Dissertationes Forestales 392

How do trees adjust water-conducting cells with distance from the treetop – possible effects of growing conditions on hydraulic architecture

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Academic dissertation

To be presented, with the permission of the Faculty of Agriculture and Forestry of the
University of Helsinki, for public examination,
in Auditorium B2, Forest Sciences Building (Viikki Campus, Latokartanonkaari 7,
Helsinki)
on 5th June 2026, at 12:00

Title of dissertation: How do trees adjust water-conducting cells with distance from the treetop – possible effects of growing conditions on hydraulic architecture

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Dissertationes Forestales 392
https://doi.org/10.14214/df.392

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ISSN 1795-7389 (online)
ISBN 978-951-651-874-2 (pdf)

Publishers:
Finnish Society of Forest Science
Faculty of Agriculture and Forestry of the University of Helsinki
School of Forest Sciences of the University of Eastern Finland

Editorial Office:
Finnish Society of Forest Science
Viikinkaari 6, 00790 Helsinki, Finland
https://www.dissertationesforestales.fi

Held, M. 2026. How do trees adjust water-conducting cells with distance from the treetop – possible effects of growing conditions on hydraulic architecture. Dissertationes Forestales 392. 47 pp. https://doi.org/10.14214/df.392.

ABSTRACT

In trees, xylem conduits transport water from roots to leaves. Conduit walls represent resistance to water transport, which accumulates with transport pathway length. To counteract, conduit dimensions increase with distance from the treetop ($d_{treetop}$). Water travels between conduits *via* pits. Little is known about the scaling of pits along the water transport pathway. Larger conduits and pits conduct more water but are more vulnerable to hydraulic failure. Furthermore, conduit formation is sensitive to environmental conditions, which may affect conduit numbers and dimensions.

This thesis aimed to a) bring evidence on within-tree variation and coordination of conduit and pit dimensions, particularly their scaling along the water transport pathway, b) explore interspecific differences, and c) better understand effects of environmental conditions on the timing of conduit formation phases and on conduit and pit dimensions.

We analyzed conduits and pits from inner to outer tree rings at breast height and from treetops to coarse roots in outer rings in Scots pine, Norway spruce, and silver birch on sites with different growing conditions. We also analyzed the timing of conduit formation phases in pine and spruce along a latitudinal gradient. We used light and scanning electron microscopy to study conduits and pits.

Conduit and pit dimensions scaled with $d_{treetop}$ indicating that their coordination along the water transport pathway is crucial for the survival of both conifers and angiosperms. Scaling but not coordination deviated in roots. In conifers, pit functional properties differed between sites and species, indicating that the relative size of torus and pit aperture may be particularly important for hydraulic safety. Lower latitudes and warmer years enabled longer conduit formation phases and a greater number of conduits. Thus, global warming may enhance wood production in boreal forests until another factor than temperature becomes limiting.

Key words: *Betula pendula*, inter-conduit pits, *Picea abies*, *Pinus sylvestris*, wood formation, xylem conduits

ACKNOWLEDGEMENTS

The trail of completing this thesis was filled with ups and downs, and alone I would have got lost in the woods. First and foremost, I would like to thank my supervisors, Anna Lintunen and Tuula Jyske, who have guided me wisely from the successful applications to both study program and funding to thesis completion and defense. Anna warmly welcomed me as her PhD student and has since been a great mentor for anything related to scientific research, but also to Finnish culture. I remember one of the first things I learned from you was how to enjoy sauna. Tuula often spotted small but important unclarities or errors in manuscripts.

I would like to express gratitude to my thesis committee members Kurt Fagerstedt and Eija Jokitalo. In our meetings, Eija gave me refreshing feedback from her non-tree researcher perspective and I learned to adjust the background information of my presentations. I am also very grateful that she offered an open ear for issues related to work-wellbeing. I thank Kurt for providing the cryo-microtome, with which I prepared some of my samples, and both him and Olga Blokhina for teaching me how to use it. I would like to thank Harri Mäkinen for providing the dataset for my third paper, contributing to the analysis, and being a helpful co-author for polishing the text. I would like to thank the pre-examiners Elina Oksanen and Georg von Arx for their valuable comments on my thesis, which helped to improve the clarity.

I would like to thank the staff at SMEAR II, particularly Silja Vuorenmaa for providing the tree corers and other tools, Juho Aalto for cutting the sample trees for me, and the kitchen, cleaning, and janitorial staff for making work at the station enjoyable. I thank the mosquitos and moose flies for giving me very authentic field work experiences.

I am grateful that I received the salaried position of the ATM-DP for four years, and would like to thank Anna Lintunen, Jaana Bäck, and Timo Vesala for covering my funding for the remaining time.

As my studies of wood anatomy started in Innsbruck, I would like to thank my master thesis supervisors Stefan Mayr and Walter Oberhuber for enabling me to explore the microscopical secrets of wood. I want to thank Werner Kofler, Adriano Losso, Andrea Ganthaler, Barbara Beikircher and Birgit Dämon for guiding me through the first steps of using microtome, microscopes, and ImageJ. I want to thank Holger Gärtner and other teachers at the wood anatomy autumn school in Klosters, where I gained further microtome skills and knowledge about wood anatomy.

I thank my colleagues and friends for walking various parts of the path from Bachelor to PhD with me, for sharing tips and tricks, deep conversations, and experiences, for the adventurous travels, birding and biodiversity excursions, and numerous rounds of games, which I will keep in good memory.

I am sending gratitude to the coaches and members of Focus Revolution for support, accountability, and community spirit. Without you, I would not have completed my thesis yet. You enabled me to keep momentum during parts of my work that I dread, and taught me to value breaks, movement, and creative activities as part of my work progress. I had fun during office work due to fancy headbands, and I learned how to cope during dark times.

Last but not least, I would like to thank my family. Without you, this journey would not have been possible. I thank my mother and my father for giving me their unlimited love and support, even when they do not fully understand my choices and problems, my grandmother for sparking my enthusiasm for trees when I could barely walk, my godmother for exchanging heartfelt letters, my cousin for meaningful conversations, and my sister for her warmheartedness, and for understanding me deeper than anyone else could. Finally, I would like to thank my nephews for many cheerful moments, but also for challenging me to find simple but interesting ways to share my knowledge, and for giving me a reason to continue.

LIST OF ORIGINAL ARTICLES

This thesis is a summary of three research articles, which are re-printed after the summary section. In the summary, these articles are referred to by the roman numerals I – III.

I Held M., Ganthaler A., Lintunen A., Oberhuber W., & Mayr S. (2021) Tracheid and pit dimensions hardly vary in the xylem of *Pinus sylvestris* under contrasting growing conditions. Frontiers in Plant Science 12: 786593. doi: 10.3389/fpls.2021.786593.

II Held M., Jyske T., & Lintunen A. (2026). Conduit and pit dimensions scale in a coordinated way from the treetop to coarse roots in three boreal tree species. Tree physiology 46. doi: 10.1093/treephys/tpag003.

III Held M., Lintunen A., Jyske T., & Mäkinen H. (under review). Duration of wood formation phases in Scots pine and Norway spruce lengthens towards the south in Nordic countries. Dendrochronologia.

AUTHOR’S CONTRIBUTION

The author conceived Study I and II with the co-authors. She also conducted the sample collection in the field, sample analysis in the laboratory, statistical analysis, and writing under guidance of the co-authors. In study III the author conducted the statistical analysis and wrote the manuscript under guidance of the co-authors.

TABLE OF CONTENTS

1. BACKGROUND

Trees are woody plants with life-long primary and secondary growth. Primary growth is the elongation of stems (incl. branches) and roots by cell division in apical meristems, whereas secondary growth is the radial thickening of stems and roots originating from cell division in the cambium, which allows trees to form new layers of wood (xylem) and living bark (phloem). Trees and forests provide many ecosystem services. The secondary growth of trees produces wood, a much-needed material for human society. Wood formation sequesters carbon from the atmosphere, as the walls of the wood cells are made of cellulose, hemicelluloses, and lignin, and thus may help to slow down global warming.

For trees, the ability to grow both in length and in diameter has many implications. Some tree species can grow stems over 100 m tall. Tree xylem needs to transport water and nutrients all the way from the soil to the treetop and distal leaves (Sperry et al., 2008), where it is needed for photosynthesis, growth, and thermoregulation (e. g., Kramer and Kozlowski, 1960; Michaletz et al., 2015). Water transport happens passively through conduits, which are walls of dead cells. It is initiated by transpiration of water from stomata in leaves, which then pulls up the chain of water molecules that connects the leaves with the roots and soil *via* the xylem (Cochard, 2006; Dixon and Joly, 1895; Venturas et al., 2017). When the leaves transpire, the water column in the xylem is under tension, which makes it susceptible to hydraulic failure, i. e., embolism (Cochard, 2006; Dixon and Joly, 1895).

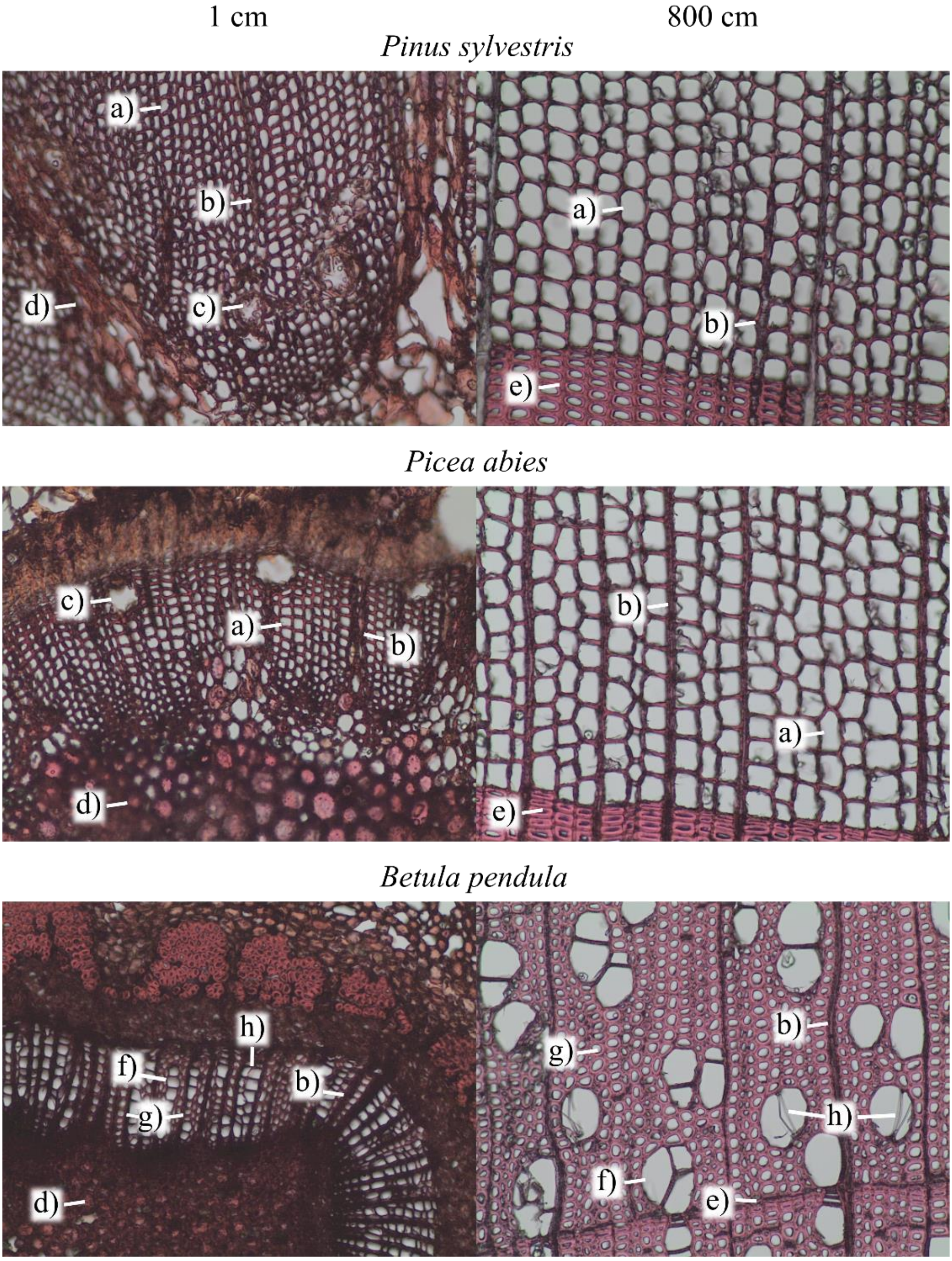
The primary growth increases the length of stems and roots, i. e., the water pathway length. To compensate the accumulating hydraulic resistance caused by conduit walls, the new xylem conduits produced by secondary growth need to adjust to their location along the water pathway length, so that a sufficient and safe water supply to all living cells is ensured (e.g., Anfodillo et al., 2013; Olson et al., 2021). This becomes further complicated when taking into account environmental conditions. For example, light, temperature, and water and nutrient availability can affect both tree growth and the tension of the water column in the wood cells.

The potential adjustability of tree growth and xylem ultra-structures to environmental conditions may be of critical importance for the productivity and survival of trees under climate change.

1.1 Structure of xylem conduits and inter-conduit pits

Xylem conduits are pipe-shaped walls of dead cells, made from cellulose and lignin (Cochard, 2006). Conifers, like our study species *Picea abies* and *Pinus sylvestris*, have only one conduit type in their xylem, tracheids (Figure 1), which are unicellular and thus restricted in their dimensions to up to 15 mm length (Kramer and Kozlowsky, 1960). Tracheids are responsible for both water transport and mechanical support of the tree.

Angiosperms, like our third study species *Betula pendula*, have several types of conduits. Vessels are multi-cellular, i. e., several cells stacked on each other, and in the case of *B. pendula*, separated by scalariform perforation plates (Figure 1 and 3). Vessels can thus be up to several meters long (Cochard, 2006), and their function is water transport. Besides vessels, many types of imperforate tracheary elements (*ITE*; Sano et al., 2011) occur in the xylem of angiosperms (Olson, 2023). *ITE* are similar to tracheids of conifers; unicellular and lack perforation plates. They are thus shorter and thinner than vessels, though the ranges of their



1 cm 800 cm

Pinus sylvestris

Picea abies

Betula pendula

Figure 1. Example images of xylem cross sections of *Pinus sylvestris*, *Picea abies*, and *Betula pendula* at 1 and 800 cm distance from the treetop. Indicated structures are a) tracheids, b) rays, c) resin ducts, d) parenchyma, e) latewood (i. e., radially flattened conduits), f) vessels, g) fiber-tracheids, h), scalariform perforation plates. All images have the same magnification.

dimensions can overlap (Figure 1). Their main task is mechanical support, though some types of *ITE* may contribute to water transport (e. g., Olson, 2023; Sano et al., 2011). The type of *ITE* found in *B. pendula* are fiber-tracheids (commonly called fibers), which are usually non-conductive (Olson, 2023). However, we do not exclude the possibility that some *ITE* in *B. pendula* contribute to water transport.

In both conifers and angiosperms, the length of one conduit is finite. Thus, to fulfill their purpose of water transport, the conduits are connected *via* bordered pits. However, pits represent a major part of the resistance to water flow within the xylem (Choat et al.,2008; Domec et al., 2006).

A bordered pit consists of two borders, i. e., oval cell-wall protrusions in each of the connected cells, with an aperture, and a pit membrane that is located in between the two pit borders (Figure 2). Conifers and angiosperms exhibit contrastingly different pit designs. First of all, the pits of conifers are larger, in study II by approximately an order of magnitude. Furthermore, in the conifer species *P. sylvestris* and *P. abies*, the pit borders and apertures are usually oval, whereas in the angiosperm species *B. pendula*, the pit apertures are elongated (Figure 2; Carlquist, 2001). Lastly, the pit membrane of our conifer species has a central thickening, the torus, which is relatively air-tight and can seal off the pit aperture, and an outer web-like part, the margo, which has large pores (Figure 2; Hacke et al., 2004; Jacobsen, 2021). Angiosperms like *B. pendula* exhibit a homogeneous pit membrane which is a sponge-like matrix made up of many layers, and thus the membrane pores are intricate networks of constrictions (Figure 3, 4; Kaack et al., 2019; Kaack et al., 2021; Zhang 2019).

1.2 Function of xylem conduits and inter-conduit pits

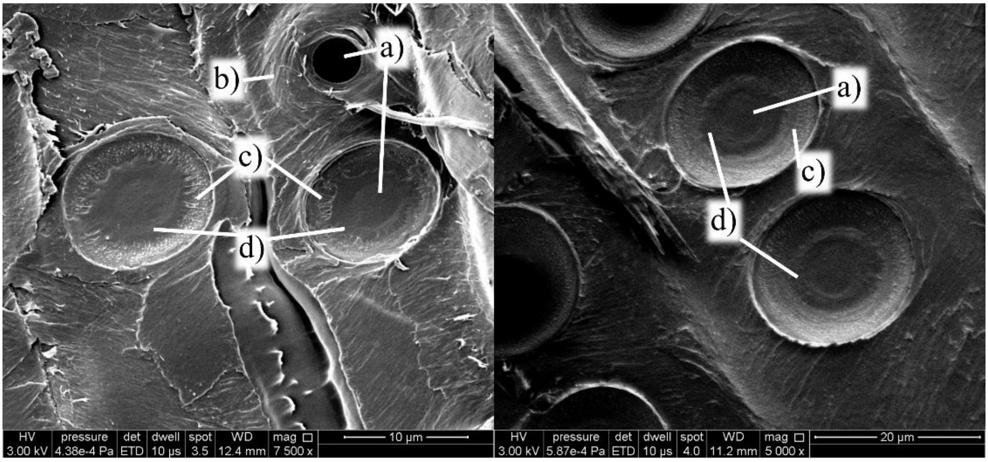
The hydraulic efficiency and safety are dependent on the number and dimensions of conduits, pits, and pit ultra-structures. According to the Hagen-Poiseulle's law, water flow changes with the 4^{th} power of diameter (Sperry and Hacke, 2004; Zimmermann, 1983). Thus, larger conduits, pit membrane pores and pit apertures enable higher water flow. However, larger conductive structures also facilitate the spread of embolism, i.e., air bubbles blocking water transport. The relationship between conduit diameter and vulnerability to embolism is likely indirect. Furthermore, the role of conduits and pits in embolism vulnerability differs depending on the environmental conditions causing embolism, i.e., between freeze-thaw included embolism and drought induced embolism.

During freezing, gasses that were dissolved in the liquid water form bubbles (Hammel, 1967). If these bubbles are large enough, they will embolize the conduit if a large enough tension is developed during thawing (Sperry & Sullivan, 1992). Thus, the higher vulnerability of wider conduits towards freeze-thaw embolism is possibly due to larger gas volumes dissolved in the water prior to freezing, which then form larger bubbles upon freezing (Pittermann & Sperry, 2003, 2006; Sperry & Sullivan, 1992). However, freeze-thaw induced embolism is not yet fully understood as recent evidence from micro-CT shows (Lintunen et al., 2022; Charra-Vaskou et al., 2023).

