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Functional xylem anatomy: intra and interspecific variation in stems of herbaceous and woody species

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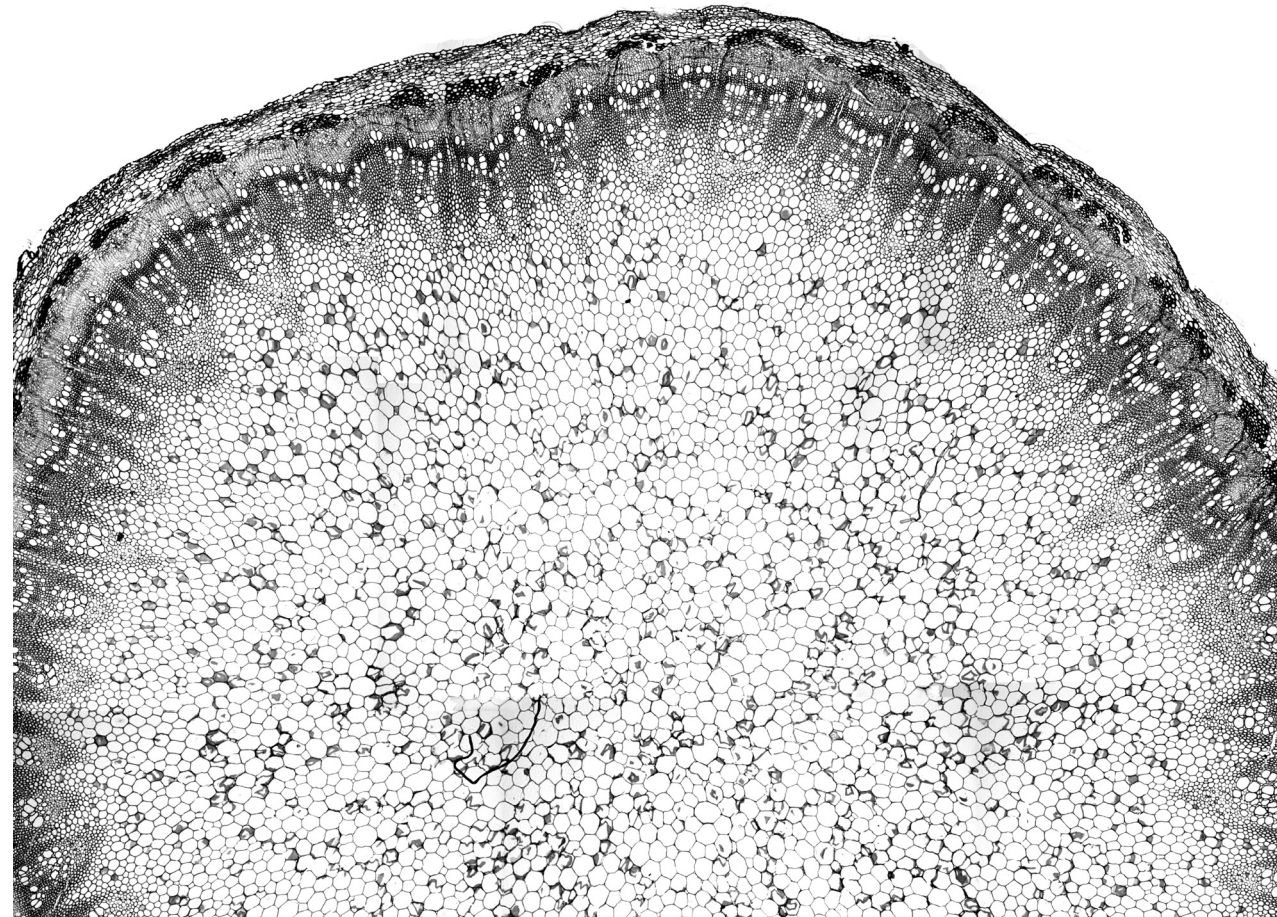
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Chapter 1

GENERAL INTRODUCTION AND THESIS OUTLINE



The evolution of a specialized, axially-arranged tissue enabling long-distance transport of sugar and water throughout the plant body, allowed the earliest woody land plants to become successful in terrestrial ecosystems since their evolution around 400 million years ago (Hartmann 2011; Ligrone *et al.* 2012). The vascular system includes xylem, that allows trees to conduct water from the soil to more than 100 m, favoring woody plants to colonize diverse environments, compete, and coexist (Koch *et al.* 2004). The physiology of water transport defines many aspects of the daily functioning of plants and it has been considered the “backbone” of the plant terrestrial ecosystems since it defines a physical limit to plant productivity and survival (Brodribb 2009). Therefore, xylem anatomy plays a central role in plant hydraulic strategies due to the interaction with the whole hydraulic continuum: from the root-rhizosphere interface to the water-air interface in the leaves (Barnard *et al.* 2011). Additionally, wood anatomy has its inherent trade-offs associated with the division of labour between water conduction and structural support functions (Chave *et al.* 2009).

This thesis is entirely dedicated to the functional aspects of xylem anatomy in woody and herbaceous species based on in-depth observations with different microscopy techniques (light, scanning and transmission electron microscopy) coupled with experimental measurements of water transport in stems as a proxy for drought stress resistance. In the two first chapters we assess the variation of the main wood anatomical characters in two co-occurring woody species in the two main seasonally dry biomes in Brazil, cerrado and caatinga, to infer wood functional roles and sampling height-related anatomical adaptations to deal with abiotic factors. In the following two last chapters, we combine hydraulic measurements with detailed stem anatomical observations to assess plant resistance to drought in woody and herbaceous species occurring in Tenerife, Canary Islands. Before going into detail of the findings of my studies, I will introduce some topics on basic xylem anatomy and long distance water transport in plants, along with a historical background of both fields. I will also give an overview about the relationship between xylem anatomy and physiology, and its relevance for ecosystems ecology.

