

Water Relations in *Salix* with Focus on Drought Responses

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Abstract

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Leaves transpire large amounts of water through pores, stomata on their surfaces, which are held open to allow CO₂ entry for photosynthesis. This loss must be balanced by water uptake in roots and further transport up the hydraulic pathway. In this thesis, water relations of willows (*Salix spp.*) were studied. Willows are used, increasingly, for biomass production on arable land where water supply limitation is more pronounced than in their native environments. Hopefully, by identifying physiological traits critical for efficient water use and high drought resistance, and by evaluating how these traits interact with traits of rapid growth, knowledge is gained that can assist breeders in improving willows for commercial use.

Clones of contrasting ecological and geographical origins were characterised in different watering regimes. This included analysis of hydraulic conductances of roots and shoots, stomatal conductance, photosynthesis, water use efficiency of growth (WUE), resistance to drought-induced leaf wilting and xylem cavitation. The lattermost trait was assessed in relation to xylem anatomy and ultrasonic acoustic emissions arising from cavitations.

The drought resistance of clones varied. It was positively related to the resistance of the xylem to cavitation, negatively related to maximal stomatal conductance and positively related to early stomatal closure, and negatively related to maximal growth rate. The resistance to xylem cavitation was positively related to wood density. The ability to acclimate to persistent, moderate drought also varied across clones. Generally, clones showed increases in WUE, whole-plant hydraulic conductance, root to shoot hydraulic conductance ratio, but decreases in stomatal conductance, osmotic potential, leaf area to leaf weight ratio, and leaf area to vessel internal cross-sectional area ratio. Drought acclimation with respect to resistance to xylem cavitation was only observed in one clone. In conclusion, water use and growth traits varied across clones and were in many cases inter-linked by trade-offs. Several of the critical traits could be assessed using rapid screening techniques.

Keywords: cavitation, drought resistance, hydraulic conductance, stomatal conductance, trade-off, ultrasonic acoustic emission, water use efficiency, willow

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Gutta cavat lapidem, non vi sed saepe cadendo

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Appendix

List of papers

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

- I.** Wikberg, J. and E. Ögren. 2004. Interrelationships between water use and growth traits in biomass-producing willows. *Trees*. 18:70-76.
- II.** Wikberg, J. and E. Ögren. 2006. Genotypic variation in drought resistance, drought acclimation and water conservation in biomass-producing willows. *Manuscript*.
- III.** Wikberg, J. and E. Ögren. 2006. Acoustic detection for the quantitative analysis of xylem cavitation in willows. *Submitted to Journal of Experimental Botany; to be accepted after revision*.

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1 Introduction

Water is the most abundant molecule on earth but its availability is nevertheless a limiting factor for global primary production (Lange *et al.* 1982). Approximately 50-60% of the yearly precipitation on land is recycled by plants (Chanine 1992). Less than 1% of the amount taken up by plants is retained in the biomass. The rest is lost to the atmosphere by transpiration through the stomata, the pores on leaf surfaces. Transpiration together with the less important evaporation from the bare ground makes up the evapotranspiration. Globally, the annual evapotranspiration shows a maximum in the tropical regions and decreases with increasing latitude together with the annual light dose and the average temperature (Köppen 1923). These climatic patterns create patterns in vegetation (Griesbach 1872). Generally, forests are the dominating vegetation type but are replaced by grass land in cold and arid regions.

Processes of photosynthesis and mineral uptake are intimately linked to transpiration. The opening of stomata will allow capture of CO₂ that is essential for photosynthesis and transpiration drives the transport of water through the plant that mediates transport of minerals from the soil. The loss of water through stomata is a purely physical event driven by the much higher concentration of water vapour within the leaf than outside. For CO₂, the corresponding concentration gradient is much smaller and this molecule is also somewhat larger and therefore moves somewhat more slowly. Taken together, as one mol of CO₂ moves into the leaf approximately 200 mol of H₂O concomitantly move out of the leaf, thus explaining the much greater rate of transpiration than photosynthesis (Fig 1). [Because a significant part of the CO₂ incorporated in carbohydrates will later on be lost by respiration, the overall balance of rates is even less favourable]. Leaves can control the rate of transpiration by regulating the aperture of stomata. By decreasing the aperture, dehydration is prevented, but the rate of photosynthesis is concomitantly reduced (Sperry 2000).

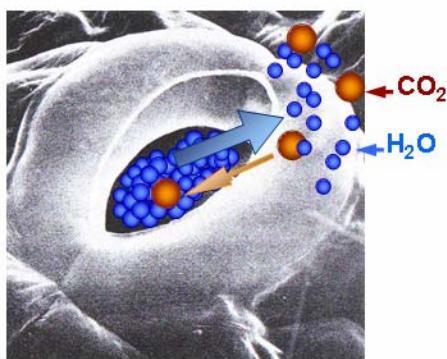


Figure 1. The diffusion of CO₂ into leaves through stomata supports photosynthesis but is associated with diffusion of H₂O out of leaves. The much smaller concentration difference across the stomatal pore for CO₂ than H₂O and the somewhat larger molecular size of CO₂ than H₂O, has the effect that the rate of CO₂ diffusion into leaves is much smaller than the rate of diffusion of H₂O out of leaves. The photograph is modified from Troughton and Donaldsson (1972).

For stomata to remain open and photosynthesis to continue, the water lost through transpiration must be replaced by water taken up and transported along the hydraulic pathway (Fig 2). A well-supported theory, the *cohesion-tension theory*, first formulated by Dixon (1914), underlies our current knowledge of water transport in plants. The theory states that sap flow is driven by *tension*, negative pressure, created by transpiration of leaves. The tension propagates along the continuous water column connecting leaves via petioles and stems to roots and thereby pulls water up the plant. The water column is contained in a series of conduits, inter-connected via pores in their walls. The theory also states that the water column can sustain tension without breaking due to the strong mutual bonding of water molecules, which is created by the hydrogen bond between dipoles, cohesion, and similar bonding, *adhesion*, between water molecules and polar groups on the cellulose molecules lining the conduit walls.

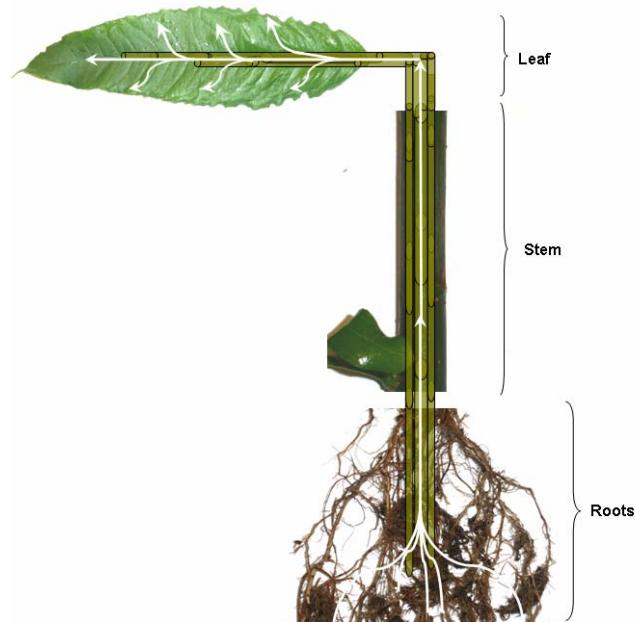


Figure 2. According to the *Cohesion-Tension* theory, water is transported as a continuous column through the plant driven by the negative pressure created by transpiration in leaves. This is possible since water is contained in inter-linked conduits.

Adhesion forces are also involved in the build up of the tension driving sap flow. The walls of the mesophyll cells of leaves, i.e. the photosynthetic cells, contain numerous fine capillaries filled with water due to adhesion. As water vapour moves from the internal air spaces of the leaf to the atmosphere through the stomata, water evaporates from the cell wall capillaries facing these spaces. The

adhesion force, also called capillary force, strives to refill the capillaries, thereby creating a tension that propagates through the capillary network extending into cell walls and connecting mesophyll cells with the nearby conduits of the hydraulic pathway.

However, the continuous water column can break, cavitate, if tension exceeds a critical value (Tyree and Sperry 1989a, Hacke *et al.* 2001a). Typically, cavitation is triggered by small air bubbles being sucked into conduits through the inter-conduit pores (Tyree and Sperry 1989a); when water under tension comes into contact with these air bubbles this can trigger spontaneous vaporization and the tension is thereby lost; the conduit is subsequently filled with air, it suffers embolism, and can no longer participate in transport (Tyree and Sperry 1989a). The critical tension initiating cavitation is typically approached when the rate of transpiration exceeds the rate of water uptake/transport during periods of drought.

In the papers of this thesis, the physiology of water use of willows (*Salix spp.*) was studied. Willows are used, increasingly, on short rotation for their potential to produce biomass for fuel use (Christersson *et al.* 1993). The maximal production, however, is rarely reached, partly because of water supply limitations (Larsson 1998, Lindroth and Båth 1999, Weih 2001, Weih and Nordh 2002). This can be related to willows' high transpiration rates (Persson and Lindroth 1994) and probably also to their high drought sensitivity related to their high vulnerability to cavitation (Pockman and Sperry 2000). However, the interplay of physiological traits controlling growth and survival under limiting and ample water supply are only partly understood. Furthering of our knowledge should help to design breeding strategies for improving willows' water use and drought resistance. Holistic studies encompassing the whole range of traits are needed because many traits are expected to be linked by trade-off relationships where improvement of one trait alone will detrimentally affect another. For instance, reducing the aperture or the number of stomata will increase the drought resistance, but at the expense of reduced photosynthesis. Whenever trade-off relationships exist, the breeding effort must be directed towards optimising rather than maximising traits. Equally important is the question whether the range of clonal variation displayed by willows is large enough to be exploited for breeding purposes. Also, willows are worth studies for themselves: they represent the pioneers, the ecologically important group that first occupies disturbed, resource-rich environments (Kuzovkina and Quigley 2005) and therefore represent a reference for comparative studies of woody plants.

Word list	
Stomata	Pore in the outer cell layer of the leaf allowing entry of CO ₂ for photosynthesis
Conduit	Water conducting cell or series of cells
g_{st}	Stomatal conductance
WUE	Water Use Efficiency
A	Net assimilation (photosynthesis) rate
A/g_{st}	Intrinsic WUE
$\delta^{13}\text{C}$	Measure of intrinsic WUE based on carbon discrimination
Hydraulic architecture	
Huber ratio	Conduit cross sectional per unit of leaf area
Hydraulic conductance	
K_S	Hydraulic conductance of shoots per unit of supported leaf area
K_R	Hydraulic conductance of roots per unit of supported leaf area
K_P	Hydraulic conductance of the whole plant per unit of supported leaf area
Methodological techniques	
HPFM	High Pressure Flow Meter
UAE	Ultra Acoustic Emission
Growth parameters	
LAR	Leaf area per unit of plant dry weight
SLA	Specific Leaf Area, leaf area per unit of leaf dry weight
LWR	Leaf weight ratio, reciprocal of SLA

1.1 Willows as a model system

In nature, willow species are preferentially found on sites where the availability of water is high, for example along ditches and watercourses. They display a large variation in morphology ranging from herblike bushes to tall trees (Fig 3). The dominance of a given species in a given environment should reflect the superior ability of this species to utilize the resources in this environment in competition with other species. If willows are dominating on humid/wet sites they might be expected to use water in a prodigal way, thereby out competing competitors. In reality, however, willows use water more efficiently for growth than most other trees species, as indicated by them having high values of the water use efficiency, WUE, i.e. the ratio between the gain of (above ground) biomass and the total loss of water by transpiration (Lindroth and Cienciala 1996, paper II). At first sight their efficient water use seems to contradict their high rates of transpiration (Persson and Lindroth 1994) but is consistent with their high rates of photosynthesis supporting growth (Ögren and Sjöström 1990, Cienciala and Lindroth 1995). The actual WUE should depend on the relation between the rate of photosynthesis and the rate of transpiration. Willows' high rates of photosynthesis suggest that they can effectively exploit resources of importance for photosynthesis, particularly light and nitrogen besides water, which should be prevalent in their native and disturbed environments. Nevertheless, their high rates of photosynthesis and high rates of growth have the effect that they consume large *amounts* of water. In fact, water consumption can exceed what is supplied by precipitation outside the watercourses even in the relatively cool and humid climate of northern Europe (Lindroth and Båth 1999). This can be problematic when growing willows on arable land for commercial purposes. Willows have been grown for biomass production on arable land for over 20 years in Sweden (Christersson *et al.* 1993) and are now being introduced in other European countries, spurred by policies to increase the production of renewable fuels (Hall and House 1994, Tuskan and Walsh 2001). Expanding willow plantations into warmer and drier climates can be expected to exacerbate the problems of water shortage. Furthering our knowledge of the physiology of water use should help breeders to create new cultivars better adapted to this new situation.

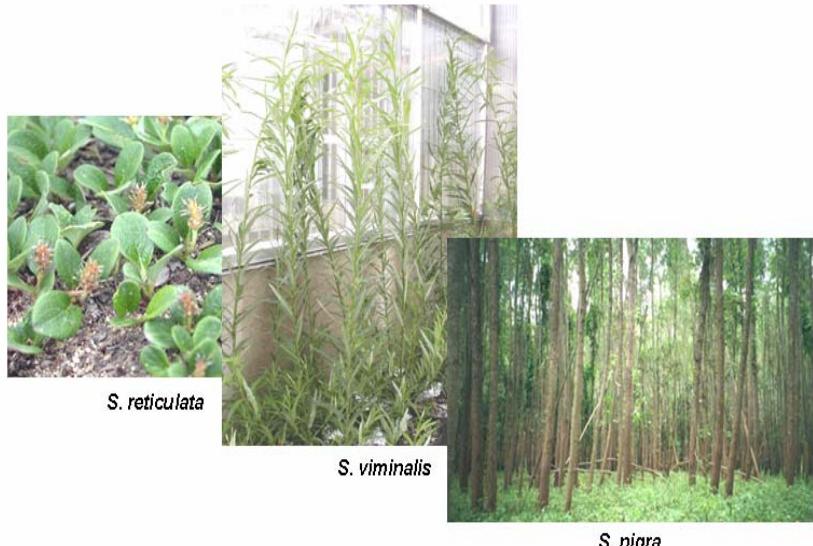


Figure 3. Examples of the morphological variation in the willow genus with sizes ranging from dwarf shrubs to trees. *S. viminalis* is the species most commonly used for biomass production. Photographs taken by Mary Ellen Hart (*S. reticulata*), Jenny Wikberg (*S. viminalis*) and Brian Lockhart (*S. nigra*).

Willows are sensitive to drought (paper I), due to their preferential occurrence in humid/wet environments. Theoretically, their high sensitivity to drought might reflect an inadequate capacity of the hydraulic pathway to supply water to balance the high rates of transpiration. However, the willows' capacity for hydraulic transport seems adequate to meet the demands by transpiration as long as the capacity is not reduced by air entering conduits causing cavitation and embolism (paper II). However, this condition arises remarkably early as drought progresses (paper I, II). Further support for cavitation playing a key role for willows comes from comparative studies of clones. In other words, the higher the resistance to cavitation of the plants, the more severe level of drought the plants can sustain without wilting (paper I). Evidently, cavitation of the hydraulic system deserves closer studies which led to paper III.

2 Objectives

In nature willows seem to be among the most sensitive of woody plants to drought (Pockman and Sperry 2000). To facilitate breeding for improved water use and drought resistance, a number of questions should be addressed: (1) What physiological traits contribute to efficient water use and high drought resistance and how do they interact with traits of rapid growth? (2) What is the range of clonal variation in critical traits setting the potential for breeding? (3) Can diagnostic tools be developed for the identification of critical traits that could serve as selection tools in breeding programs?

Paper I examines the variation in drought resistance and growth traits across willow clones and the interaction of traits. Several traits conferring drought resistance are assessed, most important of which are: (1) the ability of the hydraulic pathway to supply water to leaves, separating the pathway into roots and shoots; (2) the ability of the hydraulic pathway to resist cavitation during drought; and (3) the ability of stomata to regulate transpiration during drought. Differences found in these traits are then related to differences in traits conferring rapid growth during non-limiting water supply in order to identify possible trade-off relationships.

Paper II examines the clonal variation in the efficiency of using water for growth under both limiting and non-limiting water supply. Also, it examines the clonal differences in drought resistance traits when drought develops gradually and is less severe, as opposed to the situation in Paper I where drought is harsher. During gradual imposition of moderate levels of drought, acclimation of traits are likely to occur, but to a varying degree depending on the particular trait. Similar to Paper I, relations between traits of drought resistance and growth are sought.

Paper III deals with one particular trait of drought resistance, namely the ability of the hydraulic pathway to resist cavitation, identified as a key trait in Papers I and II. It was therefore of particular interest to develop a screening tool for this trait. The technique of monitoring acoustic emissions associated with cavitation events is tested here. Various technicalities are investigated such as the monitoring distance in wood in terms of number of centimetres and number of conduits. Functional knowledge is also sought, addressing two important questions: (i) Are there differences in cavitation resistance between different organs? (ii) Are there differences between clones and can such differences be related to differences in their wood anatomy?

3 Background and Current Research

3.1 Overview of wood anatomy

The stem of a woody plant comprises several different cell/tissue layers (Fig. 4), from the periphery and inwards: the protective outer bark; the inner bark with the phloem responsible for sugar transport from leaves to roots; the vascular cambium responsible for growth of new phloem outwards and new xylem inwards; and the mature xylem responsible for water transport. Transport occurs in conduits, comprising separate cell elements, inter-connected by pores in their walls and/or series of cell elements forming vessels; all water-conducting cell elements die after completion of secondary cell wall growth and are then filled with water. Zwieniecki *et al.* (2001) suggested that the interconnecting pores have a variable diameter, since pectin is present in the pores and acts as a hydrogel in response to variable ion concentration in the transported water.

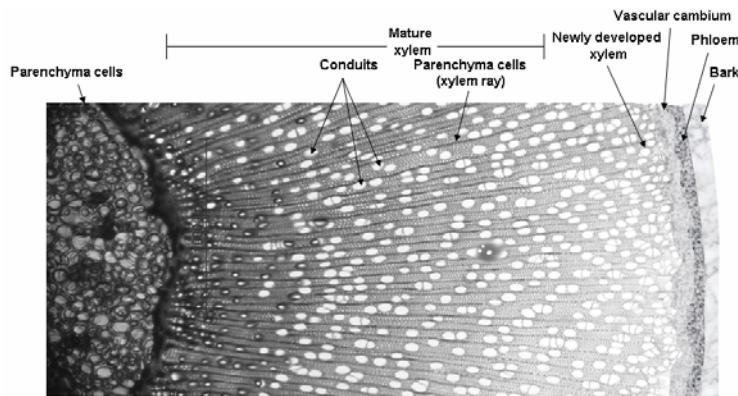


Figure 4. The general anatomy of the wood.

The xylem also contains fibres (supportive, dead cells) and xylem ray cells (undifferentiated, living cells). The length and widths of conduits varies among species, partly reflecting differences in their habitats (Comstock and Sperry 2000). Generally, the hydraulic system should be constructed for the lowest possible transport resistance while minimizing the costs of construction (West *et al.* 1999) and maintenance against cavitation. Thus, compromises are required. Longer and wider conduits impose lower resistance to transport than short and narrow ones but are typically more prone to undergo cavitation and therefore less durable; the detrimental effect of a single event of cavitation is also greater for a large than a small conduit as a larger part of the hydraulic system is affected when a large conduit cavitates (Zimmerman 1983, Comstock and Sperry 2000).

3.2 Role of stomata

By controlling the stomatal aperture, the plant balances in the short term the rate of transpiration with the rate of hydraulic supply (Franks 2004), thereby preventing water-stress induced injuries to xylem and leaves. How stomata sense water status is largely unknown but presumably phytohormone signalling is involved (Jackson *et al.* 2000) as judged from correlative observations that stomatal closure coincides with the accumulation of the abscisic acid, ABA, at stomata (Tardieu *et al.* 1992). The increased amount of ABA presumably reflects net transport along the hydraulic pathway from roots where ABA production is enhanced during drought (Luan 2002). However, the mechanistic role of ABA in stomatal regulation is not completely known (Luan 2002). Possibly, the molecular techniques now available can help to unravel this mechanism.

The promptness by which stomata close with impending water stress varies considerably among species depending on the sensitivity of their tissues to water stress (Sperry 2000) reflecting, for instance, differences in their anatomy (Asamaa *et al.* 2001, Brodribb *et al.* 2003) and for living cells, differences in their content of osmotically active solutes and osmolytes (Augé 1998, Uemura *et al.* 2004, Brodribb and Holbrook 2004, paper II). Also, the maximal conductance of stomata varies, reflecting differences in their sizes and numbers (Aasamaa *et al.* 2001). Thus, a tighter stomatal control of transpiration reduces the risk of water stress inflicting injuries to plants. It also increases the efficiency by which water is used for photosynthesis and growth (Farquhar 1989, Weih 2001, Li *et al.* 2000, paper II). However, the actual *rate* of photosynthesis and the actual *amount* of biomass produced is reduced by a reduced stomatal conductance.

The atmospheric CO₂ concentration steadily increases due to our use of fossil fuels and our conversion of forests into arable and rural land (Shiklomanov *et al.* 2000). Although this is expected to have mainly negative effects on primary production through climatic change, the elevated CO₂ concentration as such, will improve the water economy of plants: due to the increased gradient in CO₂ concentration across stomata, stomatal conductance can be reduced without impeding photosynthesis, thereby reducing water losses and improving water economy, an effect also demonstrated for willows in elevated CO₂ experiments (Johnson *et al.* 2002).

3.3 Stem hydraulics

The transpiration in leaves creates a negative pressure (suction) in the leaf end of the hydraulic system that propagates down the system to roots (Dixon 1914). This transport which was driven by suction was first demonstrated by Hales (1727) in the experiment depicted in Fig. 5. More recently, pressure probes small enough to be inserted into individual conduits have confirmed the high negative pressure within conduits (Wei *et al.* 1999a, Wei *et al.* 1999b). Already in the 1500s, da Vinci formulated what appears to be the first model describing the architecture of the hydraulic pathway (McCulloh and Sperry 2005). He suggested that the cross-sectional area of branches is preserved at intersections from roots to leaves, thereby assuring that adequate water is supplied to all the leaves of the crown. The modern approach to da Vinci's rule is the pipe model, modified to account for the fact that transport occurs in conduits that are interconnected by pores in their walls, thereby also allowing lateral transport (Shinozaki *et al.* 1964). The importance of the hydraulic pathway is convincingly demonstrated by the existence of a positive and universal correlation between the photosynthetic capacity of leaves and the hydraulic conductance of the wood supporting leaves – a correlation that is obeyed by both angiosperms and gymnosperms (Brodribb and Field 2000, Aasamaa and Söber 2001). Thus, the higher the hydraulic conductance, the higher the stomatal conductance can be maintained without risk for dehydration and the higher the capacity for photosynthesis (Lo Gullo and Salleo 1988).

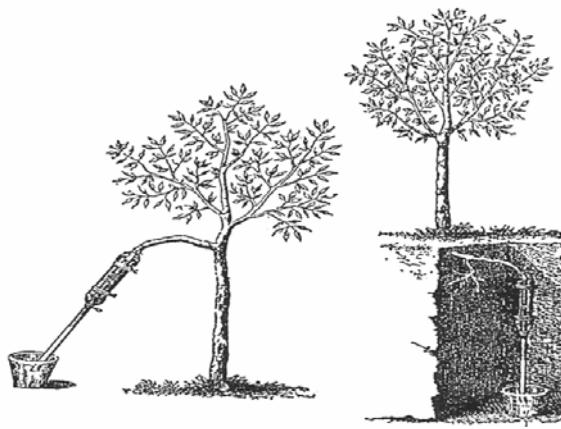


Figure 5. Hales (1727) first demonstrated that water transport of plants is driven by suction (negative pressure): a bucket of water was emptied when connected to roots and branches of a tree.

The supportive role of the hydraulic pathway for photosynthesis is also demonstrated by analysing the so-called Huber ratio, i.e. the ratio of the total cross sectional area of conduits and the total leaf area supported (Huber 1928). These analysis have revealed a somewhat higher Huber ratio for the leader shoots than the rest of

the crown because the leader shoots are more exposed to light and low humidity than the interior shoots (Huber 1928). The method, however, can only provide qualitative data as it ignores the strong dependence of the transport rate on the conduit width: a twofold increase in conduit width will increase transport fourfold per unit of conduit internal cross-sectional area (Zimmerman and Brown 1971). One useful approach for assessing the hydraulic capacity is by relating the actual rate of water transport per unit of pressure difference to the total leaf area supported in order to obtain the so-called leaf-specific conductance, K (Zimmerman 1978). In papers I and II, this was assessed for whole plants, K_p , as well as for roots, K_r , and shoots, K_s , separately. With the invention of the HPFM (high pressure flow meter), this can be relatively easily determined (Tyree *et al.* 1995).

3.4 Root hydraulics

The hydraulic system of the plant can be separated into a root component and a shoot component (paper I, II). Generally, the root component accounts for 20 to 90% of the total resistance (reciprocal of conductance) of the plant (Tsuda and Tyree 1997 and references therein). This variability largely reflects differences in the proportion of roots, their anatomy and the depths at which they grow (Steudle 2001, Rice *et al.* 2004). The resistance to water transport in roots is initially relatively high as water has to pass a complex anatomical structure before reaching the conduits of the xylem (French and Steudle 1989, Steudle and Peterson 1998 and references therein). This structure comprises from outside to inside the epidermis, the exodermis, the central cortex, the endodermis and the pericycle parenchyma, before reaching the conduits of the xylem (Fig. 6). A “composite transport model” is required to describe the lateral water transport into roots because it comprises three different pathways: (i) the apoplastic pathway exterior of cells, i.e. exterior of cell membranes, (ii) the symplastic pathway where water enters the living intracellular continuum at one place by the crossing of the cell membrane and from there on through plasmodesmata connecting cells, and (iii) the transcellular pathway where cell membranes are crossed at several places (Steudle and Peterson 1998). The rate of transport along the latter two pathways is partly controlled by the osmotic properties of the living cells, whereas the apoplastic pathway is solely controlled by the hydraulic properties, i.e. the resistance and the pressure gradient exterior to cell membranes (Steudle and Peterson 1998). With a newly developed pressure-perfusion technique for measuring hydraulic and osmotic properties of roots it has been shown that the apoplastic pathway is the predominant one (Ranathunge *et al.* 2003). The relative importance of symplastic and transcellular pathways, however, can presumably vary because of variation in the number and activity of water

channels, i.e. aquaporins, which facilitate diffusion across cell membranes; aquaporins may play a particularly important role during periods of water stress (Vandeleur *et al.* 2005, Luu and Maurel 2005).

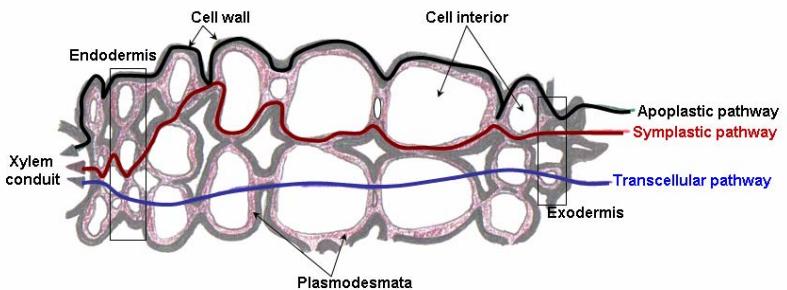


Figure 6. The three pathways for water flow across the outer part of the root before reaching the conduits: i) the apoplastic pathway in cell walls exterior to cells ii) the symplastic pathway where water enters the living intracellular continuum at one place by crossing the cell membrane and passes from cell to cell through plasmodesmata, i.e. minute cytoplasmic threads connecting cells, and iii) the transcellular pathway where water cross cell membranes several times. The various structures passed during water flow are indicated.

The importance of roots for plant water relations increases with the onset of drought for several reasons. First, root growth is typically favoured over leaf growth early on during drought, thus growth of the organ exploiting the most limiting resource is favoured (Hsiao *et al.* 2000). Second, under more severe conditions of drought, root layers may shrink or lateral roots may die from dehydration causing deteriorated contact with soil particles holding water, thus increasing the resistance of hydraulic water transport from soil to roots (Ginter-Whitenhous *et al.* 1983, Lo Gullo *et al.* 1998). Third, roots seem to be particularly prone to suffer cavitation of conduits. In many species, including poplar (Hacke and Sauter 1996, but see Hukin *et al.* 2005) and willows (paper III), roots are more vulnerable to xylem cavitation than shoots.

3.5 Cavitation and embolism

Drought imposes restrictions on water uptake by decreasing water availability and by reducing root-to-soil contact (Lo Gullo *et al.* 1998). The increased mismatch of rates of water uptake/transport and transpiration increases the tension of the xylem sap, increasing the risk of air entering conduits, triggering xylem cavitation and finally leading to air filling, embolism (Tyree and Sperry 1989a).

Such embolism of conduits during the day may be repaired by water refilling during the night, a process believed to be driven by pressurisation of conduits by osmosis (Salleo *et al.* 1996, Tyree *et al.* 1999). For herbs/grasses, this pressurisation may arise in the xylem of the root, so-called root pressure, causing an upwards flow of water purging the system (Milburn and McLaughlin 1974, Tyree *et al.* 1986). For woody plants, the pressurisation presumably occurs locally in the conduits affected: water is drawn into the embolized conduit after osmolytes (solutes) have been introduced into the embolized conduits from the surrounding living cells (Grace 1993); a prerequisite for this putative mechanism to be operative is the decoupling of the embolized conduits from the rest of the hydraulic system during the refilling phase (Zwieniecki and Holbrook 2000, and references therein). Evidence for refilling comes from measurements of positive pressures (Salleo *et al.* 1996, Hacke and Sperry 2003), and from observations using magnetic resonance imaging techniques (Holbrook *et al.* 2001) and rapid freezing techniques (Facette *et al.* 2001), although the reliability of the latter technique has been questioned (Cochard *et al.* 2000). Also, there is an ongoing discussion as to what extent the hydraulic capacity reflects the dynamic cycling of conduits between states of embolism and refilling (Meinzer *et al.* 2001). However, there seems to be an upper limit to the number of such cycles an individual conduit can undergo before mechanical fatigue locks the conduit in a permanent state of embolism (Hacke *et al.* 2001b)

Water is too strongly bound to the hydrophilic walls of the conduits for cavitation to start at the water-to-wall interface (Tyree and Sperry 1989a). Instead, cavitation presumably starts at the water-to-air interfaces in pores in conduit walls by the drawing of air into the conduits; the pressure difference required for this to happen varies between 1 to 10 MPa depending on the size of the pore; the smaller it is, the tighter the water is held in it and consequently the higher the pressure difference required for the air to enter (Sperry *et al.* 1996) (Fig 7).

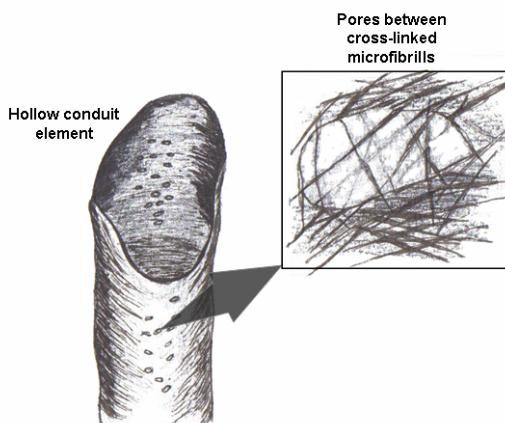


Figure 7. Pores in the conduit walls allow water to pass between conduits. However, an air bubble can be drawn from an air-filled conduit into a water-filled one and thereby seed cavitation occurs (Zimmerman 1983). Pores are formed by a relatively fragile structure of cellulose microfibrills that can be

relatively easily deformed, increasing the risk of air entry.

The size of the pore not only depends on its dimension in the relaxed state, but also on its ability to resist deformation when conduit walls start to bend inwards as tension of the xylem sap is increased (Hacke *et al.* 2001a). A whole range of anatomical factors contribute to increasing the mechanical strength of conduits and thereby help to avoid such deformation (Hacke *et al.* 2001a). However, the introduction of an air bubble into the water under high tension will cause spontaneous vaporization, subsequently leading to air-filling, embolism. With the cavitation of one conduit, the risk for another is increased as the overall conductance is reduced, thereby increasing the tension in the remaining conduits. This may start an escalating process called run-away cavitation, if not prevented by stomatal closure reducing tension (Zimmerman 1983). The cavitation events can be counted by recording counts of ultra acoustic emissions, UAEs, associated with these events (Ritman and Milburn 1991). This technique allows detailed and effortless studies of cavitation events, but unfortunately data does not reveal what cells are cavitating, whether they are conduits, fibers or living cells (Tyree and Sperry 1989a). However, in vulnerable species such as willows there may be a separation in time with conduits starting to cavitate first (paper III). For accurate measurements, particularly for the more resistant species, the vulnerability to cavitation of the hydraulic system should be assessed by direct hydraulic measurements, relating hydraulic conductance to the negative pressure within conduits to obtain vulnerability curves (Sperry *et al.* 1988a). Such measurements have revealed very large differences in the sensitivity to xylem cavitation among species with willows being among the most sensitive (Fig. 8).

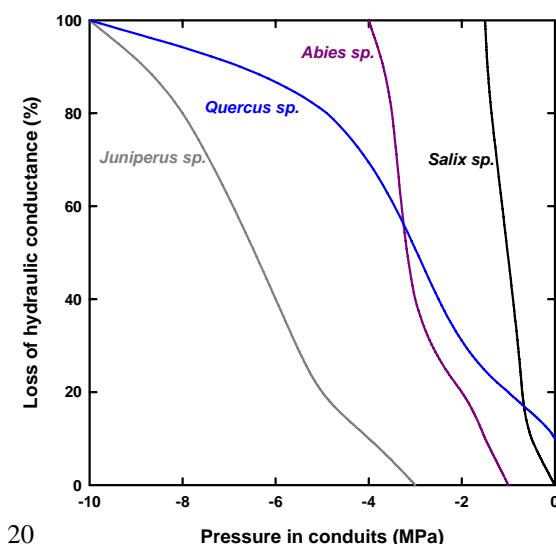


Figure 8. Vulnerability curves, expressed as percentage loss of hydraulic conductance versus the negative pressure of the conduit sap for different species. Data collected from Tyree and Sperry (1989a) for *Abies* sp. and *Juniperus* sp., Corcuera (2004) for *Quercus* sp., and paper I for *Salix* sp.

4 Materials and Methods

4.1 Plant material

To account for as much as possible of the clonal variation in *Salix* spp., clones were selected for studies that had different geographical and ecological origins. Most of these clones are used commercially for biomass production; some of them have been used previously for basketry, but others have been bred more recently at Svalöf Weibull AB. One of the clones, however, was native. The clones were pure species or hybrids of the following species: *S. schwerinii* E.L. Wolf, *S. viminalis* L., *S. dasyclados* Wimm., *S. burjatica* Nas., *S. purpurea* L., *S. repens* L. and, *S. aegyptiaca* L. Willows frequently hybridize in nature and hybrids are therefore relatively easily to create. Several of these clones have been studied in the past for water use (Lindroth and Båth 1999, Weih and Nordh 2002) and for growth and frost resistance (Nordh 1994, Larsson *et al.* 1998, Lennartsson and Ögren 2002). Names and putative geographical origins of clones are summarised in Fig. 9.

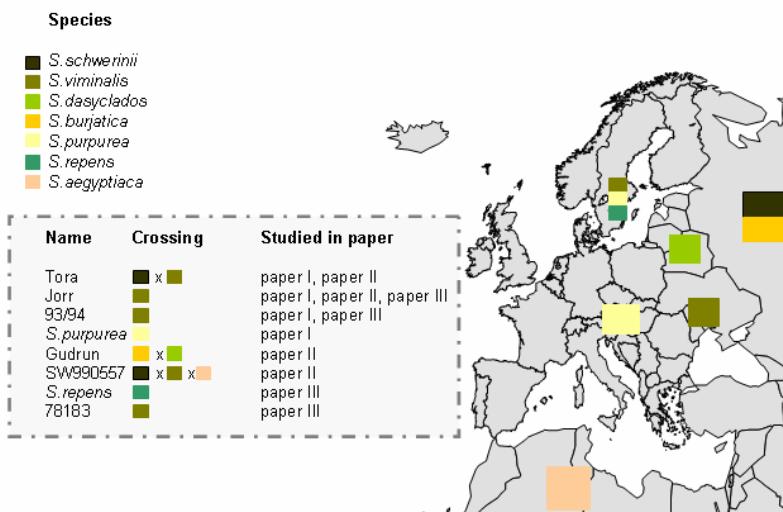


Figure 9. List of willow clones used representing pure species or crossings of species. Geographical distribution of the (parental) species is shown.

4.2 Conductance measurements

The hydraulic conductance of roots and shoots in the absence of embolisms were measured using the High Pressure Flow Meter (HPFM, Dynamax, USA), a technique that has proved reliable in critical evaluations (Tyree *et al.* 1995). The technique is rapid but destructive: the plant part to be assessed is severed from the rest of the plant and water is perfused through the cut end; the rate of perfusion as a function of the applied pressure yields the conductivity; this divided by the leaf area supported, yields the leaf-specific conductance. Potential errors of the technique are mainly related to the clogging of conduits by microbial growth and by wounding reactions, which can be minimised by adding acid/antimicrobial agents and by speeding up measurements (Sperry *et al.* 1988, Tyree *et al.* 1995). Perfusion occurs under pressure and therefore any pre-existing embolism is gradually removed. To minimise the need of removing embolisms by repeated/prolonged measurements, the plant to be assessed is re-hydrated if previously treated to drought and the plant part to be assessed is cut under water. By combining data from measurements of shoots and roots, the whole plant hydraulic conductance can be assessed.

A common response to drought is closure of stomata. A portable porometer (LCA-3; ADC BioScientific, UK) was used to assess the stomatal conductance (g_{st}) and photosynthesis (A) of attached leaves. This instrument provides instantaneous results and is easily handled. Values were related to the actual level of water stress, assessed using parallel leaves bagged in aluminium foil to equilibrate their water status with that of the stem, thereby neutralising any stem-to leaf gradient in water status that introduces unnecessary variability in data. The water status (water potential) of a bagged leaf was measured using either a pressure bomb after detachment (SKPM 1400, Skye Instruments Ltd., UK) or a psychrometer/microvoltmeter combination after cutting small disks from the leaf (HR-33T, C-52 chambers, Wescor, USA). The latter technique had the advantage that it allows repeated measurements of the same leaf to obtain longer data series without need of harvesting a large number of leaves.

4.3 Water Use Efficiency

The water use efficiency (WUE) of growth was determined by dividing the amount of dry matter produced above ground by the amount of water concomitantly used (paper II). The water use efficiency is largely a function of the exchange of CO₂ and H₂O of leaves, the so-called intrinsic water use efficiency; in paper II this was assessed from A/g_{st}, i.e. the rate of photosynthesis divided by stomatal conductance measured at one point in time. A/g_{st} is thus an

instantaneous indicator of intrinsic water use efficiency. A longer term indicator is the degree of discrimination against ^{13}C during photosynthesis, revealed by analysing the carbon isotope composition of leaf tissues (Farquhar *et al.* 1989, Panek 1996). Good agreement between water use efficiency of growth and the intrinsic indicator based on carbon isotope discrimination has been reported numerous times (Weih and Nordh 2002). Similarly good agreement with the indicator based on instantaneous gas exchange is reported in paper II.

4.4 Drought treatment

Plants were subjected to two types of drought treatment, one involving a complete withdrawal of watering finally leading to wilting (paper I) and another involving a restricted watering regime maintaining soil water content at a level imposing a relatively constant non-lethal limitation (paper II). In the former case, resistance to short-term, severe drought was studied, and in the latter case, the longer term, acclimatory responses to moderate drought were studied. The level of drought was assessed from the depression in relative soil water content by weighing pots containing pre-weighed soil or from the depression of plant water status assessed as described in *Conductance measurements*. In the severe drought treatment, the drought resistance at the whole plant level was assessed from the timing of wilting with respect to soil water content and plant water status. Only the longer term drought treatment was carried out in the field which required shielding of pots from occasional rains.

In addition, detached shoots were subjected to bench dehydration. The rate of dehydration was partly controlled by controlling levels of light and temperature but anyway was much faster than for whole plants subjected to drought. By using bench dehydration, clonal differences in resistances to xylem cavitation were assessed. The actual level of resistance, however, was overestimated using bench dehydration as compared to using intact shoots exposed to drought (paper I). This is in apparent conflict with studies showing good agreement between these two techniques (Tyree *et al.* 1992). The explanation might be that under natural drought conditions the xylem conduits undergo several air-filling/water-filling cycles that sensitize them to cavitation due to fatigue (paper I); willows may be particularly prone to such fatigue given their low wood density (Hacke *et al.* 2001b).

4.5 Cavitation measurement

Soon during studies it became evident that the resistance of the xylem to cavitation plays a key role for the survival of willow plants under drought (paper I). Much effort was therefore devoted to developing protocols for measuring the vulnerability to xylem cavitation. Three different protocols were developed to produce so-called vulnerability curves, i.e. plots of the percentage loss of hydraulic conductance versus tension of the xylem sap.

First, vulnerability curves were assessed using whole shoots, subjected to bench dehydration while detached, or subjected to drought while attached to the plant (see *Drought treatment*). After assessing the water status using bagged leaves (see *Conductance measurements*), stem segments were cut from the shoot under water and, to assess their conductance, attached to a water supply of distilled and degassed water. The conductance was obtained from the rate of perfusion assessed by collecting samples at the other cut end; the pressure was low to preserve the embolism's presence. This reading of conductance was related to a second reading after first perfusing segments at an elevated pressure to remove embolisms. The loss of conductance was assessed in relation to the tension of the xylem sap before harvest.

Second, vulnerability curves were assessed using the air pressurisation method, forcing air into conduits by applying positive pressure externally to stem segments rather than by applying negative pressure internally (Sperry and Saliendra 1994). Stem segments were harvested under water and shallow notches were cut into the xylem to facilitate equilibration with air pressure. The segment was mounted into a pressure chamber with both ends protruding. It was then perfused with water to remove any pre-existing embolism before a first reading of the conductance was taken using the protocol described in the paragraph above. Several readings were then taken at stepwise increased air pressure causing increased cavitation-induced loss of conductance. This method produced very similar results as the bench dehydration method (papers I, II), as previously observed for other species (Sperry and Saliendra 1994).

Third, vulnerability curves were assessed by counting ultra acoustic emissions, UAEs, associated with cavitations of conduits (Milburn and Johnson 1966). The expediency of this technique is the major advantage in comparison with direct hydraulic measurements (Sperry *et al.* 1988). Theoretically, each cavitation event gives rise to a discrete UAE; this high detection level can be realised using sensitive instruments with appropriately set time constants (Kikuta *et al.* 2003, paper III). Signal characteristics apart from counts, e.g.

frequencies and amplitudes, vary considerably depending on wood sturdiness, geometry and water content (Tyree and Sperry 1989b, Ritman and Milburn 1990, Kikuta *et al.* 1997). The technique has proven useful for mainly qualitative analysis, for ranking plants for relative levels of drought resistance (Jackson and Grace 1996) and for ranking treatments for relative levels of intensity (Dixon *et al.* 1984, Borghetti *et al.* 1989, Jackson *et al.* 1995). To obtain quantitative data, i.e. vulnerability curves, several prerequisites must be met. First, the listening distance must be known; for the willow stems this was estimated to be 1 cm longitudinally, similarly as reported for other soft wood species (Tyree and Sperry 1989b, Ritman and Milburn 1991). Second, the number of conduits within the listening distance must be known. Third, the xylem must be composed of conduits with similar widths. Wide conduits tend to be more vulnerable to cavitation than narrow ones (Lo Gullo *et al.* 1995, Wheeler *et al.* 2005) and wide conduits contribute more to the overall conductance than narrow ones, reflecting the rule that conductance increases by the second power of the inner diameter (Zimmerman and Brown 1971). Thus, for a mixture of wide and narrow conduits, UAEs collected early during dehydration may be associated with larger loss of conductance than those collected later on (Salleo *et al.* 2000). Fortunately, the width uniformity requirement was met for the willows, as expected for a diffuse-porous species. Fourth, for the UAE technique to provide quantitative data, it appears that the xylem must be composed of particularly vulnerable conduits. At sufficiently depressed water status, cavitations will also occur among non-conducting cells such as fibres and living cells (Tyree and Sperry 1989a). If conduits are vulnerable enough they may have completed cavitations before the non-conducting cells start to cavitate. Fortunately, this requirement seems to be met for at least some of the willows (paper III).

Qualitative, as opposed to quantitative data of cavitation vulnerability, may be obtained by recording the amplitude of UAEs: the amplitude increased with increasing dehydration presumably reflecting the gradual air-filling of the xylem (paper III). A higher rate of amplitude rise should therefore reflect a higher vulnerability of conduits to cavitation as indeed was observed in paper III. Cavitations among the non-conducting cells should have little influence in this case because of their much smaller air/water-holding volumes than conduits.

4.6 Growth and anatomy measurements

Drought resistance is linked to maximal growth rate by a trade-off relationship for the willows (Weih and Nordh 2002). Possible causes for the reduced maximal growth rate with increased drought

resistance was assessed by analyzing growth and its components, thereby assuming a linear rate of growth (paper I, II). This assumption seems justified as canopy closure occurred soon after planting which marks the transition from exponential to pseudo-linear growth (Beadle 1993). Root systems were not included in the biomass harvested because of difficulties in extracting roots from the soil, but this mass comprises nevertheless a very small part of the total biomass for willows (Cienciala and Lindroth 1995). The growth parameters assessed were: the leaf area to (above ground) plant dry weight ratio (LAR) and one of its components, the leaf area to leaf dry weight ratio (SLA) and the role of these parameters and their relations are described in (Lambers *et al.* 1998). In paper II, where variation in leaf-specific whole plant hydraulic conductance, K_p , was observed, the contribution to this variation by variation in total leaf area was assessed by estimating the leaf area to conduit cross-sectional area ratio, i.e. the reciprocal of the Huber value.

The resistance of the xylem to cavitation has been shown to be strongly and positively linked to the density of the wood (Hacke *et al.* 2001, Jacobsen *et al.* 2005, paper I, III). Wood density was assessed for stems and roots by dividing their dry mass by their volume (paper I, III).

Wood anatomy was assessed to interpret UAE data (see *Cavitation measurements*). This included determinations of the number and lengths of conduits within the UAE listening distance to allow calculations of vulnerability curves. The conduit length distribution was assessed using the paint-perfusion method (Zimmermann and Jeje 1981). In short, stem and root segments were perfused with a suspension of blue pigment particles, small enough to enter open conduits but not small enough to pass through inter-conduit pores. After prolonged perfusion, the segments were sectioned at a series of distances and the number of paint-filled conduits was counted. This finally yielded the numbers of vessels of the various lengths within the UAE listening distance (paper III). To decide whether elements shorter than conduits belonged to the hydraulic system and, hence, potentially could contribute to UAEs, a lignin staining dissolved dye was flushed through the cut ends of stems; segments were cross sectioned and the distribution of stain was analyzed under the microscope (none was found outside conduits). Concomitantly, the numbers and cross-sectional areas of conduits were assessed. Finally, in paper III fibre dimensions were analysed under the microscope after first macerating the wood according to Franklin (1945).

5 Results and Discussion

5.1 Water Use Efficiency

There are approximately 400 species of willows, many of which occur as pioneers in resource-rich, disturbed environments (Kuzovkina and Quigley 2005). In Sweden this often means wet/humid sites, along ditches and rivers, etc. Theoretically, an abundance of minerals, water and solar radiation in these places should promote the development of leaves with high photosynthetic capacities (Cowan 1982). Indeed, willows have high intrinsic capacities for photosynthesis (Ögren and Sjöström 1990) that are matched by high conductances for CO₂ uptake through stomata (paper I). On the down side, they have high rates of transpiration and for a closed stand these rates may exceed the rates of precipitation even in cool/humid climates (Lindroth and Båth 1999). Still, it seems that willows use water for photosynthesis and growth more effectively than most other woody species (Rippullone *et al.* 2004, paper II), suggesting that the negative effect of a high stomatal conductance is outweighed by the positive effect of a high photosynthetic capacity. Willows also seem to develop a small root system: roots accounted for only 14% of the total biomass in the study by Cienciala and Lindroth (1995). A small root system should be less costly to maintain than a large one, also promoting growth and water use efficiency when water is not a limiting factor. Nonetheless, a small root system and a high transpiration rate should sensitize the plants to drought. A high sensitivity to drought might not be so much of a problem in their native, often wet environments, but may seriously limit their growth on arable, often well-drained land. However, the existence within the willow genus of a substantial clonal variation in desirable traits (Larsson 1998, Weih and Nordh 2002, paper I, II) and willows willingness to interbreed, suggests that the potential exists to improve willow cultivars by breeding to suit a wide range of climatic and hydrological conditions.

A high rate of transpiration *per se* does not imply a high sensitivity to drought. What matters is the degree to which transpiration rates are balanced by the rates of supply provided by the hydraulic system linking leaves to soil water. By relating the stomatal conductance, g_{st} , to the leaf-specific whole plant conductance, K_p , this balance can be assessed. With an intact hydraulic system, i.e. in the absence of embolisms, willows show g_{st}/K_p values (paper II) that are in the order of those reported for other fast-growing plants (Mencuccini 2003 and references therein) suggesting that willows as a group are not inherently inferior with respect to the ability to maintain positive water balance. This is not to say that this ability does not vary among

willows. In fact, it varies significantly mainly because of variation in K_g (paper II). The variation in K_p is in comparison smaller, although it increases with prolonged drought because clones differ in their abilities to acclimate their hydraulic pathway (paper II).

5.2 Vulnerability to drought

Although willows' intrinsic capacity for hydraulic transport seems sufficient to meet their high demands for transpiration, willows are among the most drought sensitive of woody plants (Tyree *et al.* 1994, Tsuda and Tyree 1997). At least partly this seems to be related to them being sensitive to xylem cavitation leading to deteriorated hydraulic capacity soon after the onset of drought (paper I, III). In fact, the willows' drought resistance positively correlated with their cavitation resistance (paper I), in agreement with the view that cavitation resistance is critical to drought resistance (Sperry *et al.* 2002). Poplar species, closely related to willows, are nearly as vulnerable to xylem cavitation as are the willows (Hacke and Sauter 1996, Hukin *et al.* 2005).

In the clonal comparison of paper I, the vulnerability to cavitation was assessed for stems only. Data are conflicting as to what plant organ is the most vulnerable to xylem cavitation. Stems seem to be the most vulnerable in some species (Tsuda and Tyree 1997, Rood *et al.* 2000, Salleo *et al.* 2000, Hacke and Sperry 2001, Chocard *et al.* 2002) but more often roots are the most vulnerable (Sperry and Saliendra 1994, Alder *et al.* 1996, Hacke and Sauter 1996, Kavanagh *et al.* 1999, Martínez-Vilalta *et al.* 2002, Froux *et al.* 2003). In agreement, roots seem to be somewhat more vulnerable than stems for willows, while petioles seem to be about equally vulnerable as stems (paper III). Roots are also the most vulnerable organs in poplar (Hacke and Sauter 1996, but see Hukin *et al.* 2005).

A higher vulnerability of roots than shoots might seem in conflict with the segmentation theory stating that the peripheral parts of the crown should be affected first (Zimmerman 1983). By confining cavitations to the peripheral parts, the overall canopy size is reduced while the hydraulic supply by roots is maintained, thereby improving chances of survival. Indeed, poplar species native to arid climates sacrifice peripheral shoots during prolonged drought (Rood *et al.* 2000). In willows, the lateral shoots are the first to wilt during the summer (Sennerby-Forsse and Zuffa 1995) and they are the first to wilt during drought treatment (paper I) indicating responses according to the theory. Also in agreement with the theory, lateral shoots are the most expendable parts of the willow plant (Verwijst and Wen 1996). If, according to direct observations, lateral shoots suffer xylem cavitation first, while, according to mechanistic studies,

roots are inherently more sensitive, there is an apparent conflict of data. This conflict, however, can be solved because willows have high g_{st}/K_p ratios like other fast-growing species (paper II). Furthermore, a steep pressure gradient will develop in the peripheral shoots, presumably steep enough to overrule any moderate gradient in intrinsic vulnerability in the opposite direction, and, hence, cavitation will preferentially occur in peripheral shoots. Still, in some tree species roots are so much more vulnerable to xylem cavitation than shoots that the segmentation theory can hardly apply (Mencuccini and Comstock 1997). Their strategy might rather be to restrict cavitations to roots; thereby a rapid drop in foliar water status will result that may trigger a rapid and complete stomatal closure so that spreading of xylem failure at a fatal rate is avoided (Saliendra *et al.* 1995).

Generally, the evolutionary processes should favour the development of a xylem that is both safe and efficient (Tyree *et al.* 1994). But the maximization of both aspects may not be possible within the same plant: the hydraulic conductance will increase with increased conduit width but the sensitivity to cavitation will presumably increase as well. The somewhat greater vulnerability of roots than shoots to xylem cavitation was correlated with roots having wider conduits (paper III). Short and narrow conduits, as compared to long and wide ones, should better resist inward bending of walls caused by tension of the xylem sap; this bending must be controlled in order to avoid deformation of inter-conduits pores, widening them and allowing air entry (Wheeler *et al.* 2005). However, the correlation between conduit width and safety is neither universal nor causal (Logullo *et al.* 1995, Wheeler *et al.* 2005), at least partly because anatomical factors other than a small conduit size contribute to a high resistance to bending such as thick conduit walls (Hacke and Sperry 2001) and a large number of thick-walled fibres surrounding conduits (Jacobsen *et al.* 2005). All factors combined contribute to increasing the density of the wood. Accordingly, the resistance to xylem cavitation typically increases with increasing density of the wood, as observed for willows studied here (paper I, III) and a wide range of other species (Hacke *et al.* 2001a). In willows, however, roots were more vulnerable to cavitation than stems despite them having a denser wood, so the positive correlation between wood density and cavitation resistance broke down when combining data for stems and roots (paper III). Most likely the wide conduits having a large peripheral area also have, for biometric reasons, a large number of inter-conduit pores. A positive relationship between conduit width, total pore number and the vulnerability to cavitation has been reported for other species (Wheeler *et al.* 2005). This was attributed to an increased likelihood that there is a pore sufficiently large to allow air entry with an increased number of pores (Wheeler *et al.* 2005).

It has been suggested that ultimate limit to tree height is set by xylem cavitations (Koch *et al.* 2004). The increased gravitational pull and the increased friction of transport associated with the increased height and length of the hydraulic system both contribute to increasing the tension of the xylem sap. Partial relief is achieved by increasing stem and root diameters (Becker *et al.* 2000), by increasing sapwood porosity and by decreasing leaf area to sapwood area ratios (Zimmerman 1983). However, these changes may not be sufficient (Mencuccini and Magnani 2000) and reallocation of dry matter from shoots to roots may be required as well (paper II). Such reallocation will increase carbon costs of growth and, hence, may contribute to the trend of decreasing productivity with increasing tree height and age (Magnini *et al.* 2000).

Apart from building a mechanically strong xylem, cavitation can be effectively avoided by closing stomata. In fact, stomatal closure has been suggested to primarily serve the purpose of protecting the plant from xylem failure (Chochard *et al.* 2002, Lemoine *et al.* 2002). In agreement with this idea, stomata closed more rapidly in the more sensitive clones with respect to xylem cavitation (paper I). However, stomata did not close *early* enough to prevent these clones from suffering extensive xylem failure already at a mild water stress (paper I). Thus, the high vulnerability to xylem cavitation was nevertheless manifested in these clones.

By delaying stomatal closure upon drought a higher productivity can be realized than otherwise possible (Harvey and Driessche 1999). However, this strategy must be combined with measures to protect the xylem as well as the leaves to the depressed water status that inevitable follows. Wilting of leaves can be delayed by increasing the cellular content of solutes (osmoregulation), thereby delaying the loss of cellular turgor. Willows can osmoregulate (paper II) to about the extent as other tree species (Aspelmeier and Leushner 2004). Also, wilting can be delayed by increasing the robustness of the leaves, i.e. by decreasing their area to weight ratio, SLA (Lamont *et al.* 2002). Indeed, drought resistance of the willows was roughly inversely related to their SLA values (paper I). However, a decreased SLA will negatively affect growth under favourable conditions because carbon cost for expanding leaf area is increased (Cornelissen *et al.* 1996). Several of the above mentioned adaptive responses to drought are under developmental control of phytohormones such as abscisic acid, ABA (Aasamaa *et al.* 2002).

5.3 Interactions between water, nitrogen and temperature limitations

The plant growth rate strongly depend on nitrogen (N) availability (Öhlund and Näsholm 2001) which motivates studies on the N economy of growth, for instance growth rate per unit of plant N content (Farquhar *et al.* 1989, Weih and Nordh 2002). Weih (2001) noted that the growth rate of willow plants increased with increasing N content. However, a fast-growing hybrid clone, as compared to a slower-growing natural one, managed to acquire a higher N content at the higher N supply rates and thus could grow at a higher maximal rate. Thus, the faster-growing clone used N more effectively at the higher fertilization regimes typical for arable land. This contrasted with the efficiency of using water for growth (WUE) that was higher for the slower-growing clone throughout (Weih 2001).

Shortages in N and water supply induce similar responses in plants, most conspicuous being the relative increase in root growth (Wilson 1988). However, upon water stress, part of this effect might reflect the need of improving hydraulic capacity and cavitation resistance of roots rather than the uptake rate as such (paper II). While the former two aspects are associated with increased root diameter and wood density, the latter aspect is associated with increased root surface area. For the exploration of N, surface area is most important. This may potentially explain the observation that water stress causes decreased rate of N uptake per unit of root length (Weih 2001).

Low soil temperature in spring imposes limitations on both N uptake (Karlsson and Nordell 1996, Dong *et al.* 2001) and water uptake (Lopushinsky and Kaufmann 1984) but for different reasons. The N uptake is impeded because low temperature restricts root activity and water uptake/transport is impeded because water viscosity is increased with decreased temperature, increasing tension of the xylem sap. To avoid dehydration of leaves and xylem cavitation, stomata must remain partially closed as long as soil temperature is low, thus restricting photosynthesis (Meinzer 2002). Whatever the cause for the delay of the start of the growing season, each day of delay causes a notable loss of annual production (Cannell 1989). Accordingly, a rapid start of growth has been identified as a key criterion when selecting for high-growth yield varieties of willows (Kopp *et al.* 2001, Weih and Nordh 2002). The increase in allocations to roots have been shown to be associated with a decreased root N uptake per unit root length with the suggestion that water stress affect plant by subjecting them to nutrient stress (Weih 2001).

5.4 Trade-offs

Several trade-off relationships between growth and drought resistance traits were identified for the willows (Fig. 10). Three relationships are based on resource allocation patterns. Allocation of resources into leaf area promotes growth during establishment (Cornelissen *et al.* 1996, paper I), but is at the expense of allocation into leaf mass, wood density and/or root mass, factors which all contribute to drought resistance (paper I). First, the leaf mass to leaf area ratio, i.e. the reciprocal of SLA, positively correlated with the level of drought resistance presumably because the building of a robust leaf helps to maintain its shape when leaf turgor declines during drought, thereby delaying wilting and the mechanical injuries associated with this (Salleo and Lo Gullo 1990, Wookey *et al.* 1995, Carter *et al.* 1997, Yin 2002). Second, wood density positively correlates with the resistance to xylem cavitation and, hence, drought (paper I, III). Third, with re-allocation of resources from shoots to roots drought resistance is increased (Weihs and Nordh 2002, paper II), partly because of reduced transpiration versus supply rates, and partly because it increases uptake as such (Larcher 1995). In this thesis, the root to shoot ratio was assessed indirectly by measuring the root to shoot hydraulic conductance ratio (K_R/K_S) rather than by harvesting plants. Possibly, the potential error associated with the indirectness of the technique is offset by the advantage of not having to excavate all roots thereby avoiding the obvious risk of failing to do so. Finally, the dual nature of stomata of promoting both photosynthesis and transpiration is the basis for a functional trade-off relationship (paper I). For the willows, these four trade-offs seems to be strongly coupled to each other in that the whole set of traits, either those promoting growth or those promoting drought resistance, are represented in the same genotype. However, this may be coincidental, reflecting the fewness of clones studied. In fact, some uncoupling of trade-off relationships occurred during acclimation to moderate drought: clones that were more resistant to xylem cavitation invested lesser resources into root growth than those that were more sensitive to xylem cavitation (paper II). Nevertheless, the clones characterised by us (papers I-III), also characterised by Weihs and co-workers (Weihs 2001, Weihs and Nordh 2002) and were similarly characterized despite differences in growth conditions used.

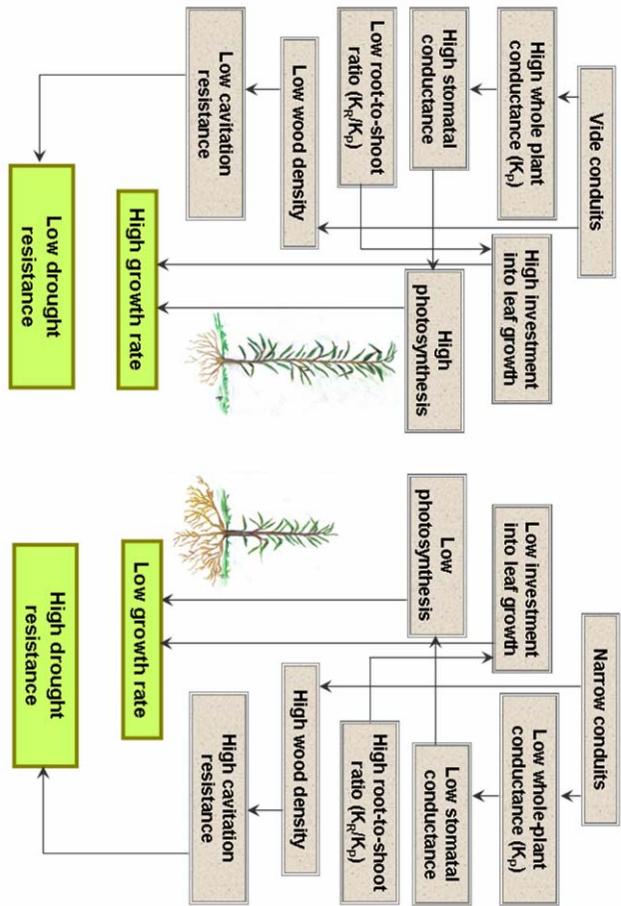


Figure 10. Trade-off relationships between traits promoting growth during favourable conditions or promoting drought resistance, studied in this thesis.

6 Conclusion

6.1 Improved drought resistance

Willows have high photosynthetic capacities partly because they have high stomatal conductances. Hence, they also have high transpiration rates that may exceed the rate of water supplied by precipitation. Because of their high photosynthetic capacities they nevertheless use water for growth with remarkably high efficiency. Willows' small root systems may contribute to this by minimising respiratory maintenance costs but will also sensitize the plants to drought. A low drought resistance for this and other reasons and a high transpiration rate may cause problems when using willows for biomass production on arable land. However, genetic improvements seem possible given the large clonal variation there exists among willows with respect to desirable traits.

6.2 Cavitation resistance: a critical trait

Willows are not inherently inferior to many other fast growing species with respect to the ability to supply water through the hydraulic system to balance the high transpiration rates as long as the system is intact. However, the hydraulic system rapidly deteriorates from xylem cavitation with the onset of drought with roots being more sensitive than stems. Nevertheless, a relatively high stomatal to hydraulic conductance ratio should produce sufficiently steep a pressure gradient at the peripheral shoots to ensure that these more expendable parts suffer xylem cavitation first. Several anatomical factors contribute to increasing the mechanical strength of the xylem, thereby improving its resistance to cavitation. This explains the correlation between wood density and resistance to xylem cavitation. Also, xylem cavitation is prevented by closing stomata, a response, however, that may not occur rapidly enough in all willow clones.

6.3 Trade-offs affecting growth

Growth capacity and drought resistance traits are inter-linked in trade-off relationships. First, increased density of the wood is associated with increased resistance to cavitation, but is at the expense of investment into leaf area promoting growth. Second, increased stomatal conductance increases both photosynthesis (growth) and transpiration (dehydration). Third, increased investment into root growth promotes drought resistance but is at the expense of leaf growth.

7 Breeding for a Purpose

Since 1987 willows have been bred for short rotation forestry (SRF) at Svalöf Weibull AB (Åhman and Larsson 1999). Breeding for resistance to diseases, insects and frosts have had high priority in addition to breeding for high growth yields. Many thousand of genotypes have been bred, mainly using *Salix viminalis* but also using other willow species originating from Siberia, central Russia, Canada and Greece to create hybrids. Willow-based SRF has the potential to contribute to the replacement of fossil fuels in Europe (Mackeschin 1999). By using perennial woody plants, a higher net energy output can be achieved than using traditional agricultural crops.

Another application of willow-based SRF is the phytoremediation of soil and recycling of nutrients from wastewater in combination with biomass production (Aronsson 2001). Many studies have indicated that willows, as compared to more conventional agricultural crops, extract more metals such as mercury, cadmium and lead from waste water and soil (Greger and Landberg 1999, Bertholdsson 2001, Hasselgren 2003). These metals can then be permanently removed by harvesting plants and filtering out the metals during combustion in furnaces. Hence, by using willows rather than conventional crops metal concentrations can be lowered in arable soils and the use of waste water for fertilization is made possible (Lundström and Hasslegren 2003).

Our studies imply that a large clonal variation exists among willows with respect to traits of importance for growth under limiting water supply. Also, potentially useful selection tools for the breeding of improved cultivars have been suggested. For instance, the intrinsic water use efficiency may be probed by gas exchange techniques and the resistance to xylem cavitation and, hence, drought may be probed by assessing wood density.

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9 References

- Aasamaa, K. and A. Söber. 2001. Hydraulic conductance and stomatal sensitivity to changes of leaf water status in six deciduous tree species. *Biologia Plantarum* 44:65-73
- Aasamaa, K., Söber, A. and M. Rahi. 2001. Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomata sensitivity to changes of leaf water status in temperate deciduous trees. *Aust. J. Plant. Physiol.* 28:765-774
- Aasamaa, K., Söber, A., Hartung, W. and Ü. Niinemets. 2002. Rate of stomatal opening; shoot hydraulic conductance and photosynthetic characteristics in relation to leaf abscisic acid concentration in six temperate deciduous trees. *Tree Physiol.* 22:267-276
- Åhman, I. and S. Larsson. 1999. Breeding for resistance in willow energy production. *Växtskyddsnotiser*. 63:17-19
- Alder, N.N., Sperry, J.S. and W.T. Pockman. 1996. Root and stem xylem embolism, stomatal conductance, and leaf turgor in *Acer grandidentatum* populations along a moisture gradient. *Oecologia*. 105:293-301
- Aspelmeier, S. and C. Leuschner. 2004. Genotypic variation in drought response of silver birch (*Betula pendula*): leaf water status and carbon gain. *Tree Physiology*. 24:517-528
- Aronsson, P. and K. Perttu. 2001. Willow vegetation for wastewater treatment and soil remediation combined with biomass production. *Tree Forestry Chronicle*. 77(2):293-299
- Augé, R.M., Duan, X., Croker, J.L., Witte, W.T. and C.D. Green. 1998. *Journal of Experimental Botany*. 49:753-759
- Beadle, C.L. 1993. Growth analysis. In: Hall DO, Scurlock JMO, Bolhár-Nordenkampf HR, Leegood RC, Long SP (eds) *Photosynthesis and production in a changing environment. A field and laboratory manual*. Chapman & Hall, London, pp 36-46
- Becker, P., Meinzer, F.C. and S.D. Wullschleger. 2000. Hydraulic limitation to tree height; a critique. *Functional Ecology*. 14:4-11
- Bertholdsson, N.-O. 2001. Fytoremidierung av tungmetaller med hjälp av *Salix*. *Sveriges Utsädesförenings tidskrift*. 2:84-90
- Borghetti, M., Raschi, A. and J. Grace. 1989. Ultra acoustic emission after cycles of water stress in *Picea abies*. *Tree Physiology*. 5:229-237
- Brodrribb, T.J and T.S. Field. 2000. Stem hydraulic supply is linked to leaf photosynthetic capacity: evidence from New Caledonian and Tasmanian rainforests. *Plant, Cell and Environment*. 23:1381-1388
- Brodrribb, T.J., Holbrook, N.M., Edwards E.J. and M.V. Gutiérrez. 2003. Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. 26:443-450
- Cannell, M.G.R. 1989. Physiological basis of Wood production: a Review. *Scand. J. For. Res.* 4:459-490
- Carter, E.B., Theodorou, M.K. and P. Morris. 1997. Responses to *Lotus corniculatus* to environmental change *New Phytologist*. 136:245-253
- Chanine, M.T. 1992. The hydrological cycle and its influence on climate. *Nature*. 359:373-380
- Christersson, L., Sennerby-Forsse, L. and L. Zsuffa. 1993. The role and significance of woody biomass plantations in Swedish agriculture. *Forestry Chronicle*. 69:687-693
- Cienciala, E. and A. Lindroth. 1995. Gas-exchange and sap flow measurements of *Salix viminalis* trees in short-rotation forest. II. Diurnal and seasonal variations of stomatal response and water use efficiency. *Trees*. 9:295-301
- Cochard H., Bodet, C., Améglio, T. and T. Cruizat. 2000. Cryo-scanning electron microscopy observations of vessel content during transpiration in walnut petioles. Facts or Artefacts? *Plant Physiol.* 124:1191-1202
- Cochard, H., Coll, L., Le Roux, X. and T. Améglio. 2002. Unravelling the effects of plants hydraulics on stomatal closure during water stress in walnut. *Plant Physiol.* 128:282-290
- Comstock, J.P. and J.S. Sperry. 2000. Theoretical consideration of optimal conduit length for water transport in vascular plants. *New Phytologist*. 148:195-218

- Corcuera, L., Camarero, J.J. and E. Gil-Pelegrín. 2004. Effects of a sever drought on *Quercus ilex* radial growth and xylem anatomy. Trees. 18:83-92
- Cornelissen, J.H.C., Diez, P.C. and R. Hunt. 1996. Seedling growth, allocation and leaf attributes in a wide range of woody plant species and types. J. Ecol. 84:755-765
- Cowan, I.R. 1982. Regulation of water use in relation to carbon gain in higher plants. In: Lange OL, Nobel PL, Osmond CB, Ziegler H (eds.) Encyclopedia of plant physiology, NS. Physiological plant ecology II, vol. 12 B. Springer, Berlin Heidelberg New York, pp 589-613
- Dixon, H.H. 1914. Transpiration and ascent of sap in plants. Macmillan, London
- Dixon, M.A., Grace, J. and M.Y. Tyree. 1984. Concurrent measurements of stem density, leaf and stem water potential, stomatal conductance and cavitation on a sampling of *Thuja occidentalis* L. Plant Cell, Environment. 7:615-618
- Dong, S., Scagel, C.F., Cheng, L., Fuchigami, L.H., and P.T. Rygiewicz. 2001. Soil temperature and growth stage influence nitrogen uptake and amino acid concentration of apple during early spring growth. Tree Physiology. 21:541-547
- Eckstein, R.L., Karlsson, P.S. and M. Weih. 1999. Life span and nutrient resorption as determinants of plant nutrient conservation in temperate-arctic regions. New Phytologist. 143:177-189
- Facette, M.R., McCully, M.E., Shane, M.W. and M.J. Canny. 2000. Measurement of time to refill vessels. Plant Physiol. Biochem. 39:59-66
- Farquhar, G.D., Ehleringer, J.R. and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Ann. Rev. Plant. Physiol. Plant Mol. Biol. 40:503-537
- Franks, P.J. 2004. Stomatal control and Hydraulic conductance, with special reference to tall trees. Tree Physiology. 24:865-878
- Franklin, G.L. 1945. Preparation of thin sections of synthetic resins and wood-resin composites, and a new macerating method for wood. Nature. 155: 51
- French, J. and E. Steudle. 1998. Axial and radial hydraulic resistance to roots of maize (*Zea mays* L.) Plant Physiology. 91:719-726
- Froux, F., Durey, M., Dreyer, E. and R. Huc. 2003. Vulnerability to embolism differs in roots and shoots and among three Mediterranean conifers: consequences for stomatal regulation of water loss? Tree structure and Function. 19:137-144
- Ginter-Whitehouse, D.L., Hickley, T.M. and S.G. Pallardy. 1983. Spatial and temporal aspects of water relations of three tree species with different vascular anatomy. Forest science. 29:317-329
- Grace, J. 1993. Refilling of embolized xylem. In: Borgetti, M., Grace, J. and A. Rachi. (Eds.) Water transport in plants under climate stress. Cambridge University Press. Cambridge. pp. 51-62
- Greger, M. and T. Landberg. 1999. Use of cillow in phytoextraction. Int. J. Phytorem. 1(2):115-124
- Griesbach, A. 1872. Die Vegetation der Erde nach ihrer klimatischen Anordnung. Wilhelm Engelmann, Leipzig, pp. 709
- Hacke, U.G. and J. Sauter. 1996. Drought-induced xylem dysfunction in petioles, branches, and roots of *Populus balsamifera* L. and *Alnus glutinosa* (L.) Gaertn.. Plant Physiology 111:413-417
- Hacke, U.G. and J.S. Sperry. 2001. Functional and ecological xylem anatomy. Perspectives in Plant evolution and systematics. 42: 97-115
- Hacke, U.G. and J.S. Sperry. 2003. Limits to xylem refilling under negative pressure in *Laurus nobilis* and *Acer negundo*. Plant, Cell and Environment. 26:303-311
- Hacke, U.G., Sperry, J.S., Pockman, W.T., Davis S.D. and K.A. McCulloh. 2001a. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. Oecologia. 126:457-461
- Hacke, U.G., Stiller, V., Sperry, J.S., Pittermann, J., and K.A. McCulloh. 2001b. Cavitation fatigue. Embolism and refilling cycles can weaken the cavitation resistance of xylem. Plant Physiol. 125:779-786
- Hales, S. 1727. Vegetable Staticks. W & J Inneys and T Woodward, London
- Hall, D.O. and J.I. House. 1994. Trees and biomass energy: carbon storage and/or fossil fuel substitution? Biomass and Bioenergy. 6:11-30

- Harvey, H.P and R. Van Den Driessche. 1999. Nitrogen and potassium effects on xylem cavitation and water-use efficiency in poplars. *Tree Physiology*. 19:943-950
- Hasselgren, K. 2003. Use and treatment of municipal waste products in willow biomass production. Rapport 3242. Lunds tekniska Högskola. Lunds Universitet
- Hsiao, T.C. and X. Liu-Kang. 2000. Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relations to water transport. *Journal of experimental Botany*. 51:1595-1616
- Holbrook, N.M., Ahrens, E.T., Burns, M.J. and M.A. Zwieniecki. 2001. In vivo observation of cavitation and embolism repair using magnetic resonance imaging. *Plant Physiol.* 126:27-31
- Huber, B. 1928. Weitere quantitative Untersuchungen über das Wasserleitungssystem der Pflanzen. *Jahrb. wiss. Bot.* 67:877-959
- Hukin, D., Cochard, H., Dreyer, E., Le Thiec, D. and M.B. Bogaert-Triboullet. 2005. Cavitation vulnerability in roots and shoots: does *Populus euphratica* Oliv., a poplar from arid areas of Central Asia, differ from other poplar species? *Journal of Experimental Botany*. 56:2003-2010
- Jackson, G.E. and J. Grace. 1996. Field measurement of xylem cavitation: are acoustic emissions useful? *Journal of Experimental Botany*. 47:1643-1650
- Jackson, G.E., Irvine, J. and J. Grace. 1995. Xylem cavitation in Scots pine and Sitka spruce samplings during water stress. *Tree Physiology*. 15:783-790
- Jackson, R.B., Sperry, J.S. and T.E. Dawson. 2000. Root water uptake and transport: using physiological processes in global predictions. *Trends in plant science*. 11:482-488
- Jacobsen, A.L., Ewers, F.W., Pratt, R.B., Paddock, III, W.A. and S.D. Davis. 2005. Do xylem fibres affect vessel cavitation resistance? *Plant Physiology*. 139:546-556
- Johnson, J.D., Tognetti, R. and P. Parisi. 2002. Water relations and gas exchange in in poplar and willow under water stress and elevated atmospheric CO₂. *Physiologica Planatarum*. 115:93-100
- Karlsson, P.S. and K.O. Nordell. 1996. Effects of soil temperature on the nitrogen economy and growth of mountain birch seedlings near its presumed low temperature distribution limit. *Ecoscience. Sainte-Foy*. 3:183-189
- Kavanagh, K.L., Bond, B.J., Aitken, S.N., Gartner, B.L. and S. Knowe. 1999. Shoot and root vulnerability to xylem cavitation in four populations of Douglas-fir seedlings. *Tree Physiology*. 19:31-37
- Kikuta, S.B., Lo Gullo, M.A., Nardini,A., Richter, P. and S. Salleo. 1997. Ultra acoustic emission from dehydrating leaves of deciduous and evergreen trees. *Plant, Cell and Environment*. 20:1381-1390
- Kikuta S.B., Hietz P. and H. Richter. 2003. Vulnerability curves from conifer sapwood sections exposed over solutions with known water potentials. *Journal of Experimental Botany*. 54: 2149-2155
- Koch, G.W., Stillett, S.C., Jennings, G.M. and S.D. Davis. 2004. The limits to tree height. *Letters to nature*. 428:851-854
- Kopp, R.F., Smart, L.B., Maynard, C.A., Isebrands, J.G., Tuskan, G.A. and L.P. Abrahamson. 2001. The development of improved willow clones for eastern North America. *Forestry Chronicle* 77:287-292
- Köppen, W. 1923. Die Klimate der Erde. Walter de Gruyter, Berlin, pp. 369
- Kuzovkina, Y.A. and M.F. Quigley. 2005. Willow beyond wetlands; Uses of *Salix* L. species for environmental projects. *Water, Air, and Soil Pollution*. 162:183:204
- Lambers, H., Chapin, F.S. and T.L. Pons. 1998. *Plant physiological ecology*. Springer, Berlin Heidelberg New York
- Lamont, B.B., Groom, P.K. and R.M. Cowling. 2002. High leaf mass per area of related species assemblages may reflect low rainfall and carbon isotope discrimination rather than low phosphorus and nitrogen concentrations. *Funct. Ecol.* 16:403-412
- Lange, O.L., Nobel, P.S., Osmond, C.B. and H. Ziegler. 1982. *Physiological plant ecology II*; water relations and carbon assimilation. *Encyclopedia of Plant physiology*, vol 12B. Springer, Berlin Heidelberg New York.
- Larcher, W. 1995. *Physiological Plant Ecology*. Springer Verlag Berlin pp.506

- Larsson, S. 1998. Genetic improvement of willow for short-rotation coppice. *Biomass and Bioenergy* 15:23-26
- Lemoine, D., Cochard, H. and A. Granier. 2002. Within crown variation in hydraulic architecture in beech (*Fagus sylvatica* L): evidence for a stomatal control of xylem embolism. *Annals of Forest Science* 59:19-27
- Lennartsson, M. and E. Ögren. 2002. Causes of variation in cold hardiness among fast-growing willows (*Salix spp.*) with particular reference to their inherent rate of cold hardening. *Plant, Cell and Environment*. 25:1279-1288
- Li, C., Berninger, F., Koskela, J. and E. Sonninen. 2000. Drought responses of *Eucalyptus microtheca* provenances depend on seasonality of rainfall in their place of origin. *Australian Journal Plant Physiology*. 27:231-238
- Lindroth, A. and A. Båth. 1999. Assessment of regional willow coppice yield in Sweden on basis of water availability. *Forest Ecology Management*. 121:57-65
- Lindroth, A. and E. Cienciala. 1996. Water use efficiency of short-rotation *Salix viminalis* at leaf, tree and stand scales. *Tree Physiology*. 16:257-262
- Lo Gullo, M.A., Nardini, A., Salleo, S. and M. Tyree. 1998. Changes in root hydraulic conductance (K_R) of *Olea Oleaster* seedlings following drought stress and irrigation. *New Phytologist*. 140:25-31
- Lo Gullo, M.A. and S. Salleo. 1988. Different strategies of drought resistance in three Mediterranean sclerophyllous trees growing in the same environmental conditions. *New Phytologist*. 108:267-276
- Lo Gullo, M.A., Salleo, S., Piaceri, E.C. and R. Rosso. 1995. Relations between vulnerability to xylem embolism and xylem conduit dimensions in young trees of *Quercus cerris*. *Plant, Cell and Environment*. 18:661-669
- Luan, S. 2002. Signaling drought in guard cells. *Plant, Cell and Environment*. 25:229-237
- Lundström, I. and K. Hasselgren. 2003. Omsättning av metaller i slamgödslad Salix odling. VA-Forskningsrapport. 46.
- Lopushinsky V. and R. Kaufmann. 1984. Effects of Cold Soil on Water Relations and Spring Growth of Douglas-fir Seedlings. *Forest Science*. 30:628-634
- Luu, D.-T. and C. Maurel. 2005. Aquaporins in a challenging environment: molecular gears for adjusting plant water status. *Plant, Cell and Environment*. 28:85-96
- MacCulloch, K.A. and J.S. Sperry. 2005. Patterns in hydraulic architecture and their implication for transport efficiency. *Tree Physiology*. 25:257-267
- Magnani, F., Mencuccini, M. and J. Grace. 2000. Age-related decline in stand productivity: the role of structural acclimation under hydraulic constraints. *Plant Cell Environment*. 23:251-263
- Makeschin, F. 1999. Short rotation forestry in Central and Northern Europe – introduction and conclusions. *Forest. Ecology Management*. 121:1-7
- Martínez-Vilalta, J. and J. Piñol. 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian peninsula. *For. Ecol. Manage.* 161:247-256
- Menizer, F.C. 2002. Co-ordination of vapour and liquid phase water transport properties in plants. *Plant, Cell & Environment*. 25:265-274
- Meinzer, F.C., Clearwater, M.J. and G. Goldstein. 2001. Water transport in trees: current perspectives, new insights and some controversies. *Environmental and Experimental Botany*. 45:239-262
- Mencuccini, M. 2003. The ecological significance of long-distance water transport: short-term regulation, long-term acclimation and the hydraulic costs of stature across plant life forms. *Plant Cell Environ.* 26:163-182
- Mencuccini, M. and J. Comstock. 1997. Vulnerability to cavitation in population of two desert species, *Hymenoclea salsola* and *Ambrosia dumosa*, from different climatic regions. *Journal of Experimental Botany*. 48:1323-1334
- Mencuccini, M. and F. Magnani. 2000. Comments on “Hydraulic limitation to tree height; a critique by Becker, Meinzer and Wulschleger”. *Functional. Ecology*. 14:135-137
- Milburn, J.A. and M.E. McLaughlin. 1974. Studies of cavitation in isolated vascular bundles and whole leaves of *Plantago major* L. *New Phytol.* 73:861-871

- Milburn, J.A and R.P.C. Johnson. 1966. The conduction of sap II. Detection of vibrations produced by sap cavitation in *Ricinus* xylem. *Planta*. 66:43-52
- Nordh, N.E. 1994. Testing of *Salix* clones in variety tests. In: Hall International Conference on Biomass for Energy and Industry, Florence, Italy, Bochum Ponte Press.
- Ögren, E. and M. Sjöström. 1990. Estimation of the effect of photoinhibition on the carbon gain in leaves of a willow canopy. *Planta*. 181:560-567
- Öhlund, J. and T. Näsholm. 2001. Growth of conifer seedlings on organic and inorganic nitrogen sources. *Tree Physiology*. 21:1319-1326
- Panek, J. 1996. Correlation between stable carbon-isotope abundance and hydraulic conductivity in Douglas-fir across a climate gradient in Oregon, USA. *Tree Physiology*. 16:747-755
- Persson, G. and A. Lindroth. 1994. Simulating evaporation from short-rotation forest - variations within and between seasons. *J. Hydrol.* 156:21-45
- Pockman, W.T. and J.S. Sperry. 2000. Vulnerability to cavitation and the distribution of Sonoran Desert vegetation. *American Journal of Botany*. 87:1287-1299
- Ranathunge, K., Steudle, E. and R. Lafitte. 2003. Control of plant water uptake by rice (*Oryza sativa* L.): role of the outer part of the root. *Planta*. 217:193-205
- Rice, K.J., Matzner, S.L., Byer, W. and J.R. Brown. 2004. Pattern of tree dieback in Queensland, Australia: the importance of drought stress and the role of resistance to cavitation. *Ecophysiology*. 139:190-198
- Rippullone, F., Lauteri, M., Grassi, G., Amato, M. and M. Borghetti. 2004. Variation in nitrogen supply changes water-use efficiency of *Pseudotsuga menziesii* and *Populus x euroamericana*; a comparison of three approaches to determine water-use efficiency. *Tree Physiology*. 24:671-679
- Ritman, K. and J.A. Milburn. 1991. Monitoring of ultrasonic and audible emissions from plants with or without Vessels. *Journal of Experimental Botany*. 42:123-130
- Rood, S.B., Patiño, S., Coombs, K. and M.T. Tyree. 2000. Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. *Trees*. 14:248-257
- Salleo, S. and M.A. Lo Gullo. 1990. Sclerophyll and plant water relations in three Mediterranean *Quercus* species. *Ann. Bot.* 65:259-270
- Salleo, S., Lo Gullo, M.A., De Paoli, D. and M. Zippo. 1996. Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism. *New Phytol.* 132:47-56
- Salleo, S., Nardini, A., Pitt, F. and M.A. Lo Gullo. 2000. Xylem cavitation and hydraulic control of stomatal conductance in Laurel (*Laurus nobilis* L.) Plant, Cell and Environment. 23:71-79
- Salleo, S., Lo Gullo, M.A., Raimