the apoplastic spaces (Larcher 1994; Neuner and Pramsohler 2006) and changes in the mesophyll conductance of CO_2.

Previous studies on leaf gas exchange have found evidence for the biochemical limitation to photosynthesis at freezing. The intracellular CO_2 concentration (c_i) of foliage has been found to increase following freezing, suggesting that changes in c_i are dominated by decreasing CO_2 consumption by the photosynthetic Calvin–Benson reactions and not so much by decreasing the CO_2 input via the stomata, i.e., stomatal closure (Strand and Öquist 1985; Strand et al. 2002; Gaumont-Guay et al. 2003). The rise in c_i has been suggested to occur due to decreased activity and regeneration capacity of ribulose-1,5-bisphosphate (Strand and Öquist 1985) in the Calvin–Benson cycle.

3.4 Embolism formation in the xylem

Water columns in the xylem are usually under negative pressure and therefore vulnerable to cavitation (Tyree and Sperry 1989). Cavitation can fill the xylem conduits with air. Excessive cavitation is unfavourable to plant function, as it decreases the hydraulic conductance of the xylem and threatens the supply of water to the leaves. If a plant does not respond by stomatal closure to prevent excessive cavitation, it would progressively spread to fill the entire xylem

in 'run-away' cavitation and the plant could die due to a shortage of water (Tyree and Sperry 1988; McDowell et al. 2008; Mencuccini et al. 2015). Various tree species have been shown to avoid stomatal conductance and the xylem water potential decreasing below critical values to avoid excess xylem cavitation (Sperry and Pockman 1993; Borghetti et al. 1998; Irvine et al. 1998; Salleo et al. 2000; Hubbard et al. 2001; Brodribb et al. 2003). Whether the stomata respond to leaf water potentials, corresponding to the onset of significant amounts of cavitation, or directly to some other signal created by cavitation events themselves, is not yet known (Whitehead et al. 1996; Salleo et al. 2000; Cochard et al. 2002).

Despite its unfavourable effect on xylem hydraulic conductance, cavitation is hypothesized to temporarily relieve water stress (Meinzer et al. 2001). Following the entry of an air bubble into a conduit, water is quickly sucked out of the embolizing conduits into adjacent xylem conduits and the surrounding tissue (Hölttä et al. 2007), and the water potential in the plant should transiently increase (Lo Gullo and Salleo 1992; Hölttä et al. 2009a). Cavitation is therefore hypothesized to act as a water release mechanism, the importance of which increases with increasing plant size (Hölttä et al. 2009a). The water tension release due to cavitation has also been observed as a temporary reversal of stem diameter shrinkage with increasing water loss in Norway spruce (*Picea abies* (L.) Karst.) stems (Rosner et al. 2009, 2010).

3.4.1 Winter embolism

Winter embolism influences tree survival and growth in all regions where sub-zero temperatures occur. Winter embolism has been observed in numerous tree species including conifers (Sperry and Sullivan 1992; Sparks et al. 2001; Mayr et al. 2002, 2007; Pittermann and Sperry 2003, 2006; Mayr and Sperry 2010) and angiosperms (Cochard and Tyree 1990; Just and Sauter 1991; Sperry and Sullivan 1992; Utsumi et al. 1998; Nardini et al. 2000). Winter embolism follows from the formation of gas bubbles during freezing and their subsequent expansion during thawing (Sucoff 1969; Ewers 1985; Sperry and Sullivan 1992; Davis et al. 1999; Mayr and Sperry 2010). Gases dissolved in the xylem sap, including CO_2, are not soluble in ice and are believed to be forced to form bubbles as the xylem sap freezes. Upon thawing, the bubbles released from the ice may expand and embolize the xylem conduits.

According to LaPlace's law, the fate of the gas bubbles during thawing, i.e. whether they collapse or expand to embolize xylem conduits, is dependent on their size and on the pressure of the surrounding xylem sap (Pittermann and Sperry 2006). The size of the bubbles formed during freezing is further hypothesized to correlate positively with conduit diameter (Sperry and Sullivan 1992; Davis et al. 1999; Pittermann and Sperry 2003, 2006). The link between conduit diameter and winter embolism has been experimentally quantified in several tree species (Sperry and Sullivan 1992; Sperry et al. 1994; Davis et al. 1999; Pittermann and Sperry 2003; Wheeler et al. 2005; Wilson and Jackson 2006), whereas the link between conduit diameter and bubble size is only theoretical (Pittermann and Sperry 2006) and, to our knowledge, has not been directly measured. The basic idea behind this relationship during freezing is that air is forced out of the freezing xylem sap, forming centrally located air bubbles in ice, the volume of which is proportional to the cross-sectional area of a xylem conduit (Sperry and Sullivan 1992; Pittermann and Sperry 2006).

4 MATERIAL AND METHODS

The effects of low apoplastic water potentials on embolism formation and the dehydration stress of living cells were studied in experiments with four different experimental setups (papers I–V). Three different tree species were used in this thesis (but not in all the experiments/papers): Scots pine (*Pinus sylvestris* L.), Norway spruce and common aspen (*Populus tremula*). Low apoplastic water potentials were caused with three different methods mimicking natural conditions; osmotic stress, desiccation and freezing.

4.1 Stem diameter change measurements

Stem diameter changes were measured (papers III-IV) with two pen-like linear variable displacement transducers (LVDT; model AX/5.0/S, Solartron, West Sussex, UK). A sensor head was placed directly on the xylem after bark had been removed from a small cross-sectional area surrounding the LVDT head (papers III–IV), or the sensor was placed on living bark (paper III) after the dead outer bark had been removed from a small cross-sectional area surrounding the LVDT head (see Figure 3). Living bark refers to the layer of phloem and vascular cambium, although the relative diameter change from vascular cambium is insignificantly small due to its small cross section. The diameter change of the living bark was calculated by subtracting the diameter change measured on the xylem from the diameter change measured on the living bark.

4.2 Gas exchange measurements

4.2.1 Lab gas exchange measurements

All gas exchange measurements in the laboratory were conducted with a portable gas exchange measuring system (Walz GFS-3000, Heinz Walz GmbH, Effeltrich, Germany). A standard cuvette was used for measuring needles (papers I and III) and a custom-made cuvette was used for measuring the stem (paper II). Photographs of needles in the standard cuvette were taken to calculate their surface area. The CO_2 flux from the stem was calculated relative to surface area. The dynamics of CO_2 assimilation rate (A), transpiration rate (E) and stomatal conductance (g_s) were estimated from needle gas exchange measurements. The custom-made cuvette was made of an opaque plastic cuvette that was 8.8 cm high and 5 cm wide and consisted of only a single intact cylinder-shaped piece. The cuvette was air-tightly fixed on a stem at a height of 10 cm using rubber socks from both open ends (see Figure 4).

Figure 3. An example how stem diameter sensors were installed to measure xylem (upper sensor) and phloem (bottom sensor) diameter changes for paper III. Upper tissue layers were carefully removed to allow placement of the sensors directly on top of the xylem or phloem.

Cuvette temperature was set to follow the conditions inside the environmental test chamber. For freezing experiments (papers II and III), air for the gas exchange system was taken from outdoors and was completely dried during the freeze–thaw treatment to avoid condensation of water inside the measurement system during the experiment at low temperatures. Ambient CO2 concentration was used for experiments conducted at room temperature. In experiments where the standard cuvette was used, cuvette light intensity was set at 200 μmol m^{-2} s^{-1} in the freezing experiments (paper II) and at 1000 μmol m^{-2} s^{-1} in the experiments conducted at room temperature (paper I). The lower light intensity in the freezing experiments was set to avoid photoinhibition development in the needles within the cuvette.

4.2.2 Field stem gas exchange measurements

Stem gas exchange measurements were conducted (for paper II) at the SMEAR II station (Hari and Kulmala 2005) located in southern Finland (61°51′ N, 24°17′ E), where stem CO_2 efflux from a mature Scots pine tree has been continuously measured since 2003 using automated flow-through gas exchange cuvettes (Kolari et al. 2009). The transparent cuvette (3.5 × 20 cm) with a 1-cm-thick opaque rubber seal was attached to the northern side of the stem on top of the bark on the main trunk. CO_2 efflux was determined from the CO_2 concentration increase in the cuvette measured with an infrared gas analyser (URAS 4; Hartmann & Braun, Frankfurt am Main, Germany) in a time frame varying from 30 to 60 min. We analysed the data from 2006 to 2009.

Figure 4. The custom made cuvette used in measuring stem CO_2 exchange was installed on the stem of a Scots pine seedling (used in paper II). The tubes were connected to the central unit of a Walz GFS-3000 gas exchange system. One tube was used to deliver air into the cuvette and the other one to remove it. The central unit calculated the net CO_2 exchange of the stem based on the CO_2 concentration between the two tubes.

4.3 Chlorophyll fluorescence measurements

Chlorophyll fluorescence is used as an indicator of photosynthetic energy conversion in higher plants. Fluorescence measurements were carried out to follow (paper III) the dynamics of fluorescence at time *t* (F_t), the operating quantum yield of photosystem II (PSII) (ΦP), as along with the non-photochemical (NPQ) and photochemical (PQ) quenching parameters in the needles in response to the freeze–thaw cycle (Maxwell and Johnson 2000; Porcar- Castell et al. 2014). The various parameters describe the portioning of absorbed light energy into different processes in the photosystem. F_t refers to energy emitted as chlorophyll fluorescence from the leaf, NPQ to energy emitted as heat to protect the photosystem from damaging excess energy, and ΦP and PQ refer to energy used in the photochemistry of photosynthesis. Measurements (see fig. 5) were conducted with a ”MONI-TORING-PAM Multi-Channel Chlorophyll Fluorometer” or MONI-PAM (Heinz Walz GmbH, Effeltrich, Germany) (Porcar- Castell et al. 2008b). The purpose of this measurement was to assess the effects of freezing on photosynthesis with an independent measurement carried out at a different set of needles, and to examine the response of the light reactions to the freeze–thaw cycle. The maximum quantum yield of PSII (Fv/Fm) was also measured prior to the experiments with a portable fluorometer (FMS-2, Hansatech Ltd, Norfolk, UK) on the dark-acclimated seedlings in the field to ascertain the background level of photosynthetic down-regulation.

Figure 5. An example of how sensor heads of the monitoring MONI-PAM fluorometer were set to measure the chlorophyll fluorescence signal from Scots pine needles (paper III).

4.4 Experimental setups

4.4.1 Role of parenchyma cells in xylem diameter changes

To understand the role of parenchyma cells in xylem diameter measurements, we compared the responses of the control and heat-injured xylem of Scots pine and common aspen with various treatments causing low apoplastic ψ by A) osmotic stress, B) desiccation and C) freezing (paper IV). Mechanisms causing xylem diameter changes were different in the three treatments (see section 3.2). Xylem diameter was expected to shrink solely due to shrinking of living cells in the osmotic and freezing stress treatments, while shrinkage in the desiccation experiment was expected to occur due to both water tension in the xylem conduits and dehydration of the living cells.

We sampled a total of 46 winter-acclimated branches from mature trees near the Helsinki University campus during the winters 2012-2014. Two 15 – 20 cm long sections were taken from each branch. One section was heated and the other one was a control. Xylem diameter changes were measured from the samples. Heat injury was inflicted by heating the sections at 60°C for an hour. We expected to see differences in the magnitude of xylem diameter change between the control and heated-injured sections. Heat-injury was confirmed by measuring the respiration rates from the sections before heat-injury treatment and 60 minutes after it. The respiration decrease averaged 75 % due to the heat-injury treatment.

Osmotic stress was caused by injecting a flow of D-mannitol solution through the branch sections using a tubing system supplied with a tap water with a hydrostatic pressure head of approximately 1 m in height (~0.01 MPa). Because of this no water tension was created in the xylem sap that could cause shrinkage of the xylem conduits. However, the D-mannitol solution leads to a difference in ψ between apoplastic xylem sap and symplastic parenchyma sap. A desiccation stress treatment was conducted by letting sections dehydrate at dry ambient room conditions (~20 °C and relative humidity of ~40 %) for 20 hours until stem ψ fell to -1.4 – -2.4 MPa. Stem ψ was estimated from the needles (enclosed in a plastic bag to prevent transpiration so needle water potential would equal the stem water potential) of a side branch on a third identically treated section with pressure chamber measurements. In the freezing treatment, sections were placed into an environmental test chamber (WK11—340/40, Weiss Umwelttechnik, Vienna, Austria) and the temperature of these sections was decreased from +10 °C to -10 °C in 40 minutes.

4.4.2 Depression of photosynthesis during freezing

The effect of the freeze–thaw cycle on photosynthesis was studied on Scots pine in an experiment that was repeated eight times (paper III). The seedlings were four-year-old crafted clones and had overwintered in large pots outdoors at the Viikki campus of the Helsinki University. The experiments were performed between 4 April and 22 April in 2009, when the seedlings were undergoing spring recovery of their photosynthetic capacity. Experiments were conducted in the environmental test chamber. Light intensity was fairly low, 200 μmol m^{-2} s^{-1}, to avoid photo oxidative damage. Needle, xylem and soil temperatures were followed using thermocouples (copper-constantan, T-type).

Each experiment consisted of three phases; (i) an overnight stabilization period in the environmental test chamber; (ii) a freeze–thaw cycle; and (iii) a 14-h recovery period (Figure 1 in paper III). ‘Cooling phase’ hereafter refers to the first half of the freeze– thaw cycle where the temperature decreased and ‘warming phase’ refers to the second half when the

temperature increased. One full experiment took ~35 h from installment in the environmental test chamber until the end of the recovery phase.

4.4.3 Embolism formation – tree response and the capacitive effect

Experiments were conducted during June and July 2010 in a laboratory to investigate how trees sense embolism and whether embolism formation can temporarily relieve water stress (paper I). Four-year-old grafted Scots pine seedlings were used as plant material. The seedlings had been grown in large pots outdoors until the beginning of the experiment and were well watered.

The seedlings were cut at the base of the stem and then re-cut under water in a container. Two double saw-cuts, running radially approximately halfway up the stem, were made at the base of the seedlings to reduce the total hydraulic conductance. This was done to decrease the water potential to ensure the occurrence of cavitation. Needle gas exchange and ψ were monitored continuously during the experiments. Needle ψ was measured with a custom-made pressure chamber. Hydraulic conductance (k, mmol m^{-2} Pa^{-1} s^{-1}) of the whole xylem pathway was calculated by dividing the transpiration rate (E, mmol m^{-2} s^{-1}) with the water potential difference over the pathway ($\Delta\psi$, Pa). The water potential difference is equal to ψ in the needles because ψ at the lower end of the xylem pathway, i.e. in the soil, was assumed to be zero. The apparent k was calculated in the same way but steady state was not assumed and therefore it does not take capacitance into account.

A surfactant (Tween 80) was used to lower the surface tension of the xylem sap. This was done to (A) see whether it affects the ψ threshold below which stomatal closure occurs and (B) to see how it affects the water status of the seedlings. The surfactant was added slightly above its micellar concentration (0.5% v/v), so that the surface tension of the water decreased to 36 N m^{-1} (Chu and So 2001), which is 50 % of the surface tension of pure water. After the initial measurement phase when the seedling had reached steady state, i.e. transpiration rate and xylem water potential remained relatively constant, the surfactant was added to the water container where the cut seedling was placed. The surfactant treatment was conducted with 11 replications (referred to hereafter as 'treatment runs'). To estimate potential errors and inaccuracies in our measurement protocol, six control runs were also performed, where no surfactant was added to the water taken up by the seedlings.

4.4.4 Gas burst from the tree stem during freezing

Effects of freezing on winter embolism were studied with observations from the field measurements and laboratory experiments (paper II). We analysed the CO_2 efflux response of the stem to the freezing events by combining the CO_2 efflux data with xylem and ambient temperature data. The laboratory measurements were conducted in February 2013 with three Scots pine and three Norway spruce saplings grown in 3-L pots. The saplings were winter acclimated as they had been kept outdoors since autumn 2012. The Scots pine saplings were three years old and their average base diameter was 0.80 cm. The spruce saplings were five years old and their average base diameter was 0.83 cm. The chamber air temperature was decreased from room temperature to –10 °C within 21–40 min.

Stem CO_2 efflux can be directly observed from our measurements, but the magnitude of the freezing-related CO_2 burst cannot, because the relative proportion of respiration (which produces CO_2) in the efflux is not known. This is because the CO_2 concentration and its radial gradient within the stem are constantly changing due to CO_2 production by respiration, radial

diffusion and CO_2 efflux into the ambient air. We used a previously published dynamic model of CO_2 mass balance and transport within the stem presented by Hölttä and Kolari (2009b) to separate the freezing-related CO_2 burst from the total stem CO_2 efflux and to estimate the amount of CO_2 within the stem just prior to freezing. The difference between the measured total CO_2 efflux and the modelled CO_2 efflux (the model does not consider freezing and the modelled respiration is only a function of temperature) represents the burst of CO_2 released from the stem due to the freezing process. Low apoplastic water potentials occur during the freezing that causes dehydration of living cells which can affect cell respiration, but this effect is not well known and not included in the model. Hence, estimates given by the model are likely over-estimated and the CO_2 bursts calculated as a difference between measured and modelled respirations, is thus likely underestimated.

5 RESULTS AND DISCUSSION

5.1 Effects of low apoplastic ψ on living cells

5.1.1 Role of parenchyma cells in xylem diameter changes (paper IV)

Branches of both Scots pine and common aspen reacted to increased apoplastic sap osmolality with xylem shrinkage (Figure 1 in paper IV). The diameter of Scots pine shrank more than that of aspen. The amount of shrinkage also responded to the strength of the osmotic solution in aspen. The heating pre-treatment reduced tissue shrinkage under osmotic stress to a minimal level.

Both species responded to the lowered water potential with xylem shrinkage in the desiccation experiment (Figure 2 in paper IV). Again, the diameter of Scots pine shrank more than that of aspen. Water tension in the tracheids dominated xylem shrinkage in Scots pine as the heated branches represented two-thirds of the control branch shrinkage. Shrinkage of the parenchyma dominated xylem shrinkage in aspen. Two different processes have been found to cause shrinking of the xylem diameter during desiccation: water tension-induced shrinkage of the conduits and shrinkage of the xylem parenchyma (Rosner et al. 2009; Zweifel et al. 2014).

Freezing in the apoplast caused xylem shrinkage in both species, but aspen shrank to a higher extent than Scots pine (Figure 3 in paper IV). This result is consistent with results from paper III (Figure 6) and with previous studies that show xylem shrinking in response to freezing (e.g. Fujikawa and Kuroda 2000, Charra-Vaskou et al. 2015). Shrinking was clearly decreased by the heating pre-treatment; heated Scots pine branches swelled in response to apoplastic freezing.

Here we showed for the first time how parenchyma-related xylem diameter changes in response to lowered apoplastic water potential due to three different phenomena: low osmotic pressure, low xylem hydrostatic pressure and low water potential of apoplastic ice. It was shown that xylem tissue in the control samples shrank significantly more in response to osmotic stress and freezing than samples that were heated in a pre-treatment to damage the living xylem parenchyma. By manipulating the osmotic pressure of the xylem sap and lowering the apoplastic water potential, we observed xylem shrinkage even in the absence of water tension within the xylem conduits.

Our interpretation of the main cause of xylem shrinkage during freezing is shrinkage of the parenchyma tissue. Samples with non-damaged xylem parenchyma shrank whereas

samples with damaged xylem parenchyma swelled in the case of Scots pine and shrank only marginally in the case of common aspen. Similarly Améglio et al. (2001) showed that when walnut tree stems were autoclaved they swelled in response to freezing because the phase change of liquid water into ice increases water volume by approximately 9%.

5.1.2 Depression of photosynthesis during freezing (paper III)

Ice nucleation occurred in needles on average at −4.3 °C and in the stem xylem at −3.9 °C (Figure 2 in paper III). Ice nucleation rapidly decreased the apoplastic water potential. The water potential of ice equivalent to the ice nucleation temperature is −5.2 MPa for needles and −4.7 MPa for xylem. The rapid shrinking of the xylem and living bark in the stem occurred after ice nucleation (Figure 6) in all experiments. Shrinking of the phloem tissue after freezing has been explained with dehydration and shrinking of the living cells that is caused by extracellular freezing (Ameglio et al. 2001) or by extraorgan freezing (Sakai 1982; Zweifel and Häsler 2000). In the former case water moves out from the living cells and freezes soon after in the extracellular space, but in the latter case water can move between various tissues after leaving the living cells. Because shrinking was observed in both phloem and xylem tissues in the stem, shrinking was more likely caused by extracellular freezing.

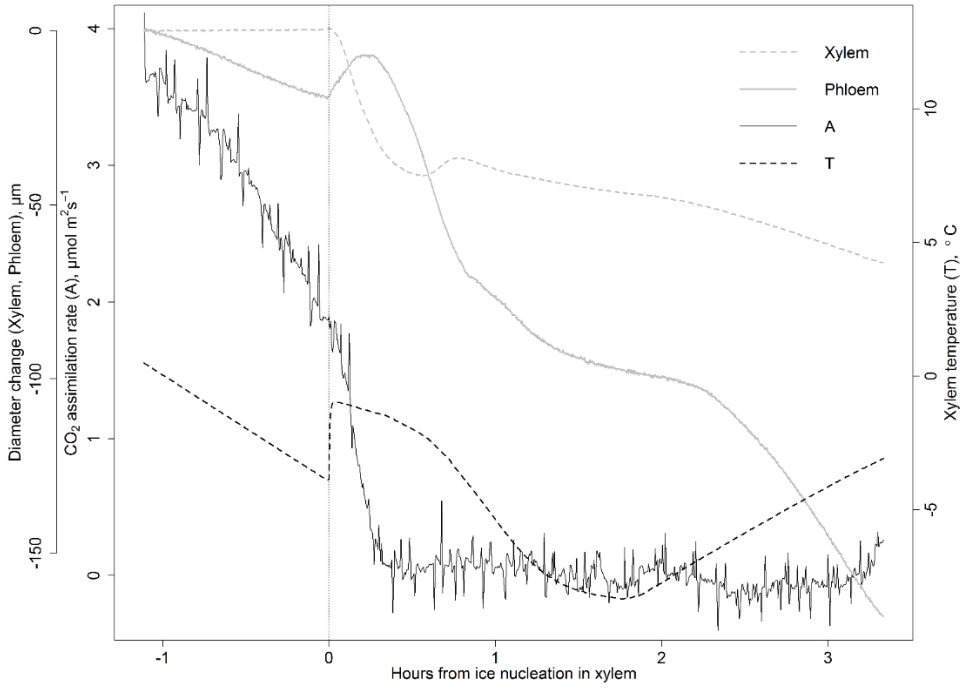

Figure 6. The average response of seedling leaf CO_2 assimilation rate, xylem and phloem diameter changes and xylem temperature (based on data from paper III, parameters are calculated based on 6 replicated experiments) on freezing, and during the rapid decrease in apoplastic water potential due to the chemical properties of ice. Dotted vertical line indicates the time of ice nucleation in the xylem. Diameter changes of xylem and phloem are normalized to zero at the beginning of the x-axis.

Fv/Fm value before the experiments was between 0.71 and 0.83, which is lower compared to values of 0.86–0.88 obtained during the summer (Porcar- Castell et al. 2008a). Gas exchange reacted to a decrease in both temperature and ice nucleation (Figure 6). A clear decrease in CO_2 assimilation rate (A) was observed before ice nucleation due to the decreasing temperature. Rapid depression of photosynthesis and increase in F_t occurred in the needles in parallel to shrinkage in xylem and the living bark. The fact that both A and F_t, measured in different needles, responded simultaneously to the freezing suggests that intracellular freezing affects photosynthesis at multiple scales and that A was not the result of any measurement artifact. Previous studies have also detected a significant increase in basic fluorescence in leaves after freezing (Neuner and Pramsohler 2006, Hacker et al. 2007). Interestingly, we observed the temperature inhibition of the NPQ regulatory mechanisms at the moment of freezing (Figure 4b in paper III), which restricted the further accumulation of NPQ. NPQ protects a photosystem from damage due to too high light intensity by releasing excess energy as heat (e.g. Eskling et al. 1997).

After ice nucleation the photosynthetic rate had fallen in ~17 min to 10% of its rate before needle ice nucleation. Ice has been suggested to block the path of CO2 into the mesophyll cells (Roden et al. 2009), thus causing depression of photosynthesis (Larcher 1994, Neuner and Pramsohler 2006). Water in the apoplastic space of the needles can be expected to freeze very rapidly and the photosynthetic rate can also drop to zero within second, e.g. when leaves stop receiving light. Hence, it does not appear likely that the path was completely blocked with ice, given how long it took for A to fall close to zero in the experiments.

Only a temporary stomatal closure was observed after ice nucleation (Figure 3f in paper III) and A remained at zero despite g_s beginning to recover even before thawing, suggesting that stomatal closure was not the primary cause for depression of photosynthesis. Similarly to previous studies (Strand and Öquist 1985; Gaumont-Guay et al. 2003), the calculated internal CO_2 concentration increased close to the ambient level during the frozen period (Figure 3d in paper III), which could be expected if the stomata were open and no photosynthesis occurred. This may result from one or more enzymes being somehow affected by the freezing in the carbon reduction cycle (Strand and Öquist 1985). I hypothesized that photosynthesis may also be affected by decreased mesophyll conductance for CO_2. Mesophyll conductance can decrease along with increasing viscosity of the cell sap as water content decreases when mesophyll cells dehydrate.

5.2 Effects of low apoplastic ψ on embolism formation

5.2.1 Embolism formation – tree response and the capacitive effect (paper I)

A clear temporary increase in leaf ψ and in the apparent k was observed in the experiments after addition of a surfactant (paper I, Figure 1). This so-called 'capacitive phase' typically reached its peak ~1 h after the addition of the surfactant, after which the leaf water potential and apparent hydraulic conductance began decreasing to a level much lower than that before addition of the surfactant. A temporary release of water stress due to cavitation was clearly observed in the experiment, as the apparent hydraulic conductance increased shortly after the surfactant was added to the transpiration stream of Scots pine seedlings. This was likely caused by an increase in water content in the needles by water that was pressurized and expelled from the xylem conduits by gas bubbles during embolism formation. The increase

in apparent hydraulic conductance during the capacitive phase was mostly due to an increase in leaf water potential (table 2 in paper I).

Physiological significance of the capacitive phase in this experiment was only marginal, due to its short duration in the small seedlings used. However, its duration can be well over a month in larger trees (Meinzer et al. 2005), as the ratio of water stored within the tree in relation to transpiration rate grows with increasing tree size (Hölttä et al. 2009a). The capacitive phase can be prolonged even further during a drought, when the transpiration rate decreases due to very strict stomatal control (Waring et al. 1979; Phillips et al. 2003; Hölttä et al. 2009).

The inability of plants to control excessive embolism in this experiment suggests that they respond to low ψ rather than sensing embolism directly. Despite of formation of embolism in the stem, rapid stomatal closure (Figure 1a,c in paper I) began only after the capacitive phase of cavitation had passed and leaf water potential and apparent hydraulic conductance had decreased below their initial values. Stomatal conductance, leaf water potential, and other variables remained relatively constant in the control runs (Figure 1e,f in paper I). Stomatal closure was initiated only at water potentials typically observed in Scots pine (approximately –1.5 MPa; Irvine et al., 1998). However, the typical xylem water potential threshold value for the onset of cavitation did not hold in our experimental set-up, as the surfactant had altered the vulnerability to cavitation, and thus rapid 'run-away' cavitation could not be prevented.

This experiment showed how the effects of low apoplastic ψ on the xylem and living cells are interconnected. Decreasing apoplastic ψ causes living cells to dehydrate, but it can also trigger embolism formation in the xylem that leading to the capacitive effect, which can temporarily increase the ψ for living cells. The capacitive effect also indicates that steep ψ gradients may persist within the tree because water is removed from one place and moved to another. While photosynthetic rate increased during the capacitive effect, it decreased steeply after it (data not shown). This appears comparable with depression of photosynthesis related to freezing (5.1.2).

5.2.2 Gas burst from the tree stem during freezing (paper II)

CO_2 bursts were clearly detected upon freezing in the laboratory experiments (Figure 1 in paper II). The freezing-related CO_2 burst followed similar dynamics in each of the three repetitions in both studied conifers. It began approximately 5 min after the freezing began and continued for 37 min.

The magnitude of the freezing-related CO_2 burst, i.e. the integral of the difference between measured CO_2 efflux and the modelled CO_2 release (Figure 1a in paper II) varied from 177 to 1003 mmol m^{-2}. The estimates of the fraction of freezing-related CO_2 burst from the stem ranged between 27 and 96% of the total CO_2 content within the stem (based on using the model described in the Materials and Methods section). On average, 71% of the CO_2 within the stem before the onset of freezing was predicted to be expelled from the stem during freezing.

The freezing-related CO_2 burst from the stem was also clearly visible in the field measurements (Figure 3 in paper II). The size of the burst in the field averaged 5276 mmol m^{-2} and lasted for nine hours. Similarly as in the laboratory experiments, stem CO_2 efflux fell close to zero after the burst. Clear CO_2 bursts related to thawing were also visible in most cases, as the CO_2 efflux was considerably high (in relation to temperature) right after thawing (Figure 3a,c in paper II).

During freezing, ice spreads rapidly within trees (Kitaura 1967; Hacker and Neuner 2007; Pramsohler et al. 2012), which can be assumed to concentrate the dissolved gases in front of the moving ice front (Sevanto et al. 2012), creating a large concentration difference between the gas within the conduits and the gas in the inter-conduit spaces and further in the ambient air. This increased concentration difference can be expected to accelerate the diffusion of gases out from the stem until ice has spread throughout the entire stem. The amount of gases trapped within the xylem conduits is crucial for the size of the bubbles formed during freezing (Sperry and Sullivan 1992; Davis et al. 1999; Pittermann and Sperry 2003, 2006). The likelihood of winter embolism during thawing should decrease if gases are able to diffuse out from the conduits during the freezing process.

The gas burst from the stem may also be detectable with stem diameter change measurements because gas pressure can increase inside the stem during the burst, which can cause the stem diameter to expand. The temporary expansion of phloem diameter after ice nucleation can be seen in the results from paper III (Figure 6) which may be caused by increase in pressure of gas passing through the phloem. If the gas burst is detectable with stem dimeter change measurements then the increase in pressure inside the stem can also be estimated based on the stem diameter change if the elasticity of the stem is known. This is because diameter change and change in pressure inside the stem are linearly related according to Hooke's law (Irvine and Grace 1997).

Further studying of the relationship between tree water status and the freezing-related gas burst would be interesting. Trees in better water status may be able to benefit more from the freezing related gas burst in terms of the reduction in winter embolism formation. Better water status means more water and less gases, and therefore the volume expansion of water during freezing may be able to expel a larger proportion of gases out from the xylem. If this is true then climate change may increase winter embolism in areas where trees experience more droughts in the autumn and undergo freezing in poor water conditions.

Pressure can change rapidly in the xylem conduits due to two phenomena; the capacitive effect and freezing. The mechanism causing the pressure increase is different in the two phenomena in that gas and water have contrasting roles. The capacitive effect involves an increase in water pressure in the xylem conduits due to the spread of embolism and an increasing amount of gas. During freezing, however, the phase change of water increases the water volume which, leads to an increase in gas pressure within the xylem vessels. While a decrease in apoplastic water potential typically increases the dehydration stress of living cells, the capacitive effect causes discontinuity in this relation. When a decrease in apoplastic ψ lead to embolism formation, it was shown to trigger the capacitive effect that expels the remaining water into the surrounding tissue, increasing its water content and water potential. Low apoplastic ψ not only causes rapid pressure changes, but can also cause a significant movement of water within the tree. Water movements typically become slower in trees when the water potential decreases because this decreases the hydraulic conductivity of the xylem. This also shows how the effects of low apoplastic ψ are interconnected between the xylem conduits and living cells. The capacitive effect releases water from the xylem for living cells that would otherwise be unavailable to them. Both these phenomena likely have high ecological significance: the capacitive effect reducing water stress in living cells during periods of drought, or even during a diurnal cycle if the xylem conduits embolize during the day and refill the embolisms during the night, and the freezing-related gas burst helping trees in spring recovery.

6 CONCLUSION

The motivation of my PhD was to understand the implications of low apoplastic ψ in trees and for living cells and for embolism formation in the xylem. Regarding living cells, particular attention was given to how low apoplastic ψ affects parenchyma cells in the xylem and mesophyll cells in the leaves. The role of living parenchyma cells in xylem diameter changes have received little attention in the past and their role has been believed to be marginal. Parenchyma cells were studied with xylem diameter change measurements. This thesis showed that parenchyma cells do have a significant effect on xylem diameter change. Dehydration of the parenchyma cells can explain the shrinking of xylem diameter, particularly when a tree freezes.

While low apoplastic ψ causes dehydration of living cells, low apoplastic ψ can also affect photosynthesis in the mesophyll cells. Depression of photosynthesis is known to occur at below-zero temperatures, while the mechanism behind this depression is not well understood. High temporal resolution leaf gas exchange measurements were used to reveal how the depression of photosynthesis occurred concurrently as freezing occurred and the stem diameter began to shrink strongly, indicating the dehydration of living cells due to a sudden decrease in ψ in the apoplast. The results suggest that the mechanism causing the depression of photosynthesis acted within the mesophyll cells because stomatal closure and blockage of CO_2 diffusion by ice in the apoplastic space were ruled out as the primary mechanism.

The aim of this thesis was also to study whether rapid pressure changes in the xylem vessels can occur due to the capacitive effect of embolism and due to freezing. A rapid change in pressure was experimentally shown to occur in the two cases, but the implications for living cells are different. Implications of the capacitive effect are that when apoplastic ψ decreases in a tree during drought, the xylem can temporarily help to increase the water status of living cells, which can particularly help large trees in surviving even long periods of drought. This thesis also experimentally revealed that a CO_2 gas burst occurs from the stem is most likely related to ice propagation when tree freezes. The gas burst from the stem may have high ecological significance in helping trees to reduce the risk of winter embolism.

Stomatal control is important for trees to prevent the water pressure from decreasing too low in the xylem. This prevents the formation of excessive embolism that decreases water conductivity in the xylem. Whether trees can sense embolism directly in the xylem conduits has previously been unknown. Experimental evidence from this thesis suggests that trees sense embolism only indirectly by the effect it mediates on the ψ of leaves. Trees in the experiment were unable to control the spread of embolism when the surface tension of water was artificially decreased, but instead responded by closing their stomata at similar ψ as in natural conditions.

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