General introduction to xylem anatomy

Starting from the basics

The vascular tissues of plants, including xylem and phloem, form a continuous system extending throughout the plant body carrying out two essential functions: delivering of resources (water, essential minerals, sugars and amino acids) to different parts of the plant body, and providing of mechanical support (Evert 2006; Lucas *et al.* 2013).

The term xylem is derived from the Greek word *xylon*, which means wood, and it is the principal water-conducting and supporting tissue of plants. The primary xylem is formed very early in the primary plant body (seedling stage) before the onset of secondary growth, and is developed from procambium strands that develop close to the stem and root apical meristems (Esau 1965; Evert 2006) (Fig. 1 A, I). Wood or secondary xylem is developed by a (secondary) meristem, called cambium, which produces wood cells towards the inside of the stem in typical radial files using periclinal divisions, while secondary phloem is produced towards the outside (Fig. 1 A, II - V). In contrast to the primary xylem, it consists of an axial and a radial system composed of three different cell types: vessel elements (and/or tracheids), fibers and parenchyma (Evert 2006) (Fig. 1 B). The primary xylem often remains functional in herbaceous species that do not undergo secondary growth (Fig. 1 A, I). However, most of the eudicot herbaceous species show a certain degree of wood formation (Schweingruber 2007; Schweingruber *et al.* 2011). It is normally confined to the base of the stems, either limited to the vascular bundle regions or somewhat expanded by a complete vascular cambium into a tiny wood cylinder (Lens *et al.* 2012a) (Fig. 1 A, III - IV). Consequently, all herbaceous non-monocot angiosperms have a limited amount of wood formation in their stems, especially at the stem base, which can continuously vary amongst these herbaceous species emphasizing that there is a fuzzy boundary between 'herbaceousness' and 'woodiness'. This makes it sometimes complex in some lineages to decide at which woodiness level a species can be considered truly woody (Lens *et al.* 2012a). Therefore, Kidner *et al.* (2016) proposed the following definition for a woody species: species that produce a distinct wood cylinder extending towards the upper parts of the stem.

Wood consists of a number of different cell types: water conducting tracheary elements (vessel elements and tracheids), non-tracheary elements (fibers) and parenchyma cells (axial parenchyma and ray parenchyma) (Fig. 1 B). Vessel elements and tracheids are both axially elongated, non-living at maturity, and have lignified secondary walls. Tracheids are generally present in the wood of gymnosperms, exerting both the function of conducting water and providing mechanical support. They are single cells ranging in size from about 0.5 - 4 mm in length and 8-80 μ m in diameter connected to each other via lateral cavities in the secondary cell wall, the bordered pits (Pittermann & Sperry 2003; Choat *et al.* 2008; Pittermann 2010). On the other hand, vessel elements are the main water-conducting cells of angiosperms and are stacked one on top of the other forming an elongated, hollow tube, called vessel, that might be up to 0.5 mm in diameter and several meters (up to 16m in some lianas; Ewers 1990) in length. They differ from tracheids by the occurrence of perforation plates, which are wide openings in the cell wall that are positioned at both end walls and axially connect the vessel elements within a single vessel.

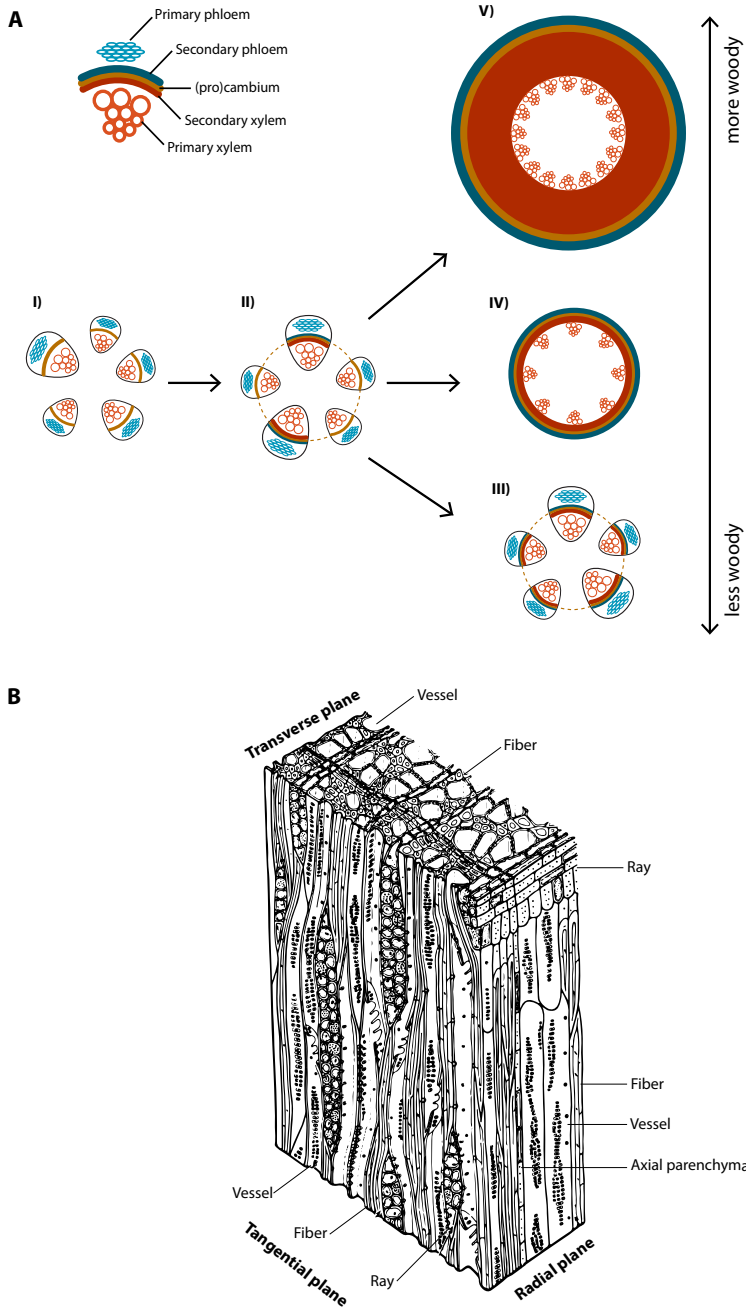


Figure 1 • Transition from primary to secondary growth and wood anatomy. A – Production of secondary xylem and phloem via the division and differentiation of cambium cells. The isolated procambium within each vascular bundles – referred to as the intrafascicular cambium – (i) gradually extends towards adjacent vascular bundles into a closed vascular cambium (ii), which produces secondary phloem to the outside and secondary xylem (wood) to the inside in a continuous range of variation (iii – v). B – 3D view of angiosperm wood with its respective cells. Adapted from Spicer (2016) and Evert (2006).

Intervessel pits are small cavities in the secondary cell wall between adjacent vessels, surrounded by overarching parts of the secondary cell wall, forming the so-called pit borders (Fig. 2 A). The intervessel pits have a crucial role in water transport, since the water movement from roots to leaves has to cross millions of pits in tall trees. Intervessel pits retain a modified porous layer derived from hydrolysis of the middle lamella and of the primary walls, called intervessel pit membrane. In angiosperms, the intervessel pit membranes consist of homogeneous microlayers of cellulose microfibrils and other partly unknown molecules, forming the nano-sized pores which allow the movement of water molecules from one vessel to another, thereby determining the flow resistance accounting for more than 50% of the total xylem hydraulic resistance in many species (Sperry *et al.* 2005; Wheeler *et al.* 2005; Choat *et al.* 2006). Therefore, there should be a trade-off between hydraulic efficiency and safety at the intervessel pit level (Choat *et al.* 2008; Jansen *et al.* 2009).

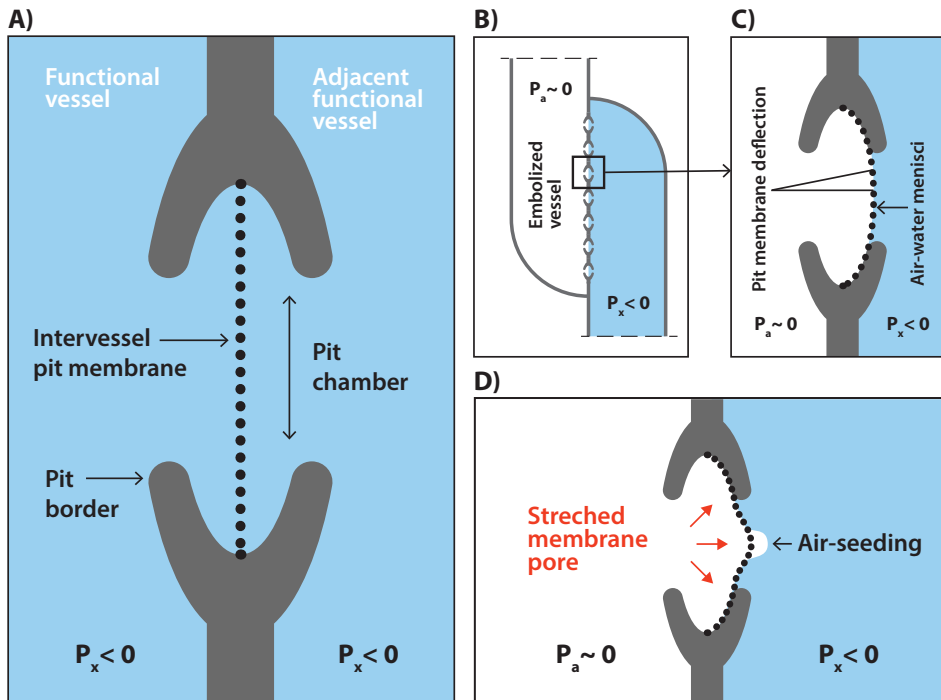


Figure 2 • Intervessel pit in angiosperms and the role of intervessel pit membrane in the air-seeding. A – View of an intervessel pit with its membrane in a relaxed state, between two adjacent functional vessels. B – Embolized vessel leading to an increase in xylem pressure (atmospheric pressure). C – The difference in pressure between adjacent vessels deflects the pit membrane. D – Increasing pressure difference might stretch the pit membrane pores leading to the spread of air bubbles inside the functional vessel via a process called air-seeding. Adapted from Venturas *et al.* (2017).

Surrounding the vessels, there is often a matrix of fibers which often have thin-to-thick secondary walls (Fig. 1 B). This functional division in labour between vessel and fibers reflects the optimized vascular strategy of angiosperms compared to gymnosperms, allowing efficient movement of water through vessels, while mechanical requirements are met by fibers (Pittermann 2010). The parenchyma cells represent the living cells in wood and are axially and radially arranged, depending on the fusiform or ray initial cell types in the vascular cambium. Both types of parenchyma have functions ranging from storage and transport of nonstructural carbohydrates and mineral inclusions (Salleo *et al.* 2004; O'Brien *et al.* 2014; Plavcová & Jansen 2015), to water storage and xylem hydraulic capacitance (Holbrook 1995; Pfautsch *et al.* 2015a), and into a lesser extent, mechanical contributions (Burgert & Eckstein 2001; Reiterer *et al.* 2002; Martinez-Cabrera *et al.* 2009).

The pioneering wood anatomists

Plant anatomy goes back to the 17th century with Hooke's microscopic observation of cell walls in the outer bark of oak trees. Hooke coined the term "cells" for what he observed and reported seeing similar structures in the wood of other plants. Almost 300 years later, Solereder published the first comparative wood anatomical study, entitled *Systematic Anatomy of the Dicotyledons* (Solereder 1908), which was mainly based on observations of twigs found in herbaria. Solereder's master piece formed the basis for the wood anatomy bible by Metcalfe & Chalk (1950): *Anatomy of the Dicotyledons*. This work is still considered as an important source of information, and was triggered at that time by the growing need for timber identification, fueled by the increasing number of unfamiliar timbers that were introduced to the market, especially from tropical countries (Metcalfe 1973).

In the field of evolutionary wood anatomy, the prominent study of Bailey & Tupper (1918), introduced evolutionary hypotheses of tracheary elements in vascular plants, now known as the Baileyan Trends. The Baileyan Trends state that lineages with vessels have evolved from tracheid bearing (i.e. vesseless) species, and this specialization was associated with evolutionary and irreversible changes in the perforation plate morphology from scalariform to simple plates, along with shorter and wider vessel element lengths, and transitions in the arrangement of interconduits pits from scalariform and opposite to alternate. Despite the importance of Bailey's work to the wood anatomy field – considered as a classical text book example to illustrate the phylogenetic signal of comparative wood anatomy – Olson (2012, 2014) offered valuable critiques about his linear view from "primitive" to "specialized" xylem, and mainly emphasized the absence for the causes behind his trends. These causes began to become more evident in the studies of ecological wood anatomy with the pioneering study of Carlquist (1966) about the environmental factors controlling evolution in wood anatomy of Compositae. The field of ecological wood anatomy was expanded later by many others who shed

more light on the importance of climatic drivers behind evolutionary patterns in wood, and also started speculations about the functional role of different xylem cell types, sizes and arrangements (Carlquist 1975, 1980, 1985; Baas 1976; Baas *et al.* 1983; Baas & Schweingruber 1987; Alves & Angyalossy-Alfonso 2000, 2002; Lens *et al.* 2004).

The general ecological trends and trade-offs in xylem anatomy

The literature in ecological wood anatomy has generated a number of global ecological trends. Wood anatomical variation has been assessed in the light of different abiotic drivers, such as latitude and altitude (van den Oever *et al.* 1981; Jansen *et al.* 2004; Lens *et al.* 2004), water availability and temperature (Carlquist 1985, Baas & Carlquist 1985; Alves & Angyalossy-Alfonso 2002; Bosio *et al.* 2010), and soil nutrients (Lupi *et al.* 2012; Dória *et al.* 2016).

A number of ecological trends associated with plants from drier areas and lower latitudes can be summarized as follows: decreasing incidence of scalariform perforations plates (Baas & Schweingruber 1987; Alves & Angyalossy-Alfonso 2000), higher incidence of vested pits (Jansen *et al.* 2004), higher occurrence of vessels with different size classes, shorter vessel elements (Carlquist 1966, 1977) and narrower vessels (Carlquist 1966; Bosio *et al.* 2010), higher vessel density, and more and larger vessel grouping patterns in association with non-conducting fibers (Baas *et al.* 1983; Carlquist & Hoekman 1985), and thicker fiber walls (Alves & Angyalossy-Alfonso 2002).

Because wood performs different functions - water transport, mechanical support and storage of water and nutrients - there are conflicting demands. This would require proficiency at one function, leading to a poorer performance at another function, which may give rise to trade-offs (Baas *et al.* 2004; Sperry *et al.* 2006) (Fig. 3). These different demands may shift depending upon the environment, and therefore, the ecological trends in wood anatomy generally support these trade-offs. A dominant hypothesis regarding the hydraulic safety versus efficiency trade-off has long been proposed (Zimmermann & Brown 1977; Carlquist 1988). Higher efficiency in conduction is linked to larger vessels and allows a more efficient photosynthesis, faster plant growth and lower xylem construction costs, i.e., less xylem tissue for a given amount of leaf area (Poorter *et al.* 2010; Gleason *et al.* 2012). However, across aridity gradients, species should develop corresponding adaptive strategies to water availability. In other words, efficient hydraulic strategy may not allow plants to operate at higher xylem tensions during high transpiration or in soils with low water potential. On the other hand, increasing safety from hydraulic failure corresponds to increased investment in conduit wall area per conduit volume and greater fiber wall thickness to withstand greater negative pressure, which would involve higher carbon construction cost (Hacke *et al.* 2001a, 2004; Jacobsen *et al.* 2007). The conflict is whether we might expect an increased cavit-

tion safety leading to decreased hydraulic efficiency. Some findings indeed support the safety x efficiency trade-off (Tyree *et al.* 1994; Hacke *et al.* 2006; Sperry *et al.* 2008; Meinzer *et al.* 2010), while others, including global meta analyses, show that many species have low efficiency and low safety, which cannot be understood by reference to a trade-off (Choat *et al.* 2005; Gleason *et al.* 2016a).

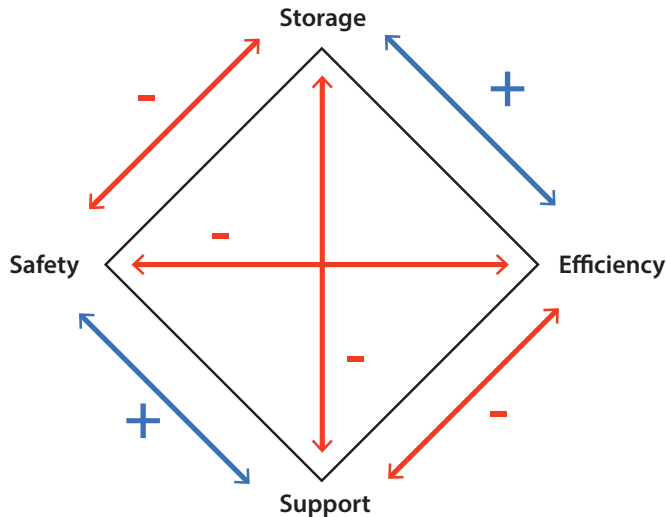


Figure 3 • Relationships (trade-offs) amongst xylem functions. Storage refers to water storage (capacitance) and carbohydrate storage; efficiency refers to the conductive efficiency (K_s in $\text{m}^2\text{MPa}^{-1}\text{s}^{-1}$); support refers to mechanical strength (vessels t/b^2 and fiber wall thickness) and safety refers to resistance to embolism (MPa). Red lines express a negative association between two functions and blue lines a positive one. Adapted from Pratt & Jacobsen (2017).

The xylem trade-off at the individual whole tree level

The majority of wood anatomical studies deal either with interspecific variation, assessing variation in floras of specific regions, or intraspecific variation evaluating individuals of the same species occurring in contrasting environments. Nevertheless, the intra-individual variation in wood anatomical characters is substantial, but often overlooked. A wide range of wood anatomical characters exhibit strong axial variation from the base towards the tip of the stem. In the literature, most of this variation is focusing on vessel widening from top to bottom in order to deal with the more negative water potentials in the upper parts of the trees and to maintain hydraulic integrity (McCulloh & Sperry 2005; Pfautsch *et al.* 2011; Olson *et al.* 2018). Indeed, the water resistance increases with longer conduits pathways as stated by the Hagen-Poiseuille's law, as well as the flow rate increases proportionally to the fourth power of the vessel diameter (Tyree & Zimmerman 2002). To overcome the higher flow resistance, the vessels need to widen basipetally as explained by the West, Brown and Enquist model (WBE model, West *et al.* 1999).

Additionally, vessel density is expected to increase upwards, as has been predicted by hydraulic models and shown by anatomical studies, in order to increase hydraulic conductance with the narrowing of vessels upwards (Höltta *et al.* 2011). In the context of hydraulic safety - efficiency trade-off, intervessel pits constitute a major proportion, roughly half, of the hydraulic resistance (Sperry *et al.* 2006; Choat *et al.* 2008), and, therefore, it is also expected to vary along the main axis of a tree. The few studies dealing with intra-individual variation at the pit level showed different results to pit aperture diameter, which either decrease (Domec *et al.* 2008) or increase (Lazzarin *et al.* 2008) with increasing tree height. Variation in the ultrastructure of intervessel pits in the angiosperm *Eucalyptus grandis* was found to be more variable across vertical gradients (Pfautsch *et al.* 2018).

General introduction to plant hydraulics

The ascent of sap and the vulnerability of xylem to cavitation

The groundbreaking publication dealing with the Cohesion Tension Theory (CTT) by Dixon and Joly (1894) explains that long distance water transport in plants is performed under tension (subatmospheric pressure), which implies that xylem sap is transported in a metastable state. The driving force is generated by evaporative demand at the leaf surfaces and is transmitted by tension in water menisci through a continuous water column that is pulled up by a difference in negative xylem pressure (Tyree & Zimmermann 2002). This mechanism is now widely accepted as the basis of xylem water transport. Under this metastable condition, if pressure drops below the minimum pressure that a water meniscus can support, capillary failure can occur, generating cavitation. This phenomenon could ultimately result in embolism (formation of air bubbles), which reduces the ability of plants to deliver water to leaves (Zimmermann 1983). The two main environmental factors that lead to xylem embolism are freeze-thaw cycles and drought. As sap freezes, dissolved gasses are forced out to the solution, forming gas bubbles in the conduits. When sap thaws, these bubbles can be dissolved or can nucleate in an embolism event (Sperry & Sullivan 1992). During drought events, as the water available in the soil decreases, the xylem tension needed to pull up water from soil to leaves increases. This increase in xylem tension will increase the probability of drought-induced embolism formation (Brodribb & Hill 2000). All the chapters of my thesis deal with drought stress, so I will pay greater attention in explaining the mechanism behind drought-induced embolism formation. The spread of embolism through conduits is explained by the air-seeding mechanism (Fig. 2 B - D) (Tyree & Sperry 1989; Tyree & Zimmermann 2002). It states that a functional xylem conduit becomes air-seeded when a gas bubble from an adjacent gas filled conduit penetrates the shared intervessel pit membrane (Brodersen *et al.* 2013). It is hypothesized that air-seeding

occurs through the largest pore of the intervessel pit membrane, which may even further enlarge when the membrane deflects due to the increasing drought-induced pressure difference between both conduits (Pockman *et al.* 1995; Choat *et al.* 2008; Jansen *et al.* 2009) (Fig. 2 B – D).

Measuring embolism resistance in plants

A vulnerability curve (VC) is a plot of the percentage loss of hydraulic conductivity (PLC) versus the xylem water potential (measured in MPa) (Fig. 4). In other words, it quantifies the plant resistance to xylem embolism (Tyree & Zimmermann 2002). There are key features in a VC that plant physiologists use as proxies for comparing species resistance to embolism: P_{12} , P_{50} and P_{88} . They refer, respectively, to the pressures inducing 12%, 50% and 88% loss of hydraulic conductivity. Physiologically, they indicate the pressure of the starting entry of air in the 3D vessel network, and the potential lethal level of hydraulic failure in conifers and in angiosperms, respectively (Brodribb & Cochard 2009; Urli *et al.* 2013). Nevertheless, P_{50} is the most common proxy used in the literature to compare levels of embolism resistance amongst species of gymnosperms and angiosperms.

There are different possible techniques to construct a VC, differing on how embolism is induced and how it is quantified (Cochard 2006; Cochard *et al.* 2013; Venturas *et al.* 2017). The methods to induce embolism are 1) bench drying, where large excised branches are put to dehydration in air and thereby best mimicking the actual plant drought-stress (Tyree *et al.* 1992); 2) air injection, where positive air pressure induces embolism (Sperry & Tyree 1988); and 3) the centrifuge-based methods that generate negative xylem pressures in the center of the sample due to centrifugal force (Pockman *et al.* 1995; Cochard *et al.* 2010). In order to quantify the relative change in hydraulic conductivity, scientists are using the conductivity apparatus where the flow is measured using a balance (Sperry *et al.* 1988) or the xylem apparatus (Cochard *et al.* 2013). In the high-throughput, custom built centrifuge called Cavitrion, the hydraulic conductance is measured during sample centrifugation, which greatly accelerates the measurement (Cochard *et al.* 2005, 2013). Additionally, it requires less plant material than the bench drying method, reducing sample variability since several pressures can be evaluated on the same segment (Martin-St Paul *et al.* 2014; Venturas *et al.* 2017). More recently, two methods to measure embolism formation were proposed, the optical vulnerability method (Brodribb *et al.* 2016) which allows the visualization of dynamic leaf (and stem and root) embolism during water stress, and the pneumatic method (Pereira *et al.* 2016) which calculates the amount of air flow out of branches under different water potentials.

The challenges of assessing plant hydraulic conductivity is mainly related to the fact that sap is under a metastable state and small perturbations can trigger cavitation. Therefore, methodological issues have always been under debate in the plant

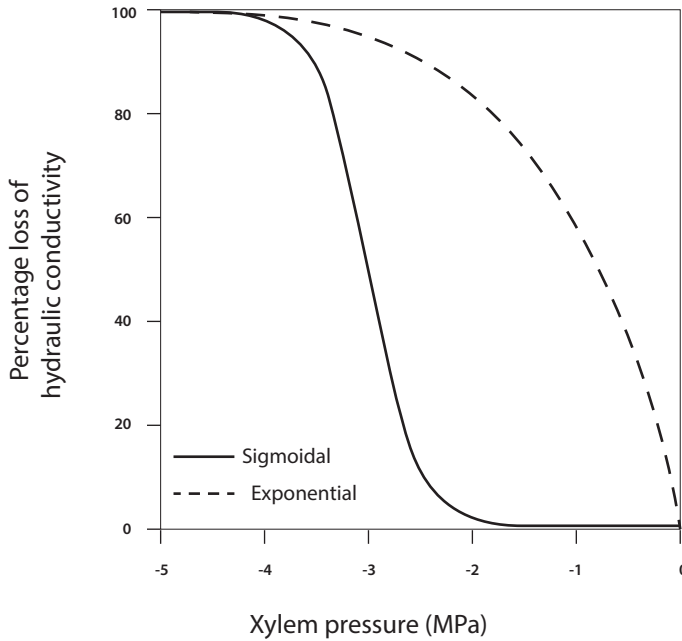


Figure 4 • Xylem vulnerability curve showing the relationship between the loss of hydraulic conductivity and xylem pressure. The sigmoidal and exponential curves are represented, showing the difference in the decline of conductivity at low xylem pressures. *Adapted from Cochard et al. (2013).*

hydraulic field due to the risk of artefacts (Cochard *et al.* 2013). One of the most debated artefacts is the biological value of the “exponential” shape of the vulnerability curves (Delzon & Cochard 2014; Martin-St Paul *et al.* 2014; Torres-Ruiz *et al.* 2014). Vulnerability curves constructed with the Cavitrone technique to species with long vessels have been shown to overestimate vulnerability to embolism (Choat *et al.* 2010; Cochard *et al.* 2010; Wang *et al.* 2014). These so-called “r” shaped curves have been attributed to the “open vessel” artefact, when vessels are long enough to extend from the cut end of the sample to its middle, which results in pre-existing embolisms caused by the open vessel end, and thereby, showing a rapid decline in conductivity at mild xylem pressures that generates the ‘r’ shape of the VC (Fig. 4). This highlights the importance of using cross-validation methods for curves having an ‘exponential’ shape (Cochard *et al.* 2013).

The functional significance of P_{50} in plants

Understanding how conductivity declines in a vessel network has become ecologically relevant in the light of increasing drought episodes under a changing climate. The accumulated embolism, as a consequence of severe water stress, may lead to plant death (Brodribb *et al.* 2010), a relationship emphasized by experimental results demonstrating the link between embolism resistance (P_{50} and P_{88}) and plant mortality (Brodribb & Cochard 2009; Uri *et al.* 2013). In this line, xylem

vulnerability to embolism has been identified as one of the major physiological factors driving reductions in forest productivity and drought-induced mortality in trees (Anderegg *et al.* 2012; Adams *et al.* 2017). In a recent commentary paper, P_{50} has been regarded as a super trait, with the capacity to globally predict species distribution and drought sensitivity (Brodribb 2017).

The importance of hydraulic failure in determining the survival of plants is exacerbated in recent meta-analyses showing that the majority of woody plants across forest biomes operate close to their hydraulic safety margins, i.e., margins observed between the minimum xylem water potential that a plant experiences in the field and the level of water stress that is likely to induce hydraulic failure (Choat *et al.* 2012). In accordance, P_{50} has been shown to be strongly correlated with species distribution in terms of water availability across a wide variety of species (Blackman *et al.* 2012; Anderegg *et al.* 2016; Larter *et al.* 2017; Trueba *et al.* 2017), making P_{50} relevant for modeling of forest die-off under climate change.

There is a vast body of literature focusing on embolism resistance for stems of hundreds of woody species (Choat *et al.* 2012). Contrastingly, stem embolism resistance remains poorly investigated for herbaceous species: it is recorded in the literature for around 30 herbaceous species, of which a minority are eudicots; most herbaceous species investigated are grasses (e.g. Rosenthal *et al.* 2010; Lens *et al.* 2013a, 2016; Nolf *et al.* 2014, 2016; Skelton *et al.* 2017; Dória *et al.* 2018; Volaire *et al.* 2018). Based on the current limited data set, most herbaceous species are considered sensitive to embolism formation. However, more recent studies have shown that some grass species (P_{50} up to - 7.5 MPa; Lens *et al.* 2016) as well as some herbaceous eudicot species belonging to Brassicaceae (P_{50} up to -4. MPa; Dória *et al.* 2019) are remarkably resistant to embolism formation, implying that both herbs and trees share the ability to support very negative water potentials during drought stress.

While the vast majority of studies examines inter-species differences in stem P_{50} , the intra-species variation is less understood. Even though, the degree of hydraulic variation in a single species could have impact on population responses to changes in climate extremes, being, therefore, also important for modeling climate impacts on vegetation (Anderegg *et al.* 2013, 2015).

Linking xylem anatomy with plant hydraulics: looking for structure-function relationships

Wood anatomy plays a central role in plant hydraulic strategies due to the inherent trade-offs associated with wood, namely water transport, mechanical support and food/water storage. Aside from the immediate physiological responses to differences in water availability, plants can adjust their hydraulic architecture over long-term responses (Hacke *et al.* 2017; Tng *et al.* in press). The degree of wood

plasticity at the individual level serves as a suitable proxy for understanding a species' ability to maintain the integrity of xylem water transport under extreme levels of xylem tension (Anderegg & Meinzer 2015).

There is a variety of wood traits that correlate with embolism resistance. Wood density, for instance, is expected to impact the long distance water transport in plants, since it is correlated with the amount of areas dedicated to conduit lumina, suggesting the presence of a mechanical-function trade-off (Pratt *et al.* 2007; Chave *et al.* 2009). The correlation between wood density and embolism resistance is often regarded as indirect and it is explained by the need to resist vessel collapse under drought-induced tension (Hacke *et al.* 2001a; Fichot *et al.* 2010; Ogasa *et al.* 2013). Additionally, increased wood density has also been shown to correlate with decreased sapwood capacitance (Scholz *et al.* 2011) and with minimum leaf water potential (Meinzer *et al.* 2008). At the tissue level, the total vessel wall thickness, as well as the fiber matrix surrounding the vessels also appear to be important in resisting vessel wall collapse under negative pressures (Hacke *et al.* 2001a; Jacobsen *et al.* 2005, 2007; Pratt *et al.* 2007; Dória *et al.* 2018). Likewise, also in herbaceous species, more lignified stems, which theoretically lead to higher stem densities, are linked with hydraulic safety in angiosperms (Lens *et al.* 2013a, 2016; Dória *et al.* 2018; Dória *et al.* 2019). The link between wood density, total degree of lignification and embolism resistance might be also explained by the ability of more lignified cells in avoiding the occurrence of microcracks or ruptures in the conduit walls, potentially nucleating embolisms (Li *et al.* 2016; Dória *et al.* 2018).

The hydraulic relevance of intervessel pit membranes in embolism resistance

Xylem conduits are much shorter than the maximum plant height, and therefore, the long distance water transport has to cross millions of intervessel pits to reach tree canopies. Hence, intervessel pits are essential in regulating hydraulic conductance. Accordingly, the air-seeding hypothesis theoretically provides a link between the ultrastructure of pit membranes and their function in limiting the spread of embolism (Sperry & Tyree 1988; Tyree & Zimmermann 2002). It states that vulnerability to drought stress-induced embolism should be dependent on the ultrastructure, thickness and chemical composition of intervessel pit membranes, since the air-seeding mechanism will depend mostly on the size of the nanometer-sized pit membrane pores (Sperry & Tyree 1988; Choat *et al.* 2008; Lens *et al.* 2011, 2013; Li *et al.* 2016). This is consistent with the fact that the hydraulic resistance of individual pits is imposed by both the average porosity and the thickness of intervessel pit membranes (Choat *et al.* 2006, 2008). The number of microfibril layers and therefore the thickness of intervessel pit membranes are likely to affect the length of the irregularly shaped pores that air-water menisci need to cross before embolism formation may occur in a neighbouring vessel (Choat *et al.* 2008; Jansen *et al.* 2009; Lens *et al.* 2011; Li *et al.* 2016). The thickness of pit membrane

(T_{PM}) ranges in between 70 - 1180 nm (Meyra *et al.* 2007; Jansen *et al.* 2009) and it has been considered the strongest predictor of drought-induced embolism resistance (P_{50}) in angiosperms across a broad taxonomic range of species when the samples are properly fixated (Jansen *et al.* 2009; Lens *et al.* 2011; Li *et al.* 2016).

Positive correlations between T_{PM} and lignification characters, such as the thickness of the vessel wall (Jansen *et al.* 2009; Lens *et al.* 2011) and the proportion of lignified area per total stem area (Dória *et al.* 2018) suggest that there could be developmental coordination between T_{PM} and lignification characters in wood. This provides a functional explanation for the indirect and positive relationship between wood density/lignification and embolism resistance (Hacke *et al.* 2001a; Lens *et al.* 2013a; Dória *et al.* 2018).

An unappreciated structure-function link: increased woodiness vs embolism resistance in otherwise herbaceous lineages

In many predominantly herbaceous families of eudicots, an evolutionary life form shift from herbaceousness towards woody life forms has occurred (Carlquist 1974). Yet, it is still not completely known why and how many times these shifts happened during the evolutionary history of flowering plants. This process is called derived woodiness and it is considered a derived phenomenon because it arises from herbaceousness, which is in turn derived from woodiness that is the ancestral character state in angiosperms (Baldwin & Sanderson 1998; Givnish 1998). The phenomenon of derived woodiness was firstly described on islands relying on the observation that several plant families with predominantly herbaceous species in the continents have woody relatives on islands (Whittaker & Fernández-Palacios 2007). For that reason, it was also referred to as insular woodiness, which is still considered as the most conspicuous aspect of (sub)tropical floras (Carlquist 1974; Lens *et al.* 2013b).

Several hypotheses have been proposed to explain why this shift from herbaceousness toward woodiness occur: 1) the competition hypothesis, proposed by Darwin (1859), states that herbaceous species reaching islands grow in denser populations, forcing plants to grow taller to capture more sunlight; one way to grow taller is to reinforce the stems and thus become woody; 2) the longevity & promotion of outcrossing hypothesis defends the idea that the development of wood would allow these plants to extend their lifespans and flower longer in insular areas that often have few pollinators (Wallace 1878; Böhle *et al.* 1996); 3) the moderate insular climate hypothesis suggests that the mild climate of islands, especially the absence of freezing, would allow these plants to grow continuously throughout the year, promoting the development of woody shrubs (Carlquist 1974); and finally, 4) the absence of large native herbivores hypothesis states that the absence of these herbivores would allow plants to continue develop and grow during their life cycle,

and thereby becoming woodier (Carlquist 1974). However, all of these hypotheses tried to explain the evolution towards derived woodiness on islands (insular woodiness). An ongoing research shows that the majority of shifts from herbs towards woody shrubs in flowering plants took place on continents (305 continental genera vs. 151 insular genera; F. Lens, ongoing review). Interestingly, these continental habit shifts are most abundant in areas with at least a few consecutive dry months per year, such as coastal mediterranean regions (55 genera), steppes (75 genera) and (semi) deserts (80 genera) (F. Lens, ongoing review). This relationship between drought and insular woodiness has also been demonstrated on the Canary Islands, where ca. 65% of the 220 insular woody species grow in markedly dry regions resistance (Lens *et al.* 2013b). Consequently, there seems to be a strong correlation between increased woodiness and increased drought. However, woody shrubs derived from herbaceous relatives do also occur in wet environments throughout the world's islands and continents (47 genera found so far; F. Lens, ongoing review), implying that drought stress is not involved in triggering woodiness in all the derived woody lineages identified so far.

Thesis outline

The aim of my thesis is to investigate the relationship and coordination amongst xylem anatomical traits, giving functional roles in relation to hydraulic conductivity, as well as the influence of abiotic factors in xylem anatomy variation. Additionally, it also brings knowledge about which xylem anatomical characters of stems of woody and herbaceous species explain differences in embolism resistance between species. Therefore, I investigated stem anatomy of woody species occurring in two seasonally dry biomes in Brazil, the cerrado and the caatinga, and of woody and herbaceous species on Tenerife (Canary Islands, Spain). The thesis contains in total 6 chapters, with the first chapter providing the general introduction and outline of the thesis, and the last chapter giving an overall summary and conclusion of my results, which are presented in 4 papers (chapters 2-5), each with their specific objectives:

Chapter 2

Based on the knowledge that embolism is a strong selective pressure and that plants need to balance between hydraulic efficiency and the risk of vulnerability to embolism, it is essential to understand the different xylem anatomical strategies that species use to deal with abiotic environmental constraints. In this chapter, I investigated xylem anatomical adaptations of two co-occurring species in the two

main seasonally dry biomes in Brazil, the cerrado and the caatinga. I tested the significance of the abiotic characters in the variation of xylem anatomical traits of both species, and the particular wood anatomical adaptations of individuals of the same species occurring in both sites.

Chapter 3

Several wood traits exhibit strong axial variation to deal with increasing height constraints, especially the increase in water resistance. In this chapter I assessed the same populations of the two species from the previous chapter, occurring in both sites. We tested how wood traits (co)vary along the main trunk of an individual tree and into what extent the differences between both sites would explain the axial variation observed in wood traits.

Chapter 4

Insular woodiness refers to the evolutionary transition from herbaceousness toward derived woodiness in island plant species. Despite several proposed hypotheses, it is still not completely known why plants have become woody. Although it is still considered an island phenomenon, these life form transitions towards derived woodiness are also common in continents where they thrive in areas with a few consecutive dry months per year. Additionally, most of the derived woody Canary Island species are native to the dry coastal regions, which points to a potential link between increased wood formation and increased drought resistance. In this chapter, I tested this drought stress hypothesis in a daisy clade, using species of the insular woody *Argyranthemum* and their herbaceous relatives native to the European continent.

Chapter 5

Hydraulic failure is one of the main physiological mechanisms associated with reductions in forest productivity and drought-induced tree mortality. Despite the ecological and economic importance of herbaceous species, knowledge about the resistance to embolism formation in herbaceous species remains negligible compared to woody species. Moreover, recent findings in embolism resistance of herbaceous monocots show that these species have a P_{50} range that is almost as extensive as the P_{50} range in woody trees, contradicting the previously idea that all herbaceous species vulnerable to embolism formation. Here, I investigated whether this is also true for a group of eudicot species belonging to Brassicaceae and Asteraceae, occurring in different vegetation zones of the Canary Islands. I also tested whether the difference in mean annual precipitation amongst the vegetation zones would explain the difference in P_{50} for populations collected in different zones.