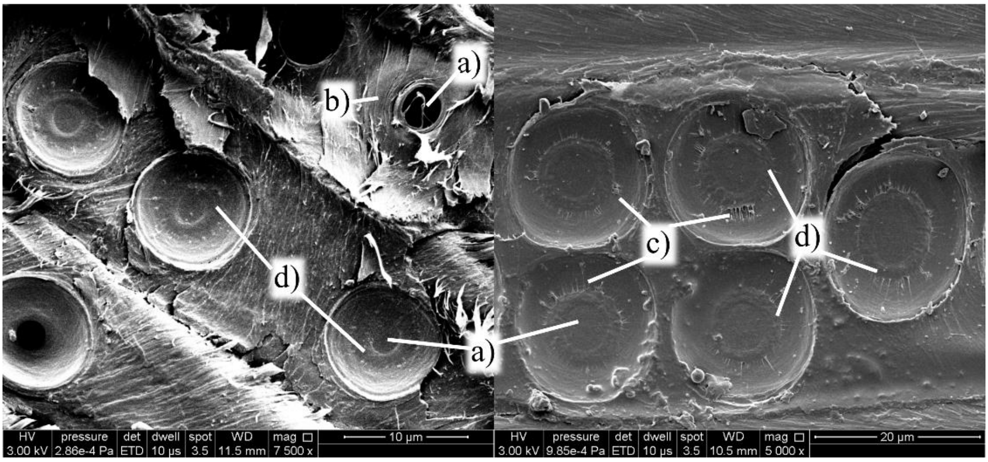
Drought-induced embolism happens when the tension in the water column of a conduit becomes so high that it ruptures. One way this could happen could be due to a collapsing conduit wall (Hacke et al., 2001). Thus, one factor for hydraulic safety against drought-induced embolism is the ratio of double cell wall thickness to conduit diameter (e.g., Bouche et al., 2014; Echeverría et al., 2022; Hacke et al., 2001). Another very important way of drought-induced embolism happens by air bubbles seeding from a previously embolized

1 cm 800 cm

Pinus sylvestris



Picea abies



Betula pendula

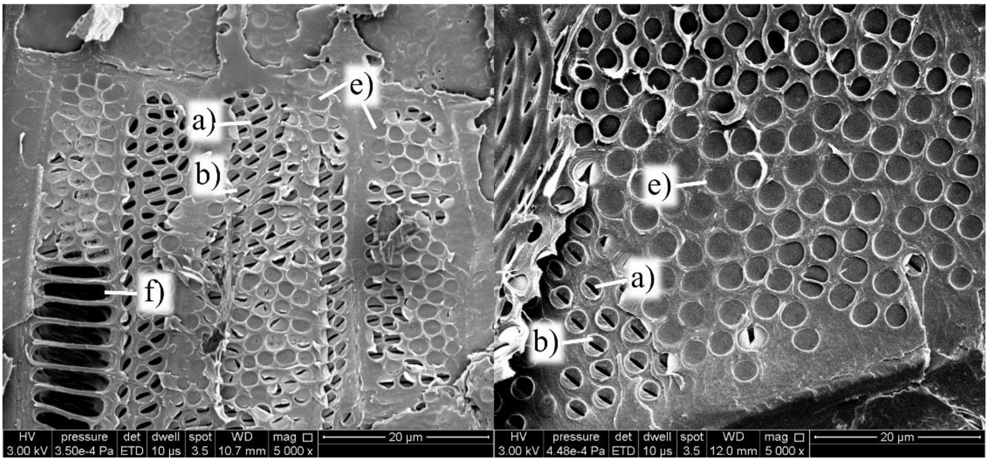


Figure 2 (facing page). Example images of xylem radial sections of *Pinus sylvestris*, *Picea abies*, and *Betula pendula* at 1 and 800 cm distance from the treetop. Indicated structures are a) pit aperture, b) pit border, c) margo (web-like part of pit membrane in conifers), d) torus (central thickening of pit membrane in conifers), e) homogeneous pit membrane (typical for angiosperm pits), f) scalariform perforation plate (typical for some angiosperms). Note that images *Pinus sylvestris* at 1 cm and *Picea abies* at 1 cm were taken at a higher magnification than the other images.

conduit into a water transporting one through the pits. The air bubble will then expand and block also this conduit from further water transport. Therefore, a possible link between drought-induced embolism and conduit diameter is the rare-pit hypothesis. Larger conduits likely have bigger (Zambonini et al., 2024) and or/more pits, and thus a higher likelihood to have a leaky pit pore, or an increased interface for air-transport between conduits (e. g., Guan et al., 2021; Jacobsen et al., 2019; Larter et al., 2017; Plavcová et al., 2013; Wheeler et al., 2005).

In both conifers and angiosperms, pits are designed to allow high water flow between functional conduits while preventing air seeding when one conduit is embolized. Though the functional groups are dealing with the efficiency-safety tradeoff in very different ways.

In conifer pits, the pit aperture and large margo pores enable high water flow between two functional conduits. If one conduit is embolized, the pit membrane moves towards the border of the functional conduit, a passive movement due to the pressure difference between the air-filled and the functional conduit, and the torus seals off the pit aperture against air seeding. However, if the pressure difference between the conduits gets too high, the seal breaks. Several ways of air-seeding through aspired conifer pits have been proposed (Cochard, 2006; Jansen et al., 2012). The air-tightness of the pit seal likely depends on the relative sizes of the margo, torus, and pit aperture, as well as on the smoothness of the pit border surface and possible pores in the torus (Bouche et al., 2014; Delzon et al., 2010; Jansen et al., 2012). A large margo relative to the torus (margo flexibility) makes the pit membrane more flexible and thus allows the torus to aspire properly, and a large torus relative to the pit aperture increases the tightness due to a higher overlapping surface (torus overlap; Delzon et al., 2010; Hacke et al., 2004). Delzon et al. (2010) found a high correlation of the valve effect (margo flexibility x torus overlap) and hydraulic safety.

Also in angiosperm pits, pit aperture size and the number and sizes of pit pores increase water flow. As the pressure difference necessary for air to seed from an embolized to a functional conduit is lower for larger pores (Mayr et al., 2014; Zimmermann, 1983), the hydraulic safety of an angiosperm pit may depend on the pore with the largest minimum restriction within the membrane layers. The likelihood of a large minimum pore restriction is affected by the pit membrane thickness (i. e., the number of membrane layers and thus restrictions in a pore), the number of pores in the pit membrane, and the pit membrane area (Kaack et al., 2021; Wheeler et al., 2005). The likelihood of a leaky pit within a vessel possibly depends on the number of pits per vessel or the pit membrane area per vessel area (i. e., again, likelihood of a leaky pore; Kaack et al., 2021; Wheeler et al., 2005). It is unknown if the pit aperture size plays a role in hydraulic safety of angiosperm pits.

Another factor for hydraulic safety in angiosperms like *B. pendula*, is the vessel lumen fraction (*VLF*). The *VLF* tells how much of a xylem cross sectional area is occupied by vessels (*versus* other cell types including fiber-tracheids; Avila et al., 2023). A high percentage of vessels means that there are larger and/or more vessels. Thus, the vessels

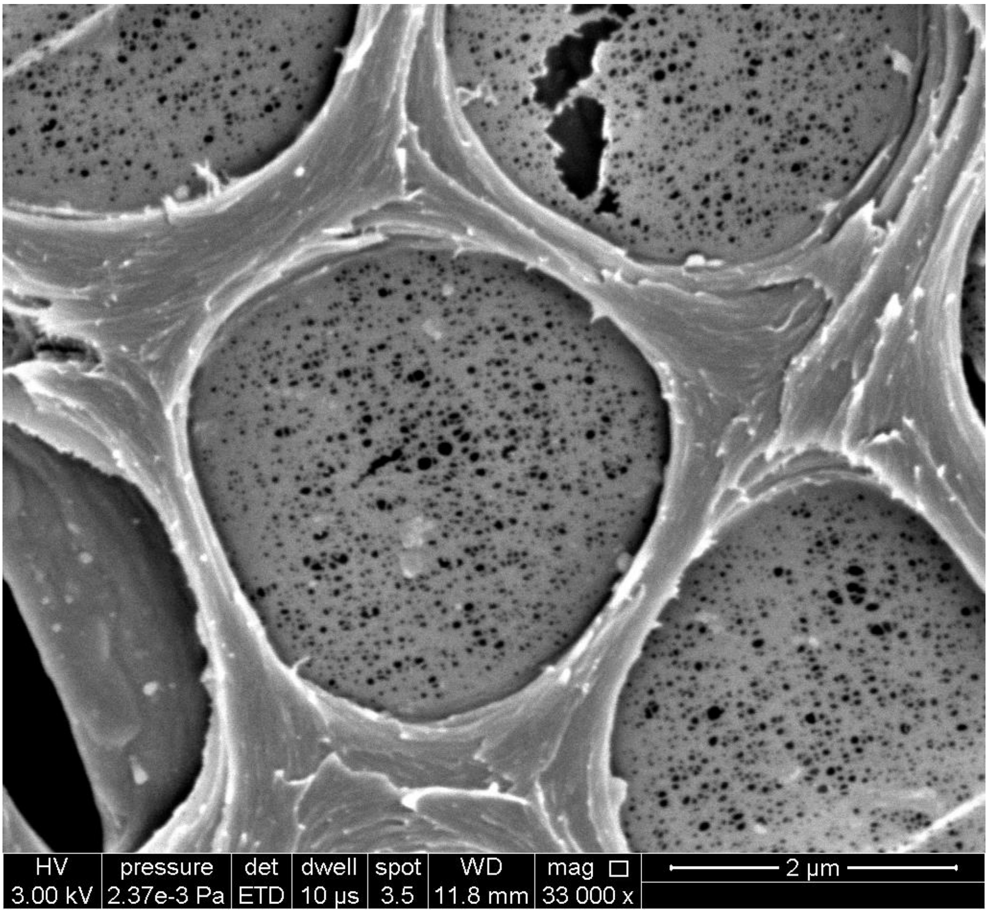


Figure 3. Close up of a pit membrane in *Betula pendula* showing pit membrane pores.

occupy more xylem area, and the probability that they are occurring in groups is higher. Therefore, a higher *VLF* is beneficial for hydraulic efficiency but may also decrease hydraulic safety. If vessels are often grouped, there are more pits and a higher likelihood of a leaky pit. If vessels are not directly connected, both water and air would need to cross fiber-tracheids to get from one vessel to the next (Avila et al., 2023; Cai et al., 2014; Guan et al., 2021). If there are low-conductive fiber-tracheids, they would be a low-efficiency but high-safety alternative to connect vessels rather than directly *via* pitted end-walls (Sano et al., 2011).

1.3 Scaling of xylem structures with distance from the treetop

Conduit walls present resistance to water flow (Ryan and Yoder, 1997), but pits can make up over 50 % of the total resistance within the xylem (Choat et al.,2008; Domec et al., 2006). These resistances accumulate as trees grow in height (Ryan and Yoder, 1997). However, trees counteract this pathway length resistance by widening their conduits with distance from the treetop ($d_{treetop}$; e.g., Kiorapostolou et al., 2020; Lazzarin et al., 2016; Lintunen and

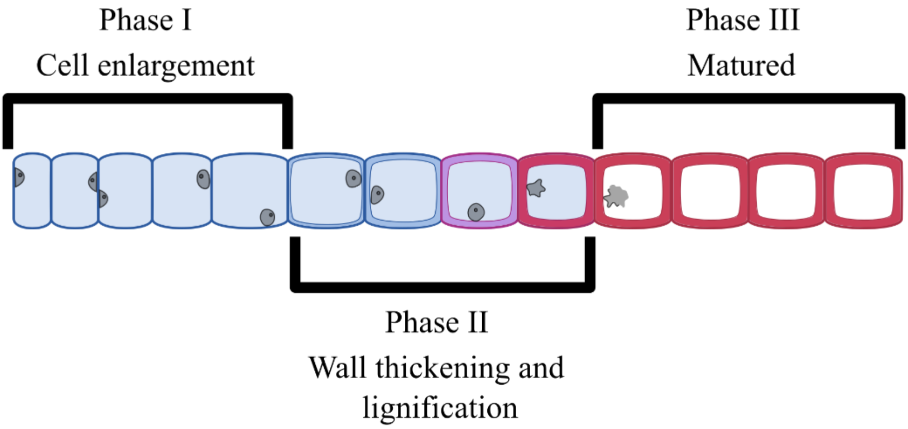


Figure 4. Schematic drawing of a stained xylem cross section indicating conduit formation phases.

Kalliokoski, 2010; Prendin et al., 2018b; Zambonini et al., 2024). Thus, there are small conduits with high hydraulic safety at the treetop, the place with the most negative water potential (Venturas et al., 2017), and larger conduits at the tree base, where high hydraulic efficiency is needed. In spite of the important role of pits for both hydraulic resistance and hydraulic safety, little is known about the coordination of conduit and pit dimensions along the water transport pathway. There are only a few studies on the coordination of conduit and pit traits at different $d_{treetop}$ (Christof et al., 2020; Losso et al., 2018). To our knowledge there are only two studies which researched the scaling of pit dimensions with $d_{treetop}$ in a total of three conifer species (Lazzarin et al, 2016; Zambonini et al., 2024), only one of them exploring inter-specific differences, and none including pit functional properties, which are crucial for hydraulic safety in conifers (Camarero et al., 2026; Delzon et al., 2010). To our knowledge, there is only one study, where the scaling of pit membrane area per conduit was analyzed in one angiosperm species (Kreinert et al., 2025). Furthermore, there is no study exploring the scaling of pits in either functional group including roots.

Furthermore, it is unclear how flexible the scaling pattern of conduit and pit dimension (and thus hydraulic functions of the xylem) is under varying environmental conditions (Petit, 2024), because anatomical and hydraulic traits are often measured without recording the distance from the sample to the treetop or branch apex. Analyzing conduits in xylem samples from breast height may lead to the conclusion that trees in unfavorable conditions for growth have smaller conduits, as they are often shorter than trees in more favorable habitats (Olson et al., 2021).

1.4 Formation of conduits

In temperate (study I) and boreal forests (study II and III), the cambium, i. e., the layer of dividable cells in the stem, has an annual cycle of active and dormant phases due to winter (Schweingruber, 2007). Thus, the tree species in our studies form annual xylem rings in which the conduits formed early in the growing season have wide lumina and thin walls, and those at the end have thin lumina but thick walls (Figure 1; Schweingruber, 2007). The width of the annual rings, i. e., the growth rate, differs between years according to variation of environmental factors, like temperature, moisture, and light (Schweingruber, 1993). However, environmental factors can have different effects on wood growth depending on the season (e.g., Lebourgeois et al., 2010; Pichler and Oberhuber, 2007). Thus, to better understand the effect of environmental conditions on conduit formation, as well as on resulting conduit numbers and conduit dimensions, it is necessary to investigate not only the final tree ring width, but the timing and duration of the different conduit formation phases. Also, environmental conditions may have different effects on shoot elongation and thus on the scaling of conduit and pit dimensions during different conduit formation phases.

Environmental conditions may affect the annual ring width *via* the conduit number and the conduit width. The conduit number is determined by the cell division in the cambium, whereas the size of a conduit is determined during the conduit formation phases that follow cell division, which are 1) cell enlargement, 2) wall thickening and lignification, and 3) matured (i. e., cell death; Figure 4). Both the timing and the rate of each phase respond to internal and external conditions in complex ways. Temperature, light, and water availability are the most important factors influencing the timing and rate of wood formation phases in Northern Hemisphere conifers (e.g., Buttò et al., 2021b; Cuny et al., 2016; Huang et al. 2020; Jyske et al., 2014; Rossi et al., 2016).

Huang et al. (2020) found for Northern Hemisphere conifers that light and temperature are the primary factors for the onset of conduit formation, whereas water availability plays a secondary role. Conduit formation occurs above a certain temperature threshold, which varies between tree species and environments (e.g., Anfodillo et al., 2012; Deslauriers et al., 2008; Jyske et al., 2014). Thus, in spring and early summer, warm temperatures affect cambial activity and phase I positively in temperature-limited regions such as high latitudes and altitudes (Wodzicki et al., 1971; Lenz et al., 2013). Furthermore, phase I needs water to create turgor pressure for cell enlargement (Cosgrove, 1997), potentially affecting conduit diameter. The timing of bud burst matches the onset of xylem differentiation, which means that shoot elongation and phase I are closely related (Buttò et al., 2021a; Fajstavr et al., 2019). Phase II is positively affected by temperature and available carbohydrates, linked to light availability, because cell wall formation is material demanding and requires high enzymatic activity (Cuny et al., 2015; 2016).

Besides the environmental factors that during the growing season, conduit formation responds also to conditions occurring outside the growing season. In winter, warmer temperatures may affect conduit formation in the following spring negatively in species which require cold temperatures to break the winter dormancy (Delpierre et al., 2019). On the other hand, warm temperatures outside the conduit formation period may enable evergreen conifers like *P. abies* and *P. sylvestris* to accumulate sugar through photosynthesis (Jyske et al., 2014; Kulmala et al., 2019), which can be used in the next growing season. To understand the environmental plasticity of xylem formation, and its effects on conduit number and size, we need to investigate the effect of growing conditions on the conduit formation phases.

2. OBJECTIVES

I investigated the dimensions of xylem conduit and inter-conduit pits, from inner to outer tree rings at breast height (Study I), and along the water transport pathway from the treetop to the coarse roots (Study II). Furthermore, I explored the intra-annual growth dynamics of conduit formation (Study III). The research was conducted on mature trees of two conifer species, *P. sylvestris* (studies I – III) and *P. abies* (studies II – III), and one angiosperm species, *B. pendula* (study II). With this thesis I aimed to bring new evidence for the scaling and coordination of conduit and pit dimensions with distance from the treetop in both conifers and angiosperms, and possible differences between species and environments (Figure 5). Furthermore, I aimed to better understand the intra-annual dynamics of conduit formation under varying environmental conditions in conifers (Figure 5).

Study I aimed to a) explore differences in conduit and pit traits between trees from two sites that were of similar height but experienced different growing conditions, and to b) better understand the within-tree variation and coordination of conduits and pits. We hypothesized that (1) trees on the growth limited site had a higher hydraulic safety than trees on the favorable site particularly thanks to adjusted pits, and (2) conduits and pits scale with distance from the treetop, i. e., at breast height from inner to the outer tree rings, increasing the hydraulic efficiency.

Study II aimed to a) shed new light onto the $d_{treetop}$-scaling and coordination of conduits and pits, b) study if the scaling of conduits and pits can adjust to growing conditions, c) explore possible differences in scaling patterns between the studied species. We hypothesized that 1) conduits and pits scale in a coordinated way with distance from the treetop, 2) that site conditions, which cause different growth rates also affect the scaling patterns, at least that of pit functional properties in conifers, and 3) that there are differences in scaling patterns of conduits between angiosperm and conifers, and of pits between the studied conifer species.

Study III analyzed a) how the dynamics of conduit formation phases vary with latitude and temperature sum, and b) the relationship between these dynamics with the final number of conduits and radial increment. We hypothesized that 1) at lower latitudes and higher temperature sums, the onset and cessation of wood formation phases occur earlier and later, respectively, resulting in longer durations, and 2) extended wood formation phases correlate with a higher final number of conduits and radial increment. This study was a follow up to Jyske et al. (2014), who analyzed the onset and cessation of conduit formation across a latitudinal gradient through Finland, but not the dynamics of each conduit formation phase.

3. MATERIAL AND METHODS

3.2 Sites and material

For Study I, we sampled mature *P. sylvestris* trees from two sites situated in the Eastern Alps west of Innsbruck (Austria; Figure 6). Due to different types of rock bed and a precipitation gradient, the sites differed in nutrient and water availability, which was visible as different growth rates of the sampled trees (tree ring width 727 ± 15 mm vs. 2,724 ± 135 mm; $p < 0.05$).

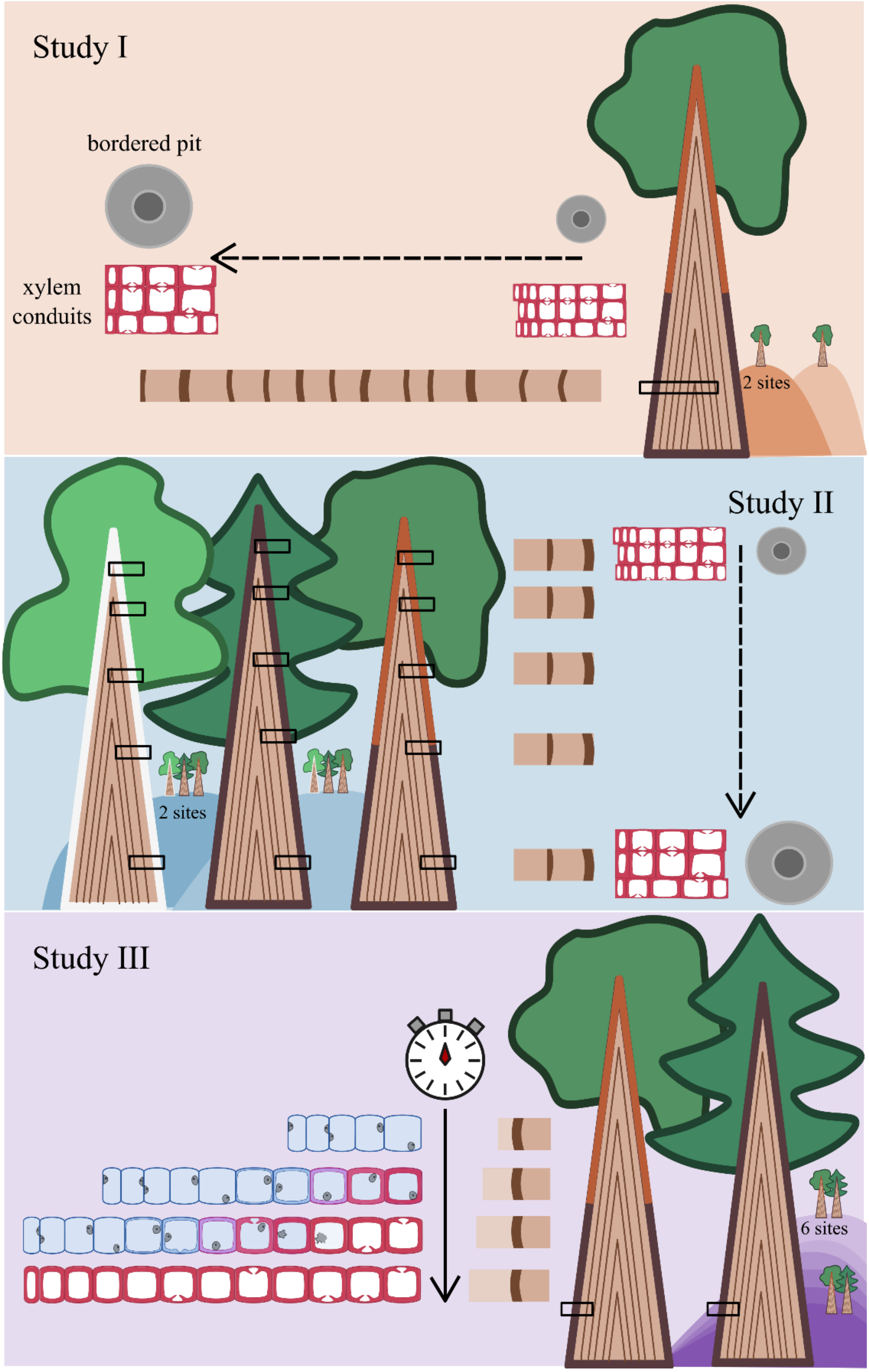

Study I
bordered pit
xylem
conduits
2 sites
Study II
2 sites
Study III
6 sites

Figure 5 (facing page). Conceptual Figure over the three studies included in this thesis. Arrows with dashed lines indicate variation of xylem traits within trees, i.e., from inner to outer tree rings in Study I, and from treetop downwards in study II. The arrow with solid line in Study III indicates temporal changes, i.e., the development of conduits. Black boxes symbolize sampling. Differently shaded hills indicate study sites with different growing conditions.

For study II, we sampled mature trees of *P. sylvestris*, *P. abies*, and *B. pendula* from two sites situated around the Hyytiälä forest station (southern Finland; Figure 6) with slightly different growing conditions as indicated by forest types as classified by Cajander (1949). The growth rate of the trees (measured as basal area increment, BAI) differed between the sites.

For study III, we worked with pre-existing data that were collected from mature *P. sylvestris* and *P. abies* trees growing on six sites along a latitudinal gradient from N 60 ° to 67.5 ° through Finland and Sweden (Figure 6). The sites furthermore differed in altitude from 30 m a. s. l. in the south to 390 m a. s. l. in the northernmost site.

3.2 Sampling design

For Study I, we chose ten *P. sylvestris* trees of similar height per site and sampled two increment cores per tree at breast height (1.3 m; Figure 5) from the south facing site of the trunk in late 2017 and early 2018. After analyzing the tree ring widths, we extracted every 5th to 10th tree ring, moving from the most recently formed tree ring inwards, for microscopical analysis of conduit and pit dimensions.

For Study II, we selected 4 trees per species and site to sample tree cores at breast height and measured the distance of those samples from the treetop in 2020. In 2021, we felled three pines, one spruce and one birch per site. From those trees we took xylem samples (stem segments, root segments, or increment cores) at several positions along the water transport pathway from the treetop to the coarse roots (Figure 5). In the stem, we sampled at distances 1 cm, 10 cm, 1 m, 4 m and 8 m from the treetop. See the publication for more details on the samples. We extracted the outermost tree rings, for which the distance from the concurrently formed treetop was known, for microscopical analysis of conduit and pit dimensions.

For study III, micro-cores were sampled at breast height with regular intervals throughout the growing season (Figure 5) for two to six years (2007 – 2012) on each site. The interval differed between sites, years, and seasons (early growing season *vs*. late growing season). From these micro-cores, cross sections were produced for microscopical analysis of conduit formation phases.

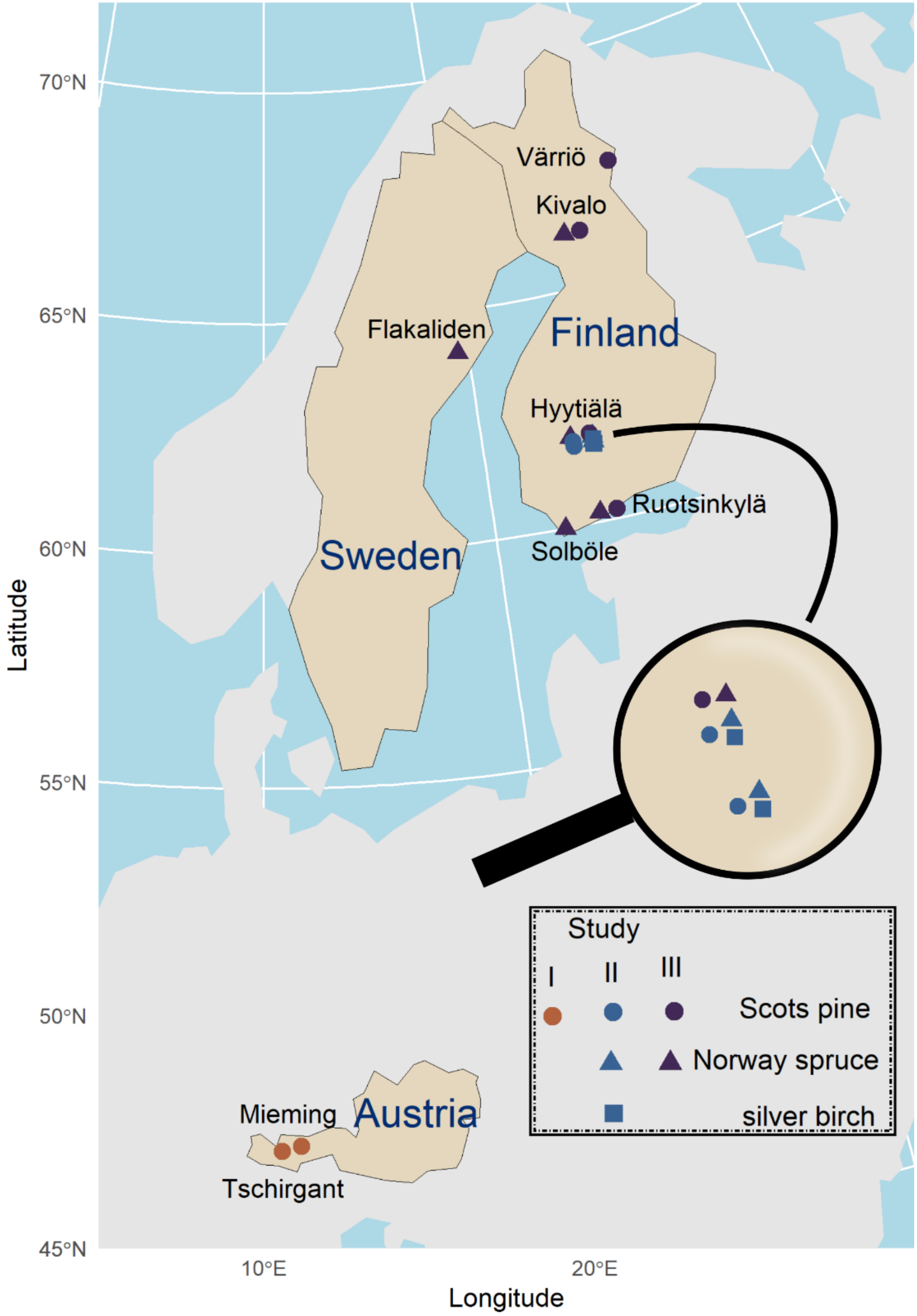


Figure 6. Map of study sites.

3.3 Analysis of conduit and pit dimensions

For both Study I and Study II, we used the xylem samples to prepare cross-sections for conduit analysis using light microscopes and radial sections for pit analysis using scanning electron microscopes (SEM). In study I, we produced cross-sections of 30 µm thickness using a sledge microtome (Sledge Microtome G.S.L. 1, Schenkung Dapples, Zurich, Switzerland), and in study II we used a cryo-microtome for the samples from 2020 (Cryostat Leica CM 3050 S, Deer Park, IL, USA) and a sledge microtome (WSL Lab-Microtome, Schenkung Dapples, Zürich, Switzerland) for the samples from 2021. After cutting, we stained the cross-sections with Etzold's or safranin solution to increase the contrast between conduit walls and lumina. Finally, we mounted the sections onto microscopic slides using water, and in study II, sealed the cover glass with nail-polish, before taking pictures using a light microscope (Study I: Olympus BX41, Olympus Austria, Wien, Austria; Study II: Olympus CX31, Olympus Optical Co., Tokyo, Japan) at 10 x magnification attached to a digital camera (Study I: ProgRes CT3, Jenoptik, Jena, Germany; Study II: Olympus SC30, Olympus Optical Co., Tokyo, Japan). The pictures taken for study I had a size of 2048 x 1536 pixels and a resolution of 3.1 pixels/µm. Those for study II had a size of 1024 x 768 pixels and a resolution of 1.58 pixels/µm.

Then, we used the software image-J 1.45 or 2.14.0 (National Institutes of Health, Bethesda, MD, United States) for image analysis. We selected a minimum of five representative radial files within the xylem ring and measured the conduit lumen areas of all conduits (Figure 7), i.e., including earlywood and latewood, and, in birch (study II), including both vessels and fiber-tracheids. From the conduit lumen areas, we calculated the mean conduit diameter, the hydraulic mean conduit diameter (D_h) as $\Sigma d^5/\Sigma d^4$, where d_c is the diameter of each analyzed conduit (Sperry and Hacke, 2004). The D_h is an important trait for indicating the hydraulic efficiency of a xylem sample cross section, because it divides the conduits of a xylem sample into 50 % share of the hydraulic conductance, i.e., half of the water is conducted by conduits wider than the D_h (Sperry and Hacke, 2004). Also, the pressure causing a 50 % loss of hydraulic conductivity is the air seeding pressure of a conduit of D_h (Sperry and Hacke, 2004). In *B. pendula*, we additionally measured the mean vessel diameter, mean fiber-tracheid diameter, and vessel-lumen-fraction (VLF) as $\sum \mathrm{v_a}/\mathrm{xylem_a} * 100$, where $\mathrm{v_a}$ is vessel area and $\mathrm{xylem_a}$ is the analyzed xylem area (Avila et al., 2023). Then, in samples of all species, we measured the conduit diameter (*B*) and double cell wall thickness (*T*) from up to 10 conduits within D_h ± 4 – 6 µm in conifer samples and D_h ± 5 – 40 µm in *B. pendula* samples (Figure 8) and calculated the cell wall reinforcement as $(T/B)^2$ (Hacke et al., 2001).

For preparing the radial surface of the sample (needed for pit analysis), we used the same microtome as for conduit analysis to produce cuboid sections of approximately 5 * 5 * 2 mm in study I. In Study II, we used the cryo-microtome to produce similar sections for some of the samples, while for the remaining samples, we used hammer and blade, to expose more pit membranes (Jansen et al., 2008). If the wood samples were large enough), we produced cuboids as in study I, but in the case of treetops and small roots, the dimensions of the sections differed. In each case, we smoothened both radial surfaces of the cuboids and mounted them onto aluminum studs. We applied conductive glue to the cross-sectional edges of the samples, and after drying, sputtered them with gold (study I) or platinum (study II). In study I, we measured the pit dimensions directly with the SEM (SEM model XL 20, Philips, Amsterdam, Netherlands) and noted the values manually and could only take a few example images. In

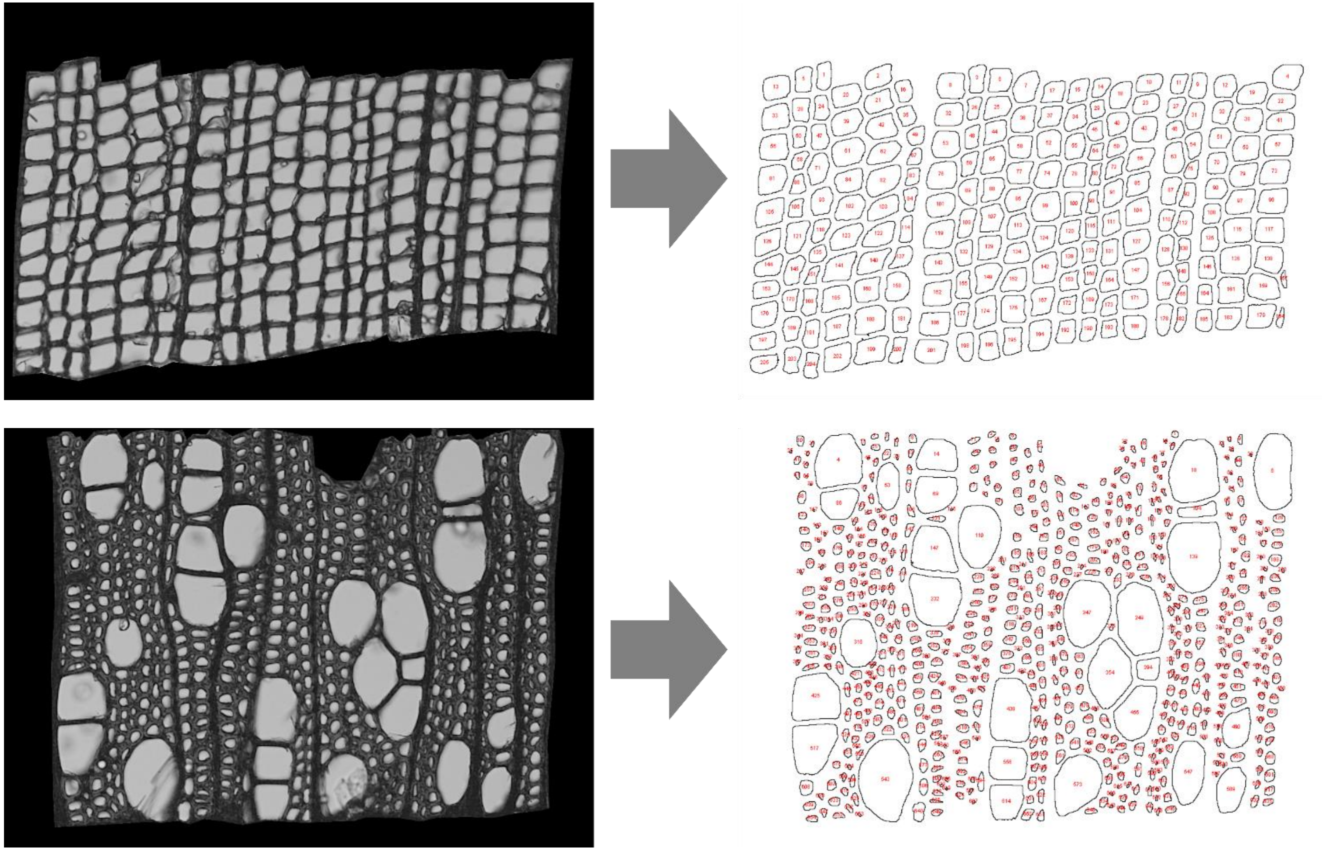


Figure 7. Xylem cross sections of *Picea abies* (representing both studied conifer species) and *Betula pendula*, and respective drawings of measured conduit lumen areas, from which the mean conduit hydraulic diameter was calculated

study II, we took images with the SEM (Quanta FEG250 SEM, FEI^{TM}, Hillsboro, OR, USA) for later measurement of the pit dimensions.

As for conduit analysis we used image-J to measure the pit dimensions. In the conifer samples, we measured the diameters of the margo (D_m), torus (D_t), and pit aperture (D_a). From these we calculated the functional properties margo flexibility ($F = (D_m - D_t) / D_m$), torus overlap ($O = (D_t - D_a), / D_t$) and valve effect ($V_{ef} = F * O$) according to Delzon et al. (2010). In the birch samples, we measured, depending on what we saw, the pit border area (A_b), pit aperture area (A_a), and pit aperture length (L_a), or the pit membrane area (A_m). We also measured the cell wall area covered by the analyzed pits and then calculated the ratios of pit border area per vessel wall area ($\sum A_b/A_v$), pit membrane area per vessel wall area ($\sum A_m/A_v$; Li et al., 2019), or pit aperture area per vessel wall area ($\sum A_a/A_v$; Li et al., 2019). See Figure 9 for examples of the measured dimensions. See table 1 for variable names and abbreviations.

For more example images of conduits and pits analyzed in Study II, see https://doi.org/10.5281/zenodo.15007559.

Table 1. Acronyms and description of terms.

Acronym	Full term
$d_{treetop}$ [cm]	distance from the treetop
D_h [µm]	hydraulic mean conduit diameter (a), in this study calculated from tracheids in conifers, vessels and fiber-tracheids in *Betula pendula*)
D_v [µm]	vessel diameter
D_f [µm]	fiber-tracheid diameter
ITE	imperforate tracheary element
VLF	vessel lumen fraction (b)
T [µm]	double cell wall thickness
$(T/B)^2$	cell wall reinforcement (c)
D_m [µm]	diameter of margo
D_t [µm]	diameter of torus
D_a [µm]	diameter of pit aperture
F	margo flexibility (d)
O	torus overlap (d)
V_{ef}	valve effect (d)
A_m [µm²]	pit membrane area (e)
A_b [µm²]	pit border area
A_a [µm²]	pit aperture area (e)
L_a [µm]	pit aperture length
$\sum A_m/A_v$	pit membrane area per vessel wall area
$\sum A_b/A_v$	pit border area per vessel wall area
$\sum A_a/A_v$	pit aperture area per vessel wall area
DoY	day of the year
ts_{ann}	annual temperature sum (f)
phase I	cell enlargement
phase II	wall thickening and lignification
phase III	matured

References: a) Sperry and Hacke (2004), b) Avila et al. (2023), c) Hacke et al. (2001), d) Delzon et al. (2010), e) Li et al. (2019), f) Jyske et al. (2014).

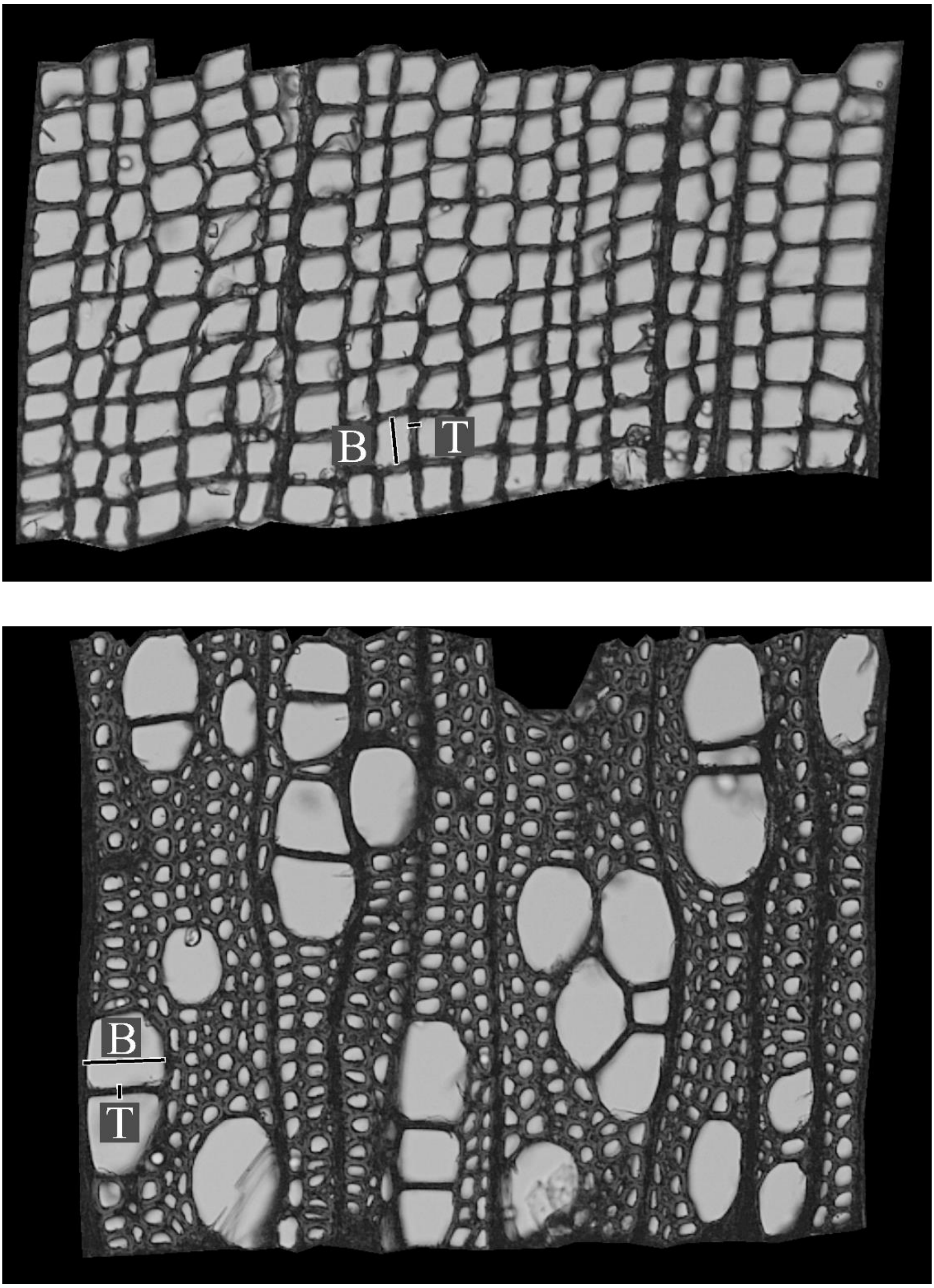


Figure 8 (facing page). Xylem cross sections of *Picea abies* (representing both studied conifer species) and *Betula pendula*, indicating measurement of double cell wall thickness (T) and conduit diameter (B) to calculate cell wall reinforcement.

3.4 Analysis of conduit formation phases

In study III, the samples were embedded in paraffin before preparing the cross sections using a rotatory microtome (Leitz 1516, Leica Microsystems, Wetzlar, Germany). The sections were then double stained to mark the lignin. Polarized light was used during light microscopy to distinguish the cellulose of primary and secondary cell walls.

In one radial file per sample, all cells were counted, their development phase determined, and their radial cell width (including the cell wall) measured. The developmental phases were distinguished as follows (Figure 4): phase I – cell enlargement: cells are wider than cambial cells in the radial direction and their primary cell walls show no birefringence under polarized light; phase II – wall thickening and lignification: the cell walls of the conduits are birefringent and exhibit red and blue colors; phase III – matured: cell walls are completely red, and thicker (Savidge, 2003) than during the previous phase.

3.5 Statistical testing

In Study I, we applied the Shapiro-Wilk Normality test to all data before further analyses. We then tested differences in conduit and pit traits between the two sites using the Welch's *t*-test if the data were normally distributed, otherwise the Mann-Whitney *U* test. To test the change of conduit and pit dimensions along tree rings from the pith to the bark, we used either the Pearson moment coefficient or Spearman's rank correlation coefficient.

In study II, we first log-transformed all data to comply with the assumption of normality and homoscedasticity (Zar, 1999) and to prepare them for allometric analysis (Kerkhoff and Enquist, 2009). We tested the allometric relationships of conduit and pit traits with the distance from the treetop and relationships of conduit traits with pit traits, and the effects of site, species (only among the conifers), and functional group on those relationships by applying linear mixed effect models fitted with restricted maximum likelihood (REML).

In Study III, we applied GAMs (generalized additive models) to estimate the timing of onset and cessation of each conduit formation phase with the accuracy of 1 day independently of the sampling interval. Then, we again log-transformed the data to comply with the assumption of normality and homoscedasticity (Zar, 1999) and applied REML to analyze the effects of latitude and annual temperature sum (ts_{ann}; calculated according to Jyske et al., 2014) on the conduit formation dynamics, i. e, onset, duration, and cessation on the conduit formation phases and total xylogenesis. We also tested the effect of conduit formation dynamics on the total number of conduits and the radial increment formed in the respective year.

For the figures shown in this thesis summary, we employed REML to fit regression lines. As we measured the same conduit and pit dimensions in conifers of Study I and II, we pooled the data from both studies, both species, and all sites, in these figures. In case of the conduit and pit dimensions measured in *B. pendula* in Study II, we pooled the data from both sites. We then applied the Shapiro-Wilk Normality test on both the conifer and the birch datasets, log-transformed the data, and applied REML to test the relationships between distance from the treetop and conduit and pit dimensions, and between the mean hydraulic diameter and pit dimensions. We included site and tree number as random effects. In case of the conifer dataset, we also included species as a random effect. For the figures derived from Study III, we averaged the data among tree individuals and performed all tests for the two species separately. Also here, we applied the Shapiro-Wilk Normality test and log-transformed the data. Then we applied REML to test the relationship between latitude and conduit width, conduit number, and radial increment, or between conduit width or conduit number and radial increment. We included year as a random effect.

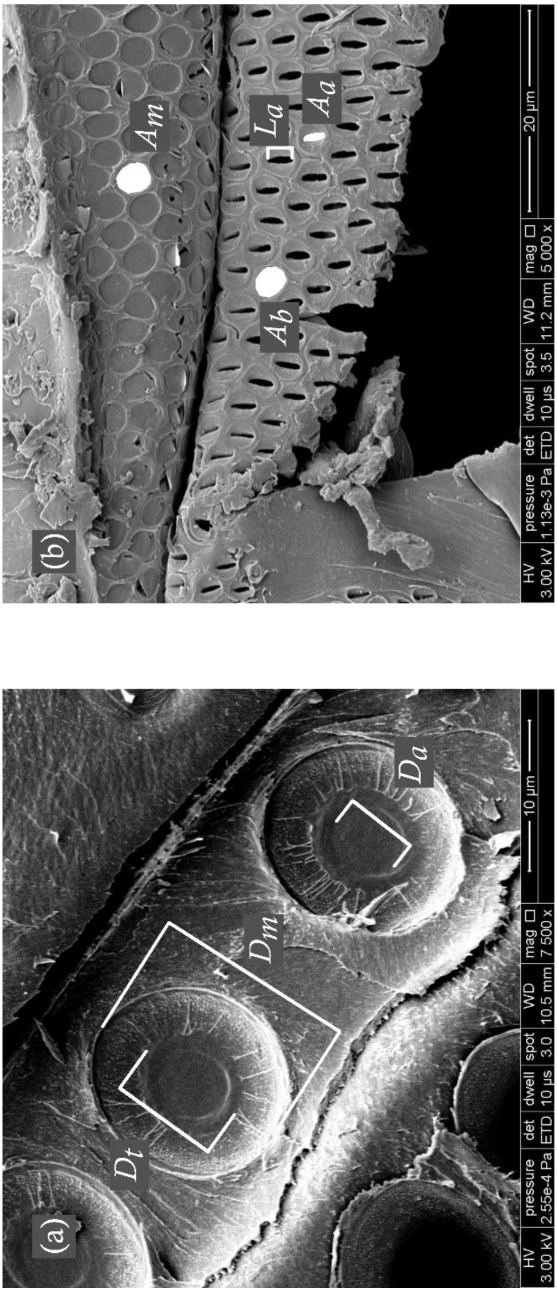


Figure 9. Xylem radial sections showing pits of *Pinus sylvestris* (representing both studied conifer species) and *Betula pendula*, with measured structures indicated.

4. RESULTS

4.1 Within-tree variation and coordination of conduit and pit dimensions

In the *P. sylvestris* trees growing in Austria (Study I), we found that at breast height, the hydraulic mean conduit diameter (D_h) and the pit dimensions diameter of margo (D_m), torus (D_t), and pit aperture (D_a) increased from inner towards outer tree rings. In Finland (study II), we found that in both *P. sylvestris* and *P. abies*, the D_h and all pit dimensions increased with distance from the treetop ($d_{treetop}$), though the root samples showed deviations from the scaling patterns. D_h and pit dimensions measured from the outermost tree ring (with known $d_{treetop}$) in the Austrian *P. sylvestris* trees complement the scaling patterns found in the *P. sylvestris* trees from Finland (Figure 10). Overall, the relationships of D_h and pit dimensions with $d_{treetop}$ showed strong trends across analyzed environments and conifer species (Figure 10).

In the Austrian *P. sylvestris* trees (Study I), the pit functional properties torus overlap (O) and valve effect (V_{ef}) decreased from inner towards outer tree rings at breast height, whereas the margo flexibility (F) showed no such relationship. In the Finnish *P. sylvestris* trees (Study II), we found that all pit functional properties increased with $d_{treetop}$, whereas in the *P. abies* trees, both O and V_{ef} decreased with distance from the treetop. Figure 11 shows, that some of the Austrian *P. sylvestris* trees exhibited a relatively low F and V_{ef} at breast height, whereas O complements the pattern of the Finnish *P. sylvestris* trees. Although the generalized lines fitted to the relationships of pit functional properties with $d_{treetop}$ show a significant slope ($p < 0.01$), there was no common trend in those relationships across environments and species, as shown by the low marginal R^2.

In both the Austrian *P. sylvestris* trees (Study I) and the Finnish *P. sylvestris* and *P. abies* trees (Study II), all pit dimensions increased with increasing D_h. Figure 12 shows that the relationship is similar across the analyzed environments and conifer species. In the Finnish *P. sylvestris* trees, both O and V_{ef} increased with increasing D_h, whereas in the *P. abies*, they decreased (Study II).

In the *B. pendula* trees from study II, the D_h and the pit border area (A_b) and pit membrane area (A_m) increased with $d_{treetop}$, whereas the pit aperture area showed no such relationship (Figure 13). A_m and A_b furthermore increased with increasing D_h (Figure 14).

4.2 Environmental effect on conduit and pit traits

Study I and II provide evidence that the effect of growing conditions on conduit and pit dimensions at similar $d_{treetop}$ are small.

In the Austrian *P. sylvestris* trees (Study I) we found that in the outermost tree ring (i. e., at known distance from the treetop), the O and V_{ef} were higher in trees from the limited site than in those from the favorable site (Figure 11). On average, the O was 0.519 ± 0.035 on the limited site *versus* 0.447 ± 0.006 on the favorable site, and the V_{ef} was 0.211 ± 0.013 on the limited site *versus* 0.188 ± 0.003 on the favorable site.

In study II (Finland), *P. sylvestris* tended to show higher intercepts in the scaling of D_m ($p = 0.07$) and D_a ($p = 0.06$) with $d_{treetop}$, and of D_a ($p = 0.06$) with D_h in trees from the limited site compared to those from the favorable site, when root data were included. Excluding

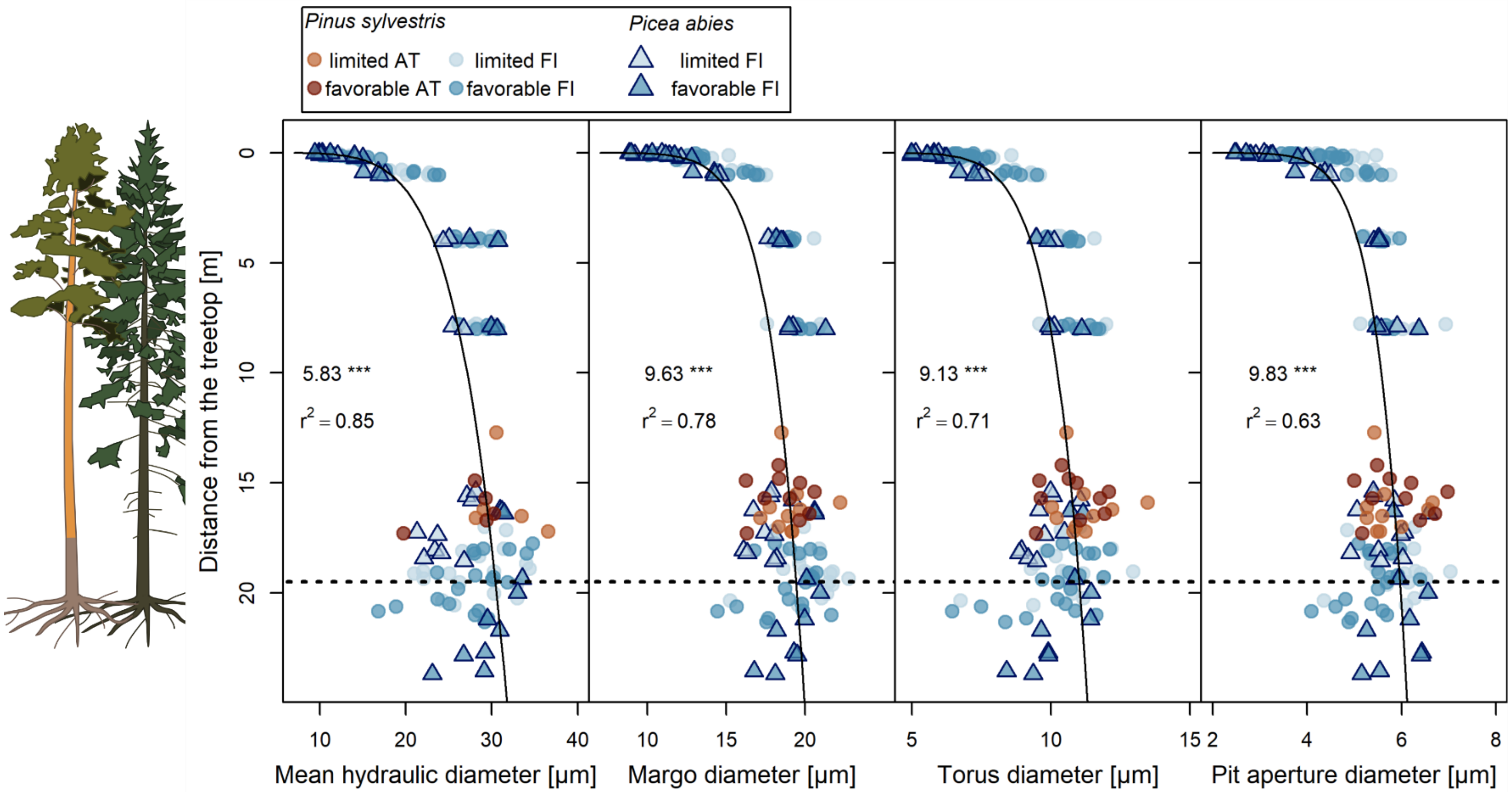


Figure 10. Scaling of mean hydraulic conduit diameter and pit dimensions (diameter of margo, torus, and pit aperture) with distance from the treetop in conifers of study I and II. The lines were fitted with parameters resulting from REML models and indicate $p < 0.05$. The formulas show parameter b with its p-value (* < 0.05, ** < 0.01, *** < 0.001) and the marginal R^2. The dashed line indicates average distance of soil surface from the treetop.

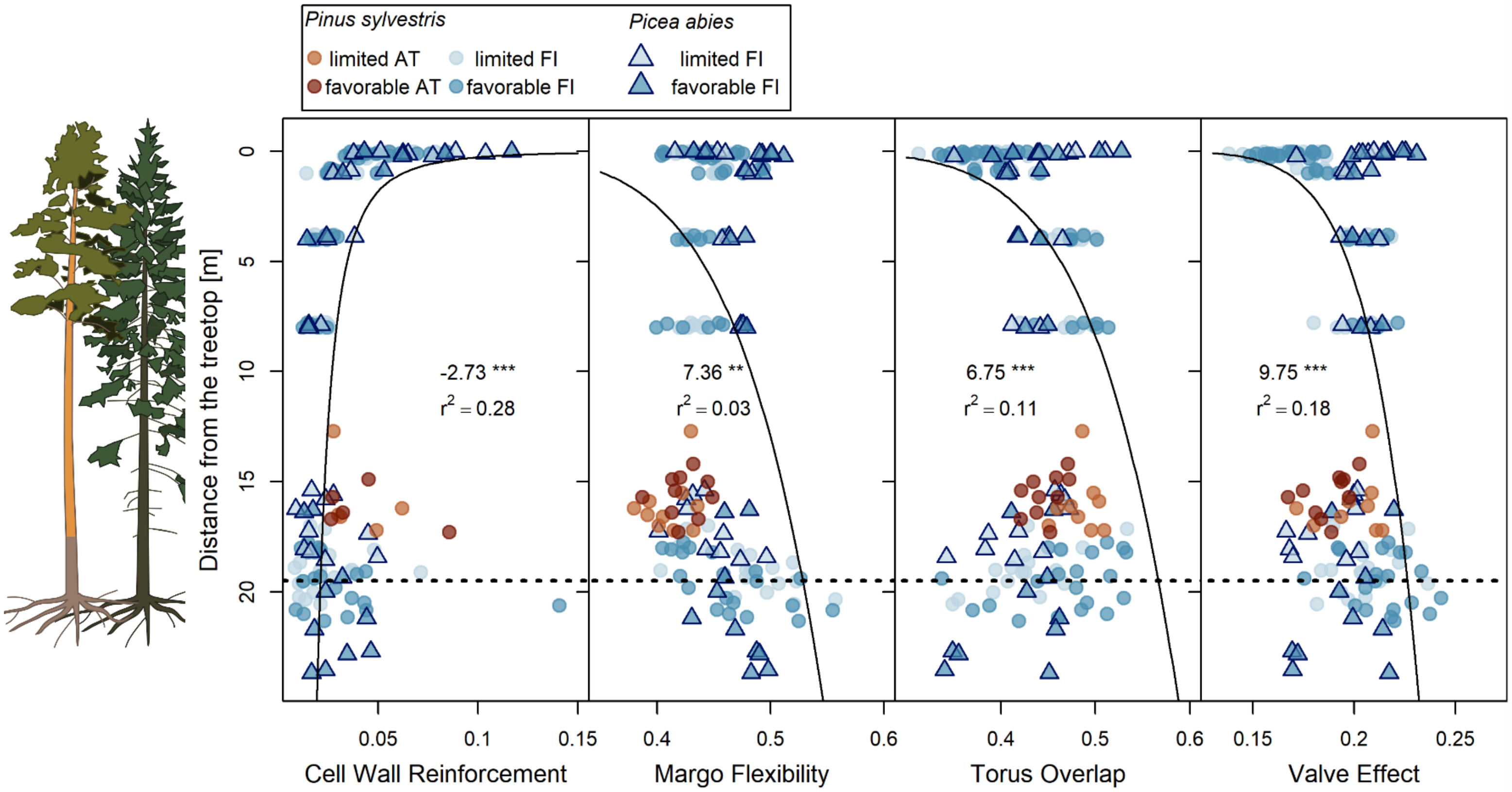


Figure 11. Scaling of cell wall reinforcement and pit functional traits (margo flexibility, torus overlap, and valve effect) with distance from the treetop in conifers of study I and II. The lines were fitted with parameters resulting from REML models and indicate $p < 0.05$. The formulas show parameter *b* with its *p*-value (* < 0.05, ** < 0.01, *** < 0.001) and the marginal R^2. The dashed line indicates average distance of soil surface from the treetop.

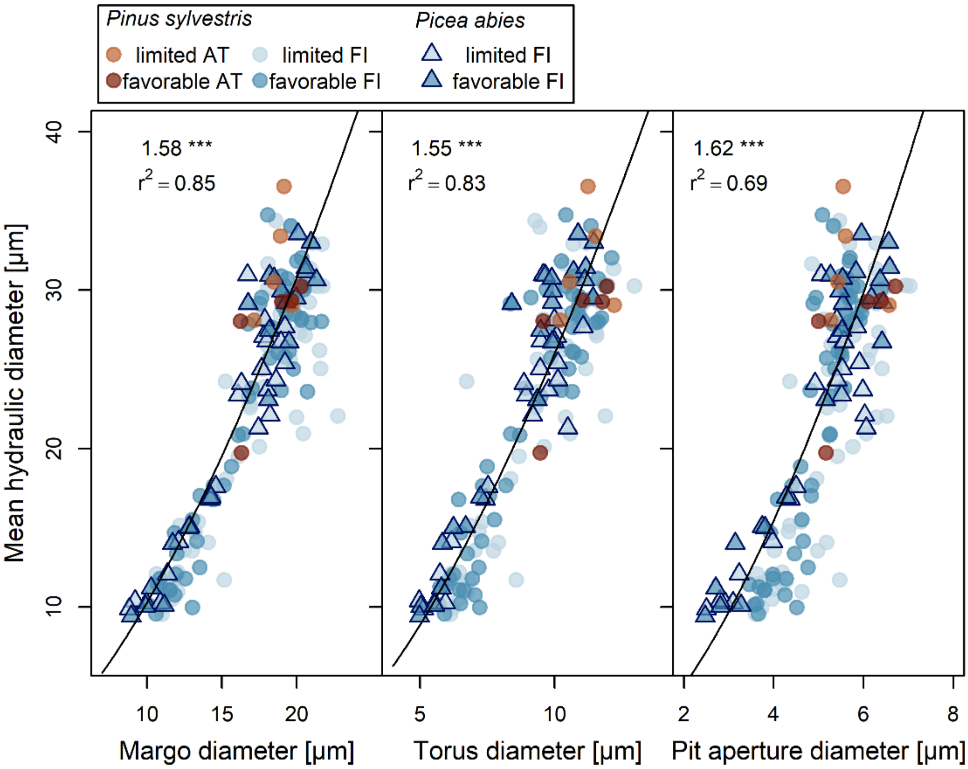


Figure 12. Scaling of pit dimensions margo diameter, torus diameter, and pit aperture diameter with mean hydraulic conduit diameter in conifers of study I and II. The lines were fitted with parameters resulting from REML models and indicate $p < 0.05$. The formulas show parameter *b* with its *p*-value (* < 0.05, ** < 0.01, *** < 0.001) and the marginal R^2.

roots, only the relationship of D_a with $d_{treetop}$ showed a higher intercept in trees from the limited site ($p < 0.05$).

In the same study, *P. abies* tended to show a lower intercept in the scaling relationship of D_h ($p = 0.05$) and V_{ef} ($p = 0.07$) with $d_{treetop}$ in trees from the limited site compared to those on the favorable site, when root data were included. Excluding roots, the relationship of double cell wall thickness (T) with $d_{treetop}$ tended to have a lower intercept (p = 0.07) on the limited site. Also, the relationship V_{ef} with $d_{treetop}$ had a lower intercept ($p < 0.05$) on the limited site. In the relationships of D_t with D_h, the intercept was higher in trees from the limited site compared to those from the favorable ($p < 0.05$) site when root data were included. Also, the relationship of D_a with D_h tended to have a higher intercept ($p = 0.1$) in trees from the limited site compared to those from the favorable site, when root data were included. Excluding root data, there were no such differences. For the relationship of V_{ef} with D_h, *P. abies* exhibited a lower intercept in the limited site (including roots $p = 0.06$; excluding roots $p < 0.05$).

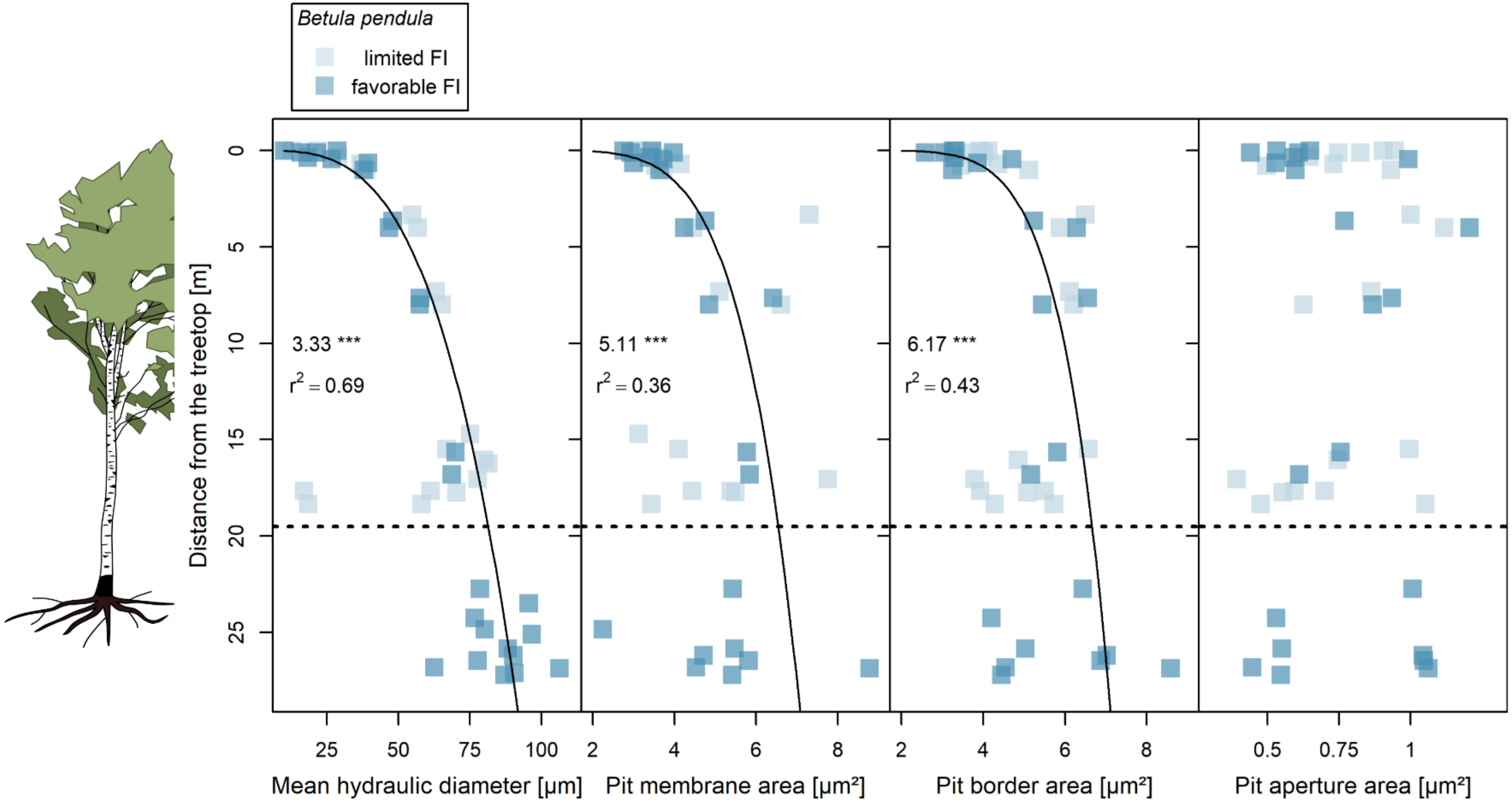


Figure 13. Scaling of mean hydraulic conduit diameter and pit dimensions pit membrane area, pit border area, and pit aperture area with distance from the treetop in birch of study II. The lines were fitted with parameters resulting from REML models and indicate $p < 0.05$. The formulas show parameter b with its p-value (* < 0.05, ** < 0.01, *** < 0.001) and the marginal R^2. The dashed line indicates average distance of soil surface from the treetop.

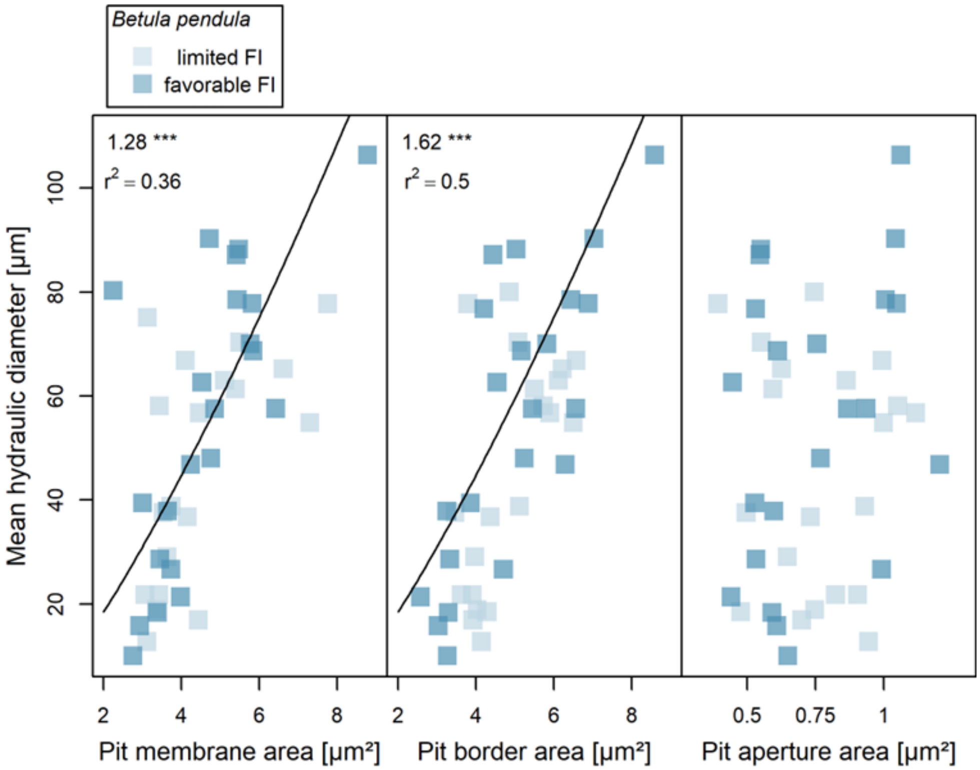


Figure 14. Scaling of pit dimensions pit membrane area, pit border area, and pit aperture area with mean hydraulic conduit diameter in birch of study II. The lines were fitted with parameters resulting from REML models and indicate $p < 0.05$. The formulas show parameter *b* with its *p*-value (* < 0.05, ** < 0.01, *** < 0.001) and the marginal R^2.

In *Betula pendula* (study II), including roots, the scaling of D_h ($p = 0.1$), and double cell wall thickness ($p = 0.08$) with $d_{treetop}$ tended to have a steeper slope on trees from the favorable site. Also, the mean vessel diameter ($p < 0.05$), mean fiber-tracheid diameter ($p < 0.05$), cell wall reinforcement ($p < 0.05$) and A_b ($p < 0.05$) with $d_{treetop}$ had a steeper slope on trees from the favorable site, whereas the scaling of VLF ($p = 0.05$) and pit membrane area per vessel wall area ($p < 0.05$) with $d_{treetop}$ had a steeper slope in trees from the limited site.

Also, the scaling of VLF ($p = 0.05$) with $d_{treetop}$ tended to have a steeper slope in trees from the limited site. The relationship of $(T/B)^2$ with $d_{treetop}$ furthermore had a higher scaling exponent on the favorable site ($p < 0.05$). Excluding roots, the relationships of mean vessel diameter ($p < 0.05$), and mean fiber diameter ($p < 0.05$) with $d_{treetop}$ had lower intercepts, but no differences in slope, on the favorable site. Also, A_b tended to have a lower intercept ($p = 0.06$) on the favorable site. $(T/B)^2$ had a higher intercept ($p < 0.05$) but tended to have a lower slope ($p = 0.05$) in the relationship with $d_{treetop}$. In the relationship of pit aperture length with D_h, the trees from the favorable site exhibited a steeper slope but lower exponent ($p < 0.05$) when including root data.

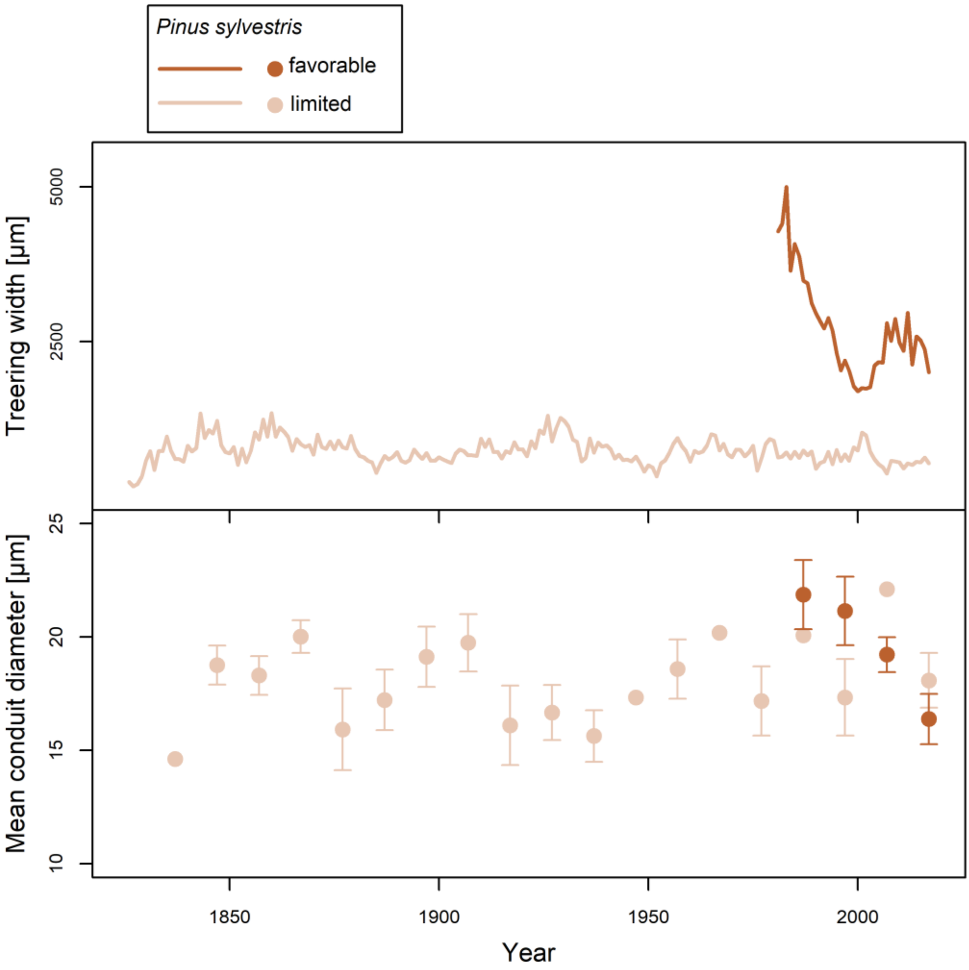


Figure 15. Change of tree ring width and mean conduit diameter along years in pines of study I. Tree ring width was averaged from 10 trees per site, conduit diameter was averaged from 1 or 5 trees on the limited and 5 trees on the favorable site.

4.3 Environmental effect on conduit formation

In study III, we found that in lower latitudes and sites with higher ts_{ann}, all conduit formation phases in *P. sylvestris*, and phase II and III in *P. abies* start at an earlier *DoY*. Both species exhibited a later cessation of all conduit formation phases. Thus, all conduit formation phases in *P. sylvestris* and phases II and III in *Picea abies* had longer durations in lower latitudes and higher ts_{ann}. The timing of onset and cessation, and the duration of all conduit formation phases correlated with the annual number of conduits produced and the annual radial increment in *P. sylvestris*. This was similar in *P. abies*, except that there were no such correlations for the duration of phase I.

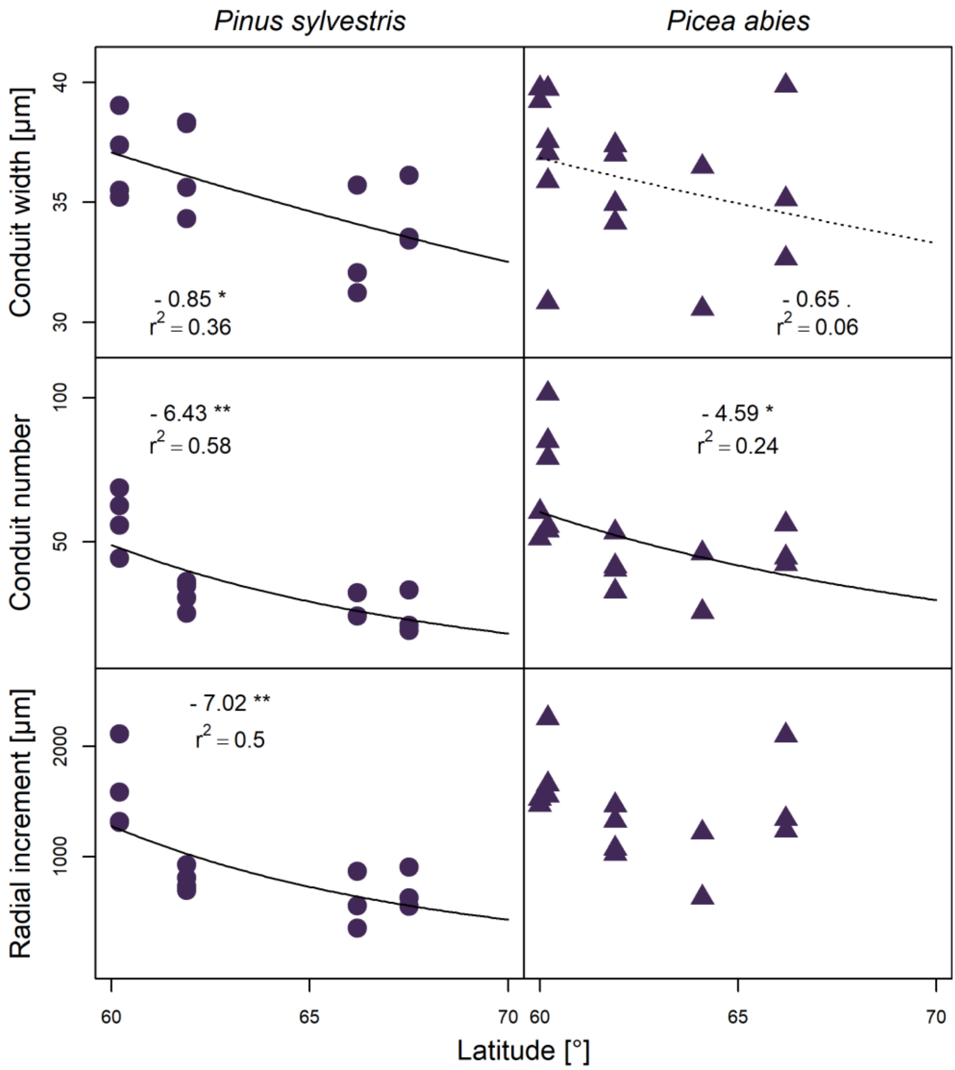


Figure 16. Change of mean annual conduit width, mean annual conduit number, and mean annual radial increment with latitude in conifers of study III. The data were averaged among trees for each year and site. The lines were fitted with parameters resulting from REML models and indicate $p < 0.05$ (solid) or $p < 0.1$ (dashed). The formulas show parameter *b* with its *p*-value (* < 0.05, ** < 0.01, *** < 0.001) and the marginal R^2.

4.4. Environmental effect on conduit number, conduit size, and tree ring width

In study I, we found that on average, the tree ring width was 2724 ± 135 mm on the favorable site and 727 ± 15 mm on the limited site ($p < 0.05$). Furthermore, the mean tree ring width in the trees from the favorable site was constantly higher than that in those from the limited site throughout the time span covered by the sampled tree rings (Figure 15). Throughout the

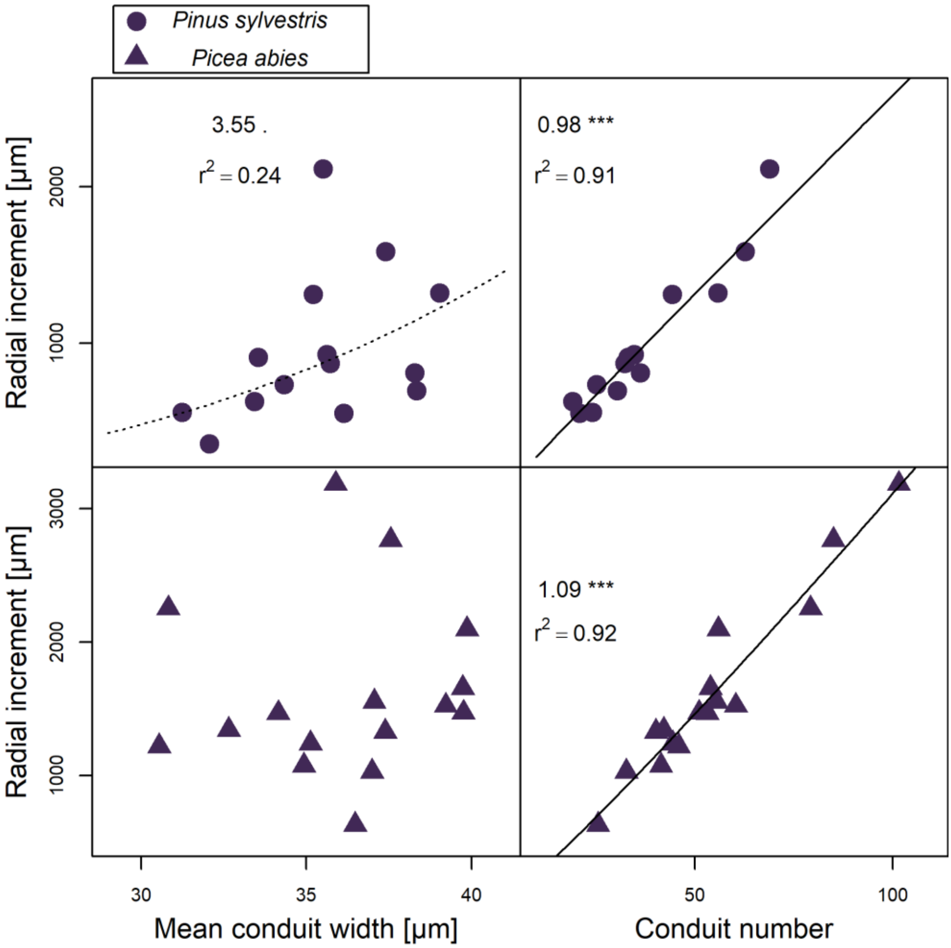


Figure 17. Effect of mean conduit width and conduit number on radial increment. The data were averaged among trees for each year and site. The lines were fitted with parameters resulting from REML models and indicate $p < 0.05$ (solid) or $p < 0.1$ (dashed). The formulas show parameter *b* with its *p*-value (* < 0.05, ** < 0.01, *** < 0.001) and the marginal R^2.

analyzed tree ring chronology, the mean conduit diameter was similar on both sides (Figure 15), with an average of 19.65 ± 1.0 on the favorable and 17.20 ± 0.9 on the limited site ($p > 0.05$).

Figure 16 produced with data of study III reveals that the latitude affects mean conduit width, conduit number, and radial increment in some way, in both *P. sylvestris* and *P. abies*. Figure 17 shows that in both species, there is a clearly visible relationship between conduit number and radial increment, but not between mean conduit width and radial increment.

5. DISCUSSION

5.1 Importance of coordinated scaling of conduit and pits for hydraulic efficiency

Studies I and II indicate, that a coordinated scaling of conduit and pit dimensions with distance from the treetop ($d_{treetop}$) is important for the water transport in both conifer and angiosperm trees. This has been reported so far only for three conifer species (Lazzarin et al., 2016; Zambonini et al., 2024), and to our knowledge, there is only one study analyzing pits along the water transport pathway (Kreinert et al., 2025), that did not report the variation of pit dimensions with $d_{treetop}$ as in our study II. Thus, our results bring new understanding on the xylem hydraulic structure of our study species. Our findings indicate that, along with hydraulic conductivity, embolism resistance may vary greatly along the water transport pathway (see also Zambonini et al., 2024). In study II, the stronger relationship of pit dimensions with the hydraulic mean conduit diameter (D_h) than with $d_{treetop}$ indicates that the conduit and pit dimensions are well coordinated also when deviating from the $d_{treetop}$-scaling pattern.

5.1.1 Pits scale with distance from the treetop in diffuse-porous angiosperm Betula pendula

In *B. pendula*, the increase of A_b and A_m with $d_{treetop}$ and D_h (Figures 13, 14) indicates that the pit membranes increased the hydraulic efficiency by having more space for more or larger membrane pores. Interestingly, the pit apertures (A_a and L_a) did not increase with $d_{treetop}$ or D_h. As our study II is the first to investigate the variation of pit border, pit membrane, or pit aperture size along the water transport pathway in an angiosperm species, it is unknown how generalizable these results are for angiosperms. The ratios of pit border area and pit membrane area per vessel wall area showed little or no change with $d_{treetop}$ or D_h, which means that while the individual pit size increased, the relative pit density stayed constant. The pit aperture area per vessel wall area decreased with $d_{treetop}$ and D_h, which means that the hydraulic efficiency decreased, even more as the T increased with $d_{treetop}$, which furthermore decreased pit aperture conductivity (Sperry and Hacke, 2004). This reduction in hydraulic efficiency may be compensated by wider vessels having more wall area that can be occupied by pits. Indeed, Kreinert et al. (2025) found that the inter-vessel pit membrane area increased with distance from the apex along with vessel diameter and hydraulic function in a *Fagus sylvatica* L. sapling (Kreinert et al., 2025).

An interesting observation in Study II was that the mean fiber-tracheid diameter (D_f) scaled with $d_{treetop}$, though with a lower slope than the mean vessel diameter (D_v) or D_h. This could mean that some of the fiber-tracheids play a role for the hydraulic functions of the xylem. Fiber-tracheids are usually non-conductive (Olson, 2023). However, Sano et al. (2011) found that *Betula japonica* had imperforate tracheary elements (*ITE*) in contact with vessels, which may have been conducting water, although those *ITE* were very rare. Another indication of conductive *ITE* is the shape of their pits (Olson, 2023). Some of the pits we observed in the *ITE* of *B. pendula* (Figure 18) looked similar to the bordered pits in vessels (Figure 3) and to those of true tracheids in the schematic Figure 1 in Olson (2023), though their functionality cannot be clearly determined from our images. Conductive *ITE* may be a low-efficient but high-safety alternative to connect vessels than directly *via* pitted end-walls, and they may even represent a back-up system for water transport when the big vessels are embolized (Sano et al., 2011).

5.1.2 Scaling of conduit and pit dimensions well-coordinated in conifers

The increase in D_h and pit dimensions from inner to outer tree rings in Study I and with $d_{treetop}$ in study II shows that we can reach the same conclusion with both methods, i.e., that D_h and pit dimensions scale along the water transport pathway in *P. sylvestris*. Similarly, Petit et al. (2023) found that D_h increased at tree base from pith to bark, and with $d_{treetop}$ in *P. abies*.

Figure 10 shows that at similar $d_{treetop}$, the D_h and pit dimensions are similar across the species and countries analyzed in Studies I and II. This means that conduit and pit dimensions, and thus hydraulic efficiency and safety, depend largely on $d_{treetop}$. The coordination of conduit dimensions with pit dimensions may be even more general across the analyzed environments (Figure 12) than the scaling of both conduit and pit dimensions with $d_{treetop}$. However, there are differences in the coordination of conduit lumen area with cell wall thickness (Figure 11) and of pit dimensions with each other between species and countries (Figure 11), which will be discussed in the sub-sections 5.1.3 and 5.2.1.

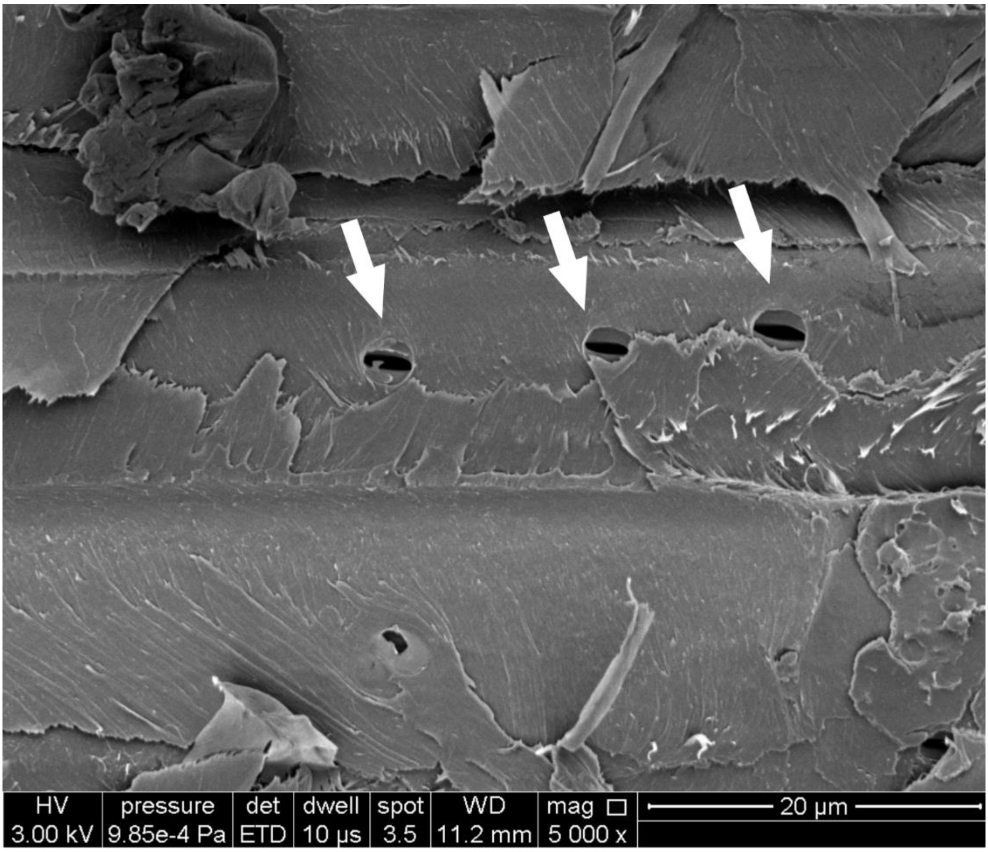


Figure 18. Examples of pits in imperforate tracheary elements (fiber-tracheids) in the xylem of *Betula pendula*.

5.1.3 Scaling of pit functional properties differs between conifer species Pinus sylvestris and Picea abies

P. sylvestris had larger pit dimensions close to the treetop, but lower scaling exponents in the relationship of pit dimensions with $d_{treetop}$, compared to *P. abies*. Particularly the larger pit aperture diameter (D_a; Figure 6 in Study II) indicates a higher hydraulic efficiency (Hacke et al., 2004) in *P. sylvestris* compared to *P. abies* at the treetop. Also, the higher conduit number (larger xylem cross sectional area) at 1 cm $d_{treetop}$ in *P. sylvestris* (Figure S1 in Study II) indicates a higher hydraulic conductivity close to the treetop, though without reduced hydraulic safety. It is possible that the larger conduits are linked to longer needles, i.e., higher leaf area, of *P. sylvestris*, which needs more water than that of *P. abies*. However, the resulting higher functional properties torus overlap (O) and valve effect (V_{ef}) indicated also a higher vulnerability of the xylem to hydraulic failure at the treetop in *P. sylvestris* compared to *P. abies* (Bouche et al., 2014; Delzon et al., 2010; Hacke et al., 2004). A lower torus overlap means that in an aspired pit there is less overlapping surface between the torus and the pit border, and thus the pit seal might be less tight against air seeding (Delzon et al., 2010). Camarero et al., (2026) found that *Pinus pinaster* trees which declined during drought had larger D_a and smaller D_m and D_t at breast height than non-declining trees, even though the declining trees were shorter, i. e., the distance from breast height to the treetop was shorter. This is evidence for the importance of D_a and functional properties O and V_{ef} for xylem hydraulic vulnerability. The hydraulic vulnerability of the xylem at the treetop, where the xylem water potential is typically the lowest (Venturas et al., 2017) may, together with leaf function (Camarero et al., 2026; Salmon et al., 2015), be important for crown dieback during drought. In our study, O and V_{ef} scaled with opposite exponents with $d_{treetop}$ in *P. sylvestris* versus *P. abies*, and thus at breast height, both species had similar O and V_{ef}, indicating similar hydraulic vulnerability. Potential differences in vulnerability to drought between these two conifer species thus seems to be confined to the crown.

Besides the difference in pit functional properties between the two conifer species, we also observed a difference in the smoothness of the pit border (Figure 19). In study I and II, we found that the pit borders of *P. sylvestris* exhibit many warts, as observed previously by Liese and Johann (1954). In study II, we found that *P. abies* has fewer warts (though we did not quantify that). We are unaware about possible roles of warts concerning the hydraulic safety of the pits. It can be speculated that the rougher surface of the pit border allows the torus to seal off the pit aperture less tightly (Cochard, 2006).

5.1.4 Steeper scaling of the hydraulic mean conduit diameter with distance from the treetop in angiosperm than conifers

The steeper slope in the relationship between $d_{treetop}$ and D_h in *B. pendula* compared to the conifers (Study II) indicates a steeper increase in hydraulic efficiency with $d_{treetop}$, as the conifers experience a higher cumulative resistance to water flow (Olson et al., 2021). However, a steeper scaling of D_h indicates that also the hydraulic safety reduces faster with $d_{treetop}$ in *B. pendula*. Our *B. pendula* trees exhibited a D_h of over 40 µm in stem samples from beyond 1 m $d_{treetop}$ (Figure 13), which means that 95 % of the conduits may become embolized in a single freeze-thaw event (Davis et al., 1999). In spring, positive xylem sap pressure refills embolized conduits of *B. pendula* trees with water (Salmon et al., 2014; Hölttä et al., 2018), which may allow this species to have a steeper scaling exponent of D_h with $d_{treetop}$.

5.2 Geographical variation of xylem anatomy

5.2.1 Comparing conduit and pit dimensions of Pinus sylvestris between a boreal forest and an alpine valley

Overall, the *P. sylvestris* trees from Austria (Study I) and Finland (Study II) had similar conduit and pit dimensions in the outermost tree ring at breast height, due to a similar $d_{treetop}$ (Figure 10, 11). Though, , some of the Austrian *P. sylvestris* trees had had a higher $(T/B)^2$ than those from Finland, indicating a higher safety against wall implosion and higher mechanical stability (Hacke et al., 2001), and a lower F, which could mean that the margo may allow the torus to seal off the pit aperture against air seeding less well (Delzon et al., 2010). However, Bouche et al. (2014) found that F plays a smaller role for hydraulic safety than O. Also, the hydraulic safety of pits may be less crucial at breast height, i.e., 15 – 20 m $d_{treetop}$ in the sampled trees, as the most negative pressures within the xylem occur at the treetop (Venturas et al., 2017). It remains to be tested if there are intra-specific differences in the scaling patterns of pit dimensions and pit functional properties across larger spatial ranges, like different climate regions.

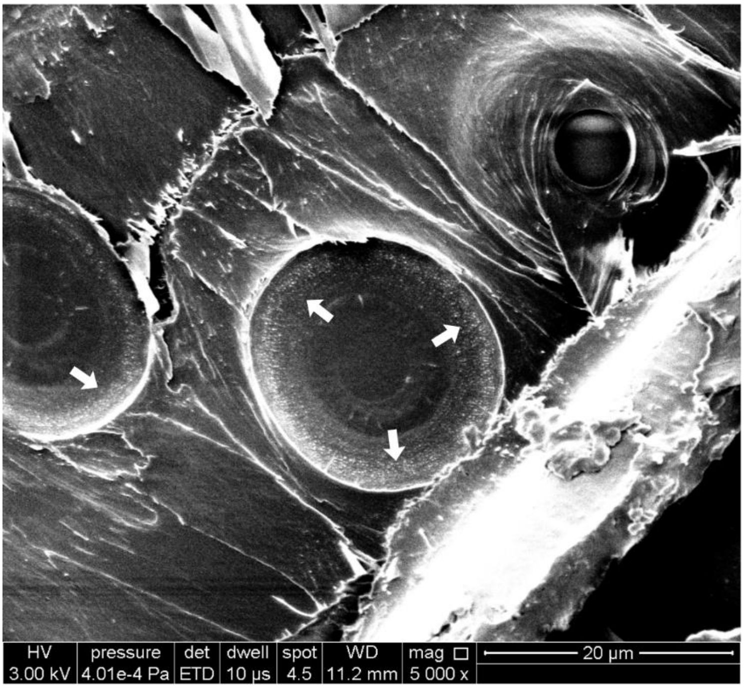
5.2.2 Small differences in xylem anatomy between sites within studies

The small differences in conduit and pit traits that we found between sites with different growing conditions in study I and II are evidence for the strict control of the distance from the treetop on the hydraulic structure of the xylem and that a rather similar water supply is needed by the distal living cells of trees under different growing conditions.

In study I, the difference in O and V_{ef} between the sites indicates that at breast height, the *P. sylvestris* trees on the growth limited site had a higher hydraulic safety compared to those on the site more favorable for growth (Delzon et al., 2010). We did not find differences in O or V_{ef} between the sites in *P. sylvestris* of study II as in *P. sylvestris* of study I. However, *P. abies* of study II had a higher V_{ef}, i. e., higher pit hydraulic safety, on the site more favorable for growth than on the growth limited site. These findings indicate that conifers may be able to slightly adjust pit level hydraulic functions to site conditions *via* changing the relative sizes of margo, torus, and pit aperture.

In study II, the roots were responsible for many of the site differences in scaling exponent and intercept between the sites in all analyzed species. While stems grow vertically and are self-supporting, roots are more horizontally oriented, supported by the soil, and thus experience different mechanical requirements (Christensen-Dalsgaard et a., 2008). Also, the hydraulic challenges of roots differ from those in stems, as they are at the lower end of the water potential gradient that stretches along the water transport pathway (e.g., Venturas et al., 2017). Furthermore, the contribution of a root to the total water supply of the main stem depends on the water sources in the soil, which may have a high micro-site variability, and thus conduit and pit dimensions (and numbers) may vary greatly among roots. Furthermore, the root branching order may influence conduit size (Valenzuela-Estrada et al., 2008), as a root of higher order transports the water collected from several roots of lower order. Thus, in future studies about the scaling of conduit and pit dimensions in the roots, it may be useful to record traits like the root order, as well as soil characteristics.

Pinus sylvestris



Picea abies

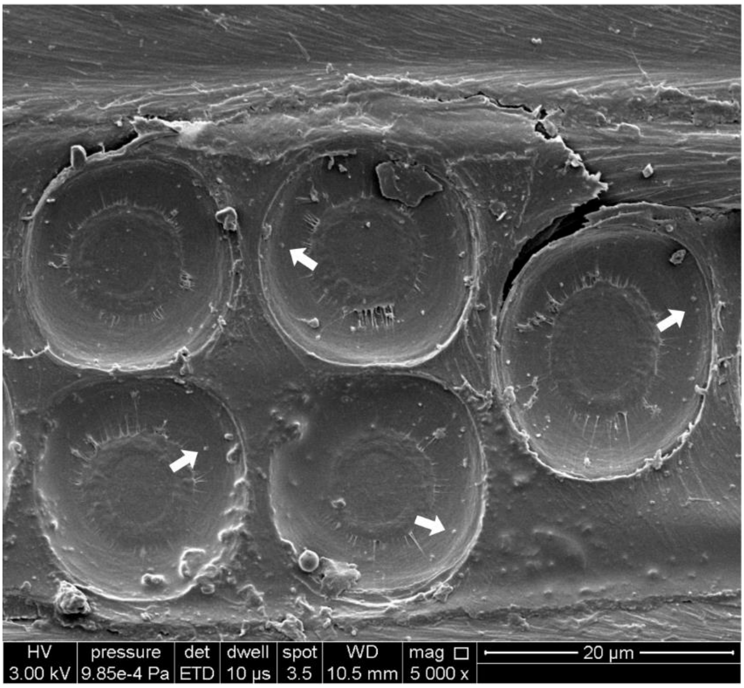


Figure 19. Example images of pits in *Pinus sylvestris* showing many warts and *Picea abies* showing few warts.

Some of the site differences in scaling patterns in the stem may be due to differences in tree height, as well as leaf and crown traits (Anfodillo and Olson, 2024). For example, different intercepts between sites in the scaling of conduit and pit dimensions could be due to different leaf sizes, at least in the angiosperm *B. pendula*. Lechthaler et al. (2020) found for other angiosperm species that conduits scale also within the leaf, whereas they found no scaling of conduits in the needles of *P. abies*. Thus, for future studies aiming to compare conduit and pit scaling patterns between different growing conditions we recommend recording, in addition to the distance from the treetop, also leaf traits.

5.2.3 Response of conduit formation timing and conduit size to environmental conditions

In study I, we found that while overall the D_h increased from inner to outer tree rings at breast height, i.e., with increasing distance from the treetop, the change of D_h along the tree rings was inconsistent (Figure 15). This may indicate that interannual variation of environmental conditions can slightly affect conduit dimensions independent of the distance from the treetop. Similarly, Fonti et al. (2013) found that conduit lumen diameter shows interannual variation in *Larix sibirica* Ldb.

In study III, we found that overall latitude had a prolonging effect on total xylogenesis and on each conduit formation phase in both *P. sylvestris* and *P. abies*. However, the onset of phase I (cell enlargement) in *P. abies* was more sensitive to annually changing conditions. The sensitivity of the onset of phase I to ts_{ann} was similar in both species. Though, the species may respond differently to climate variables calculated from shorter time periods than the ts_{ann}. For example, the onset of xylogenesis in *P. abies* is more sensitive to frost during the preceding winter than in *P. sylvestris* (Lebourgeois et al., 2010). Also, the temperature and moisture availability in the spring months may play a crucial role for the onset of xylogenesis (Wodzicki et al., 1971; Lenz et al., 2013). Pichler and Oberhuber (2006) found that temperature and precipitation from different three-monthly periods during the growing season had different effects on total tree ring width and late wood width in *P. sylvestris* and *P. abies*. Thus, the timing of the conduit formation phases may show higher correlations with climate variables calculated from periods of a few months than with ts_{ann}, which may also reveal more details about inter-specific differences of the timing and environmental sensitivity of conduit formation phases.

Like the onset of phase I, also the conduit width at breast height shows a clearer response to latitude, i. e., constant conditions like the annual light cycle, in *P. sylvestris* than in *P. abies* (Figure 16). It is unclear, however, how much of the variation in conduit width with latitude is due to tree height, as the distance between the sample and the treetop that was formed along with the sampled annual growth ring was not recorded. The onset of phase I and primary growth are coordinated, so that shoot formation and cell enlargement of earlywood happens simultaneously (Buttò et al., 2021a; Fajstavr et al., 2019). The adjustment of the D_h along the water transport pathway to the newly forming treetop and branch apexes likely happens during this time, and environmental conditions could affect tree height and conduit dimensions then. After phase I, the cells enter phase II, during which conduit wall traits, like for example wall thickness and pits, are determined. To learn more about the development of pits and potential influences of tree-internal and environmental conditions on pit dimensions, it may be useful to observe radial sections of micro-cores taken regularly during phase II with a SEM.

5.2.4 Favorable growing conditions increase tree diameter growth via conduit numbers

Study I (Figure 15) and study III (Figure 17) show that temporal and spatial variation of annual growth ring width is largely determined by conduit number and less by conduit size. Conduit diameter is adjusted to the water transport pathway length, i. e., $d_{treetop}$ (Study I, II; e.g., Anfodillo et al., 2013; Olson et al., 2021). Anfodillo et al. (2012) reported that the duration of phase I (but not phase II) increased with $d_{treetop}$, and Cuny and Rathgeber (2016) found no climatic influence on phase I.

In both study I and study III we found that more favorable conditions for growth enabled higher conduit numbers within a tree ring, i.e., higher cambial productivity. In Study I (Austria), the higher cambial activity was enabled by a higher water and nutrient availability on the favorable site. In study III (Finland), both temporally constant environmental factors, like the annual light cycle, and temporally variable environmental factors like the ts_{ann} affected cambial productivity by timing the onset of cell division. We analyzed the onset of phase I and not of cambial activity itself in Study III, however, an earlier onset of phase I implies an earlier onset of cell division (Rossi et al., 2016).

The sensitivity of conduit formation phases and conduit number to ts_{ann} indicates that global warming could advance the timing of these phases and related processes, such as primary growth (shoot and needle formation; Buttò et al., 2021a) and peak carbon sequestration. An advanced start of xylogenesis may enable trees to produce more conduits (i. e., more wood). However, an earlier onset of spring phenology may lead to frost damages (e. g., Ray et al., 2025; Zohner et al., 2020). Also, photoperiod may limit this advancement by becoming the dominant regulator. Indeed, Huang et al. (2020) found for conifers in the Northern Hemisphere that while temperature plays an important role, the photoperiod is the key driver for the onset of wood formation. Furthermore, *P. abies* and *P. sylvestris* need both chilling and forcing temperatures to break winter dormancy and start growing, thus higher winter temperatures may have ambivalent effects on the onset of wood formation (Delpierre et al., 2019).

Adjusting the hydraulic efficiency by changing the conduit size would be less costly (less carbon for wall material needed), however, changing the conduit number may be a hydraulically safer alternative. Smaller conduits, which likely have smaller (Study I and II; Zambonini et al., 2024) and/or fewer pits, are safer against embolism under drought and freezing conditions (e. g., Guan et al., 2021; Jacobsen et al., 2019; Larter et al., 2017; Pittermann & Sperry, 2003, 2006; Plavcová et al., 2013; Sevanto et al., 2012; Sperry & Sullivan, 1992; Wheeler et al., 2005) and more numerous conduits mean more alternative water transport routes to embolized conduits. Forming more conduits in favorable years or sites, instead of bigger ones may make sense for trees in places with a long frost season and variable water availability like in the boreal forests in high latitudes in Finland or the lower montane forests in the alpine valleys of Austria.

Environmental conditions can indeed restrict the dimensions of conduits, however, their influence independent of $d_{treetop}$ seems to be limited (see also Olson et al., 2021). Hydraulic constraints of conduit sizes limit primary growth, and trees at harsh growing conditions at timberlines are thus short (Petit et al., 2009). Environmental conditions that limit cell enlargement thus limit tree height.

6. CONCLUSIONS

Our studies I and II show that inter-conduit pits scale with transport distance. We provide evidence that a coordinated scaling of conduit and pit dimensions with distance from the treetop is crucial for sufficient water transport in both conifers and angiosperms. Differences in scaling patterns between sites were small.

Study II indicated species specific adjustments of the scaling patterns. The angiosperm *B. pendula* had a steeper scaling exponent in the relationship of the hydraulic mean conduit diameter with distance from the treetop than the conifers *P. abies* and *P. sylvestris*, and thus a steeper increase in hydraulic efficiency with transport distance. Between the conifers, there were differences in pit hydraulic efficiency and safety as indicated by the pit functional properties torus overlap and valve effect, meaning that the pits of *P. sylvestris* at the treetop are more vulnerable to embolism than those of *P. abies*.

Study III shows that in boreal forests of the Nordic countries, lower latitudes and warmer years allow for longer conduit formation phases and the production of a higher conduit number. Our findings indicate that in cold environments, global warming may increase wood production through prolonged growing seasons, until another factor becomes limiting.

As conduit size is determined largely by distance from the treetop, making more conduits under favorable conditions may be a more costly but safer way to increase water transport than making larger conduits.

For future comparative studies about xylem hydraulic structure and function we recommend recording the distance of samples from the treetop, and to take several samples along water transport pathway, preferably with higher sampling density close to the treetop. As the hydraulic pathway starts in the leaf and not the treetop, it may be useful to also record leaf traits.

7. References

Anfodillo T, Deslauriers A, Menardi R, Tedoldi L, Petit G, Rossi S (2012) Widening of xylem conduits in a conifer tree depends on the longer time of cell expansion downwards along the stem. J Exp Bot 63: 837–845. https://doi.org/10.1093/jxb/err309.

Anfodillo T, Giai P, Crivellaro A (2013) Axial conduit widening in woody species: a still neglected anatomical pattern. IAWA J 34: 352–364. https://doi.org/10.1163/22941932-00000030.

Anfodillo T, Olson ME. 2024. Stretched sapwood, ultra-widening permeability and ditching da Vinci: revising models of plant form and function. Ann Bot 134: 19–41. https://doi.org/10.1093/aob/mcae054.

Avila RT, Kane CN, Batz TA, Trabi C, Damatta FM, Jansen S, McAdam SAM (2023) The relative area of vessels in xylem correlates with stem embolism resistance within and between genera. Tree Physiol 43:75–87. https://doi.org//10.1093/treephys/tpac110.

Bouche PS, Larter M, Domec J-C, Butlett R, Gasson P, Jansens S, Delzon S (2014) A broad survey of hydraulic and mechanical safety in the xylem of conifers. J Exp Bot 65: 4419–4431. https://doi.org/10.1093/jxb/eru218.

Buttò V, Khare S, Drolet G, Sylvain J-D, Gennaretti F, Deslauriers A, Morin H, Rossi S (2012a) Regionwide temporal gradients of carbon allocation allow for shoot growth and latewood formation in boreal black spruce. Global Ecol Biogeogr 30:1657–1670. https://doi.org/10.1111/geb.13340.

Buttò V, Rozenberg P, Deslauriers A, Rossi S, Morin H (2012b) Environmental and developmental factors driving xylem anatomy and micro-density in black spruce. New Phytol 230:957–971. https://doi.org/10.1111/nph.17223.

Cai J, Li S, Zhang H, Zhang S, Tyree MT (2014) Recalcitrant vulnerability curves: methods of analysis and the concept of fibre bridges for enhanced cavitation resistance. Plant Cell Environ 37: 35–44. https://doi.org/10.1111/pce.12120.

Cajander AK (1949) Forest types and their significance. Suomen metsätieteellinen seura.

Camarero JJ, Colangelo M, Valeriano C, Gazol A, González de Andrés E, Alonso-Forn D, Voltas J, Torres-Ruiz J M, Delzon S, Badel E, Gil-Pelegrín E (2026) Xylem Pit Anatomy and Minimum Leaf Conductance Drive Drought Mortality in *Pinus pinaster*. Plant Cell Environ 49: 279–294. https://doi.org/10.1111/pce.70211.

Carlquist S (2001) Comparative wood anatomy: systematic, ecological, and evolutionary aspects of dicotyledon wood. Berlin: Springer.

Charra-Vaskou K, Lintunen A, Améglio T, Badel E, Cochard H, Mayr S, Salmon Y, Suhonen H, van Rooij M, Charrier G (2023) Xylem embolism and bubble formation during freezing suggest complex dynamics of pressure in *Betula pendula* stems. J Exp Bot 74: 5840–5853. https://doi.org/org/10.1093/jxb/erad275.

Choat B, Cobb AR, Jansen S (2008) Structure and function of bordered pits: new discoveries and impacts on whole-plant hydraulic function. New Phytol 177: 608–626. https://doi.org/10.1111/j.1469-8137.2007.02317.x.

Christensen-Dalsgaard KK, Ennos AR, Fournier M (2008) Interrelations between hydraulic and mechanical stress adaptations in woody plants. Plant Signal Behav 3: 463–5. https://doi.org/10.4161/psb.3.7.5673.

Christof A, Ræbild A, Thygesen LG (2020) Pit and pit aperture dimensions in plantation-grown Douglas fir as affected by local growth conditions and height in stem. IAWA J 41:131–140. https://doi.org/10.1163/22941932-bja10003.

Cochard H (2006) Cavitation in trees. Comptes Rendus Physique 7: 1018–1026. https://doi.org/10.1016/j.crhy.2006.10.012.

Cosgrove D (1997) Relaxation in a High-Stress Environment: The Molecular Bases of Extensible Cell Walls and Cell Enlargement. The Plant Cell 9: 1031–1041. https://doi.org/10.1105/tpc.9.7.1031.

Cuny HE, Rathgeber CBK, Frank D, Fonti P, Mäkinen H, Prislan P, Rossi S, del Castillo EM, Campelo F, Vavrčík H, et al. (2015) Woody biomass production lags stem-girth increase by over one month in coniferous forests. Nature Plants 1: 5160. https://doi.org/10.1038/nplants.2015.160.

Cuny HE, Rathgeber CBK (2016) Xylogenesis: Coniferous trees of temperate forests are listening to the climate tale during the growing season but only remember the last words! Plant Physiol 171: 306–317. https://doi.org/10.1104/pp.16.00037.

Davis SD, Sperry JS, Hacke UG (1999) The relationship between xylem conduit diameter and cavitation caused by freezing. Am J Bot 86: 1367–1372. https://doi.org/10.2307/2656919.

Delpierre N, Lireux S, Hartig F, Camarero JJ, Cheaib A, Čufar K, Cuny H, Deslauriers A, Fonti P, Gričar J, et al. (2019) Chilling and forcing temperatures interact to predict the onset of wood formation in Northern Hemisphere conifers. Glob Change Biol 25: 1089–1105. https://doi.org/10.1111/gcb.14539.

Delzon S, Douthe C, Sala A, Cochard H (2010) Mechanism of water-stress induced cavitation in conifers: bordered pit structure and function support the hypothesis of seal capillary-seeding. Plant Cell Environ 33: 2101–2111. https://doi.org/10.1111/j.1365-3040.2010.02208.x.

Deslauriers A, Rossi S, Anfodillo R, Saracino A (2008) Cambial phenology, wood formation and temperature thresholds in two contrasting years at high altitude in southern Italy. Tree Physiol 28: 863–871. https://doi.org/10.1093/treephys/28.6.863.

Dixon HH, Joly J (1895) On the ascent of sap. Philosophical Transactions of the Royal Society of London. B, 186: 563–576. https://doi.org/10.1098/rspl.1894.0126.

Domec J-C, Lachenbruch B, Meinzer FC (2006) Bordered pit structure and function determine spatial patterns of air-seeding thresholds in xylem of douglas-fir. *Pseudotsuga menziesii*; Pinaceae. trees. Am J Bot 93: 1588–1600. https://doi.org/10.3732/ajb.93.11.1588.

Echeverría A, Petrone-Mendoza E, Segovia-Rivas A, Figueroa-Abundiz VA, Olson ME (2022) The vessel wall thickness-vessel diameter relationship across woody angiosperms. Am J Bot 109: 856–873. https://doi.org/10.1002/ajb2.1854.

Fajstavr M, Bednářová E, Nezval O, Giagli K, Gryc V, Vavrčík H, Horáček P, Urban J (2019) How needle phenology indicates the changes of xylem cell formation during drought stress in *Pinus sylvestris* L. Dendrochronologia 56: 125600. https://doi.org/10.1016/j.dendro.2019.05.004.

Fonti P, Bryukhanova MV, Myglan VS, Kirdyanov AV, Naumova OV, Vaganov EA (2013) Temperature-induced responses of xylem structure of Larix sibirica (Pinaceae) from the Russian Altay. Am J Bot 100:1332–1343. https://doi.org/10.3732/ajb.1200484.

Guan X, Pereira L, McAdam SAM, Cao K, Jansen S (2021) No gas source, no problem: proximity to pre-existing embolism and segmentation affect embolism spreading in angiosperm xylem by gas diffusion. Plant Cell Environ 44: 831–845. https://doi.org/10.1111/pce.14016.

Hacke UG, Sperry JS, Pittermann J (2004) Analysis of circular bordered pit function II. Gymnosperm tracheids with torus-margo pit membranes. Am J Bot 91: 386–400. Https://doi.org/10.3732/ajb.91.3.386.

Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. Oecologia 126: 457–461. https://doi.org/10.1007/S004420100628.

Hammel HT (1967) Freezing of xylem sap without cavitation. plant physiol (Bethesda) 24: 55–66. https://doi.org/10.1104/pp.42.1.55.

Held M, Lintunen A, Jyske T (2025) Exemplaric microscopical images of publication "Conduit and pit dimensions scale in a coordinated way from the treetop to coarse roots in three boreal tree species". Zenodo. https://doi.org/10.5281/zenodo.15007559

Hölttä T, Dominguez Carrasco MDR, Salmon Y, Aalto J, Vanhatalo A, Bäck J, Lintunen A (2018) Water relations in silver birch during springtime: How is sap pressurized? Plant Biol 20: 834–847. https://doi.org/10.1111/plb.12838.

Huang J-G, Ma Q, Rossi S, Biondi F, Deslauriers A, Fonti P, Liang E, Mäkinen H, Oberhuber W, Rathgeber CBK, et al. (2020) Photoperiod and temperature as dominant environmental drivers triggering secondary growth resumption in Northern Hemisphere conifers. P Natl Acad Sci USA 117: 20645–20652. https://doi.org/10.1073/pnas.2007058117.

Jacobsen AL (2021) Diversity in conduit and pit structure among extant gymnosperm taxa. Am J Bot 108: 559–570. https://doi.org/10.1002/ajb2.1641.

Jacobsen AL, Pratt RB, Venturas MD, Hacke UG (2019) Large volume vessels are vulnerable to water-stress-induced embolism in stems of poplar. IAWA J: 40:4–22. https://doi.org/10.1163/22941932-40190233.

Jansen S, Pletser A, Sano Y (2008) The effect of preparation techniques on SEM-imaging of pit membranes. IAWA Journal 29: 161–178. https://doi.org/10.1163/22941932-90000177.

Jansen S, Lamy J-B, Burlett R, Cochard H, Gasson P, Delzon S (2012) Plasmodesmatal pores in the torus of bordered pit membranes affect cavitation resistance of conifer xylem. Plant Cell Environ 35: 1109–1120. https://doi.org/10.1111/j.1365-3040.2011.02476.x.

Jyske T, Mäkinen H, Kalliokoski T, Nöjd P (2014) Intra-annual xylem formation of Norway spruce and Scots pine across latitudinal gradient in Finland. Agr Forest Meteorol 194: 241–254. https://doi.org/10.1016/j.agrformet.2014.04.015.

Kaack L, Altaner CM, Carmesin C, Diaz A, Holler M, Kranz C, Neusser G, Odstreil M, Schenk HJ; Schmidt V, Weber M, Zhang Y, Jansen S (2019). Function and three-dimensional structure of intervessel pit membranes in angiosperms: a review. IAWA J 40: 673–702. https://doi.org/10.1163/22941932-40190259.

Kaack L, Weber M, Isasa E, Karimi Z, Li S, Pereira L, Trabi CL, Zhang Y, Schenk HJ, Schuldt B, Schmidt V, Jansen S (2021) Pore constrictions in intervessel pit membranes provide a mechanistic explanation for xylem embolism resistance in angiosperms. New Phytol 230: 1829–1843. https://doi.org/10.1111/nph.17282.

Kerkhoff AJ, Enquist BJ (2009) Multiplicative by nature: Why logarithmic transformation is necessary in allometry. Journal of Theoretical Biology 257: 519–521. https://doi.org/10.1016/j.jtbi.2008.12.026.

Kiorapostolou N, Camarero JJ, Carrer M, Sterck F, Brigita B, Sangüesa-Barreda G, Petit G (2020) Scots pine trees react to drought by increasing xylem and phloem conductivities. Tree Physiol 40: 774–781. https://doi.org/10.1093/treephys/tpaa033.

Kramer JK, Kozlowski TT (1960) Physiology of trees. McGraw-Hill Book Company, Inc., New York, Toronto, London.

Kreinert S, Kaack L, Pereira L, Miranda M T, Mäck S, Schuler J, Jansen S (2025) Xylem vessel anatomy and hydraulic function scale in concert along the tip-to-base axis of an angiosperm tree. AoB PLANTS 17: plaf072. https://doi.org/10.1093/aobpla/plaf072.

Kulmala L, Pumpanen J, Kolari P, Dengel S, Berninger F, Köster K, Matkala L, Vanhatalo A, Vesala T, Bäck J (2019) Inter- and intra-annual dynamics of photosynthesis differ between forest floor vegetation and tree canopy in a subarctic Scots pine stand. Agr Forest Meteorol 271:1–11. https://doi.org/10.1016/j.agrformet.2019.02.029.

Larter M, Pfautsch S, Domec J-C, Trueba S, Nagalingum N, Delzon S (2017) Aridity grove the evolution of extreme embolism resistance and the radiation of conifer genus *Callitris*. Ney Phytol 215: 97–112. https://doi.org/10.1111/nph.14545.

Lazzarin M, Crivellaro A, Williams CB, Dawson TE, Mozzi G, Anfodillo T (2016) Tracheid and pit anatomy vary in tandem in a tall *Sequoiadendron giganteum* tree. IAWA J 37: 172–185. https://doi.org/10.1163/22941932-20160129.

Lebourgeois F, Rathgeber CBK, Ulrich E (2010) Sensitivity of French temperate coniferous forests to climate variability and extreme events (*Abies alba*, *Picea abies* and *Pinus sylvestris*). J Veg Sci 21:364–376. https://doi.org/10.1111/j.1654-1103.2009.01148.x.

Lechthaler S, Kiorapostolou N, Pitacco A, Anfodillo T, Petit G (2020) The total path length hydraulic resistance according to known anatomical patterns: What is the shape of the root-to-leaf tension gradient along the plant longitudinal axis? J. Theor. Biol. 502, 110369. https://doi.org/10.1016/j.jtbi.2020.110369.

Lenz A, Hoch G, Körner C (2013) Early season temperature controls cambial activity and total tree ring width at the alpine treeline. Plant Ecol Divers 6:365–375. https://doi.org/10.1080/17550874.2012.711864.

Li S, Hao G-Y, Niinemets Ü, Harley PC, Wanke S, Lens F, Zhang Y-J, Cao K-F (2019) The effects of intervessel pit characteristics on xylem hydraulic efficiency and photosynthesis in hemiepiphytic and non-hemiepiphytic Ficus species. Physiol Plantarum 167: 661–675. https://doi.org/10.1111/ppl.12923.

Liese W, Johann I (1954) Elektronenmikroskopische Beobachtungen über eine besondere Feinstruktur der verholzten Zellwand bei einigen Coniferen. [Electron microscopical observations about a particular ultrastructure in the lignified cell wall of some conifers]. Planta, 44:269–285. https://doi.org/10.1007/BF01929519.

Lintunen A, Kalliokoski T (2010) The effect of tree architecture on conduit diameter and frequency from small distal roots to branch tips in *Betula pendula*, *Picea abies* and *Pinus sylvestris*. Tree Physiol 30: 1433–1447. https://doi.org/10.1093/treephys/tpq085.

Lintunen A, Salmon Y, Hölttä T, Suhonen H (2022) Inspection of gas bubbles in frozen Betula pendula xylem with micro-CT: Conduit size, water status and bark permeability affect bubble characteristics. Physiol Plantarum 174. https://doi.org/10.1111/ppl.13749.

Losso A, Anfodillo A, Ganthaler A, Kofler A, Markl Y, Nardini A, Oberhuber W, Purin E, Mayr S (2018) Robustness of xylem properties in conifers: analyses of tracheid and pit dimensions along elevational transects. Tree Physiol 00: 1–11. https://doi.org/10.1093/treephys/tpx168.

Mayr S, Kartusch B, Kikuta S (2014) Evidence for Air-Seeding: Watching the Formation of Embolism in Conifer Xylem. J Plant Hydraul 1: e004. https://doi.org/10.20870/jph.2014.e004.

Michaletz ST, Weiser MD, Zhou J, Kaspari M, Helliker BR, Enquist BJ (2015) Plant Thermoregulation: Energetics, Trait–Environment Interactions, and Carbon Economics. Trends in Ecology & Evolution 30: 714–724. https://doi.org/10.1016/j.tree.2015.09.006.

Olson ME (2023) Imperforate tracheary element classification for studies of xylem structure-function relations. IAWA Journal: 44: 439–464. https://doi.org/10.1163/22941932-bja10125.

Olson ME, Anfodillo T, Gleason SM, and McCulloh KA (2021) Tip-to-base xylem conduit widening as an adaptation: causes, consequences, and empirical priorities. New Phytol 229: 1877–1893. https://doi.org/10.1111/nph.16961.

Petit G, Anfodillo T, De Zan, C (2009) Degree of tapering of xylem conduits in stems and roots of small Pinus cembra and Larix decidua trees. Botany 87: 501–508. https://doi.org/10.1139/B09-025.

Petit G, Mencuccini M, Carrer M, Prendin AL, and Höltta T (2023) Axial conduit widening, tree height and height growth rate set the hydraulic transition of sapwood into heartwood. J Exp Bot 74: 5072–5087. https://doi.org/10.1093/jxb/erad227.

Petit G (2024). An appreciation of apex-to-base variation in xylem traits will lead to more precise understanding of xylem phenotypic plasticity. New Phytol 244: 1175–1180. https://doi.org/10.1111/nph.20109.

Pichler P, Oberhuber W (2007) Radial growth response of coniferous forest trees in an inner Alpine environment to heat-wave in 2003. Forest Ecology and Management 242: 688–699. https://doi.org/10.1016/j.foreco.2007.02.007.

Pittermann J, Sperry J (2003) Tracheid diameter is key trait determining the extent of freezing-induced embolism in conifers. Tree Physiol 23: 907–914. https://doi.org/10.1093/treephys/23.13.907.

Pittermann J, Sperry J (2006) Analysis of freeze-thaw embolism in conifers. The interaction between cavitation pressure and tracheid size. Plant Physiol 140: 374–382. https://doi.org/10.1104/pp.105.067900.

Plavcová L, Jansen S, Klepsch M, Hacke UG (2013) Nobody's perfect: can irregularities in pit structure influence vulnerability to cavitation? Frontiers in Plant Science 4: 453. https://doi.org/10.3389/fpls.2013.00453.

Prendin AL, Petit G, Fonti P, Rixen C, Dawes MA, von Arx G (2018) Axial xylem architecture of *Larix decidua* exposed to CO2 enrichment and soil warming at the tree line. Funct Ecol 32: 273–287. https://doi.org/10.1111/1365-2435.12986.

Ray D, Fung F, Pirret J, Plenderleith F, Trotter F, Marchi M (2025). Risk of extreme climate impacts on European Norway spruce forest: drought and frost in the climate emergency. Climatic Change 178: Article 114. https://doi.org/10.1007/s10584-025-03954-z.

Rossi S, Anfodillo T, Čufar K, Cuny HE, Deslauriers A, Fonti P, Frank D, Gričar J, Gruber A, Huang J-G, et al. (2016) Pattern of xylem phenology in conifers of cold ecosystems at the Northern Hemisphere. Glob Change Biol 22: 3804–3813. https://doi.org/10.1111/gcb.13317.

Ryan GM, Yoder BJ (1997) Hydraulic limits to tree height and tree growth. BioScience 47: 235–242. https://doi.org/10.2307/1313077.

Salmon Y, Lintunen A, Lindfors L, Suhonen H, Sevanto S, Vesala T, Hölttä T (2014) Silver birch ability to refill fully embolized xylem conduits under tension. Acta Hortic 1222: 67–74. https://doi.org/10.17660/ActaHortic.2018.1222.10.

Salmon Y, Torres-Ruiz J M, Poyatos R, Martinez-Vilalta J, Meir P, Cochard H, Mencuccini M (2015) Balancing the risks of hydraulic failure and carbon starvation: a twig scale analysis in declining Scots pine. Plant Cell Environ 38: 2575–2588. https://doi.org/10.1111/pce.12572.

Sano Y, Morris H, Shimada H, Ronse De Craene LP, Jansen S (2011) Anatomical features associated with water transport in imperforate tracheary elements of vessel-bearing angiosperms. Ann Bot 107: 953–964. https://doi.org/10.1093/aob/mcr042.

Savidge RA (2003) Tree Growth and Wood Quality. In: Barnett JR, Jeronimidis GG, editors. In: Wood Quality and Its Biological Basis. Oxford (UK): Blackwell Publishing Ltd. p. 1–29.

Schweingruber F H (1993) Trees and wood in dendrochronology. Springer, Berlin, New York.

Schweingruber F H (2007) Wood structure and environment. Springer, Berlin, New York.

Sevanto S, Holbrook NM, Ball MC (2012) Freeze/thaw-induced embolism: probability of critical bubble formation depends on speed of ice formation. Frontiers in Plant Science 3: 107. https://doi.org/10.3389/fpls.2012.00107.

Sperry JS, Meinzer FC, McCulloh KA (2008) Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. Plant Cell Environ 31: 632–645. https://doi.org/10.1111/j.1365-3040.2007.01765.x.

Sperry JS., Hacke UG (2004) Analysis of circular bordered pit function I. Angiosperm vessels with homogenous pit membranes. Am. J. Bot. 91: 369–385. https://doi.org/10.3732/ajb.91.3.369.

Sperry JS, Sullivan JEM (1992) Xylem embolism in response to freeze-thaw cycles and water stress in ring-prous, diffuse-porous, and conifer species. Plant Physiol 100: 605–613. https://doi.org/10.1104/pp.100.2.605.

Valenzuela-Estrada LR, Vivianette V-C, Ruth LE, Eissenstat DM (2008) Root anatomy, morphology, and longevity among orders in *Vaccinium corymbosum*. Ericaceae. Am J Bot 95: 1506–1514. https://doi.org/10.3732/ajb.0800092.

Venturas MD, Sperry JS, Hacke UG (2017) Plant xylem hydraulics: what we understand, current research, and future challenges. JIPB 59: 356–389. https://doi.org/10.1111/jipb.12534.

Wheeler JK, Sperry JS, Hacke UG, Hoang N (2005) Inter-vessel pitting and cavitation in woody Rosaceae and other vesselled plants: a basis for a safety versus efficiency trade-off in xylem transport. Plant Cell Environ 28:800–812. https://doi.org/10.1111/j.1365-3040.2005.01330.x.

Wodzicki TJ (1971) Mechanism of xylem differentiation in *Pinus silvestris* L.. J Exp Bot 22:670–687. https://doi.org/10.1093/jxb/22.3.670.

Zambonini D, Savi T, Rosner S, Petit G (2024) Consistent decrease in conifer embolism resistance from the stem apex to base resulting from axial trends in tracheid and pit traits. Front Plant Sci 15: 1414448. https://doi.org/10.3389/fpls.2024.1414448.

Zar JH (1999) Biostatistical analysis, 4th edition. Upper Saddle River (NJ) USA: Prentice-Hall

Zhang Y, Carmesin C, Kaack L, Klepsch MM, Kotowska M, Matei T, Schenk HJ, Weber M, Walther P, Schmidt V, Jansen S (2019) High porosity with tiny pore constrictions and unbending pathways characterize the 3D structure of intervessel pit membranes in angiosperm xylem. Plant Cell Environ 43:116–130. https://doi.org/10.1111/pce.13654.

Zimmermann MH (1983) Xylem Structure and the Ascent of Sap. Berlin, Germany: Springer.

Zohner CM, Mo L, Renner SS, Svenning J-C, Vitasse Y, Benito BM, Ordonez A, Baumgarten F, Bastin J-F, Sebald V, et al. (2020) Late-spring frost risk between 1959 and 2017 decreased in North America but increased in Europe and Asia. P Natl Acad Sci USA 117: 12192–12200. https://doi.org/10.1073/pnas.1920816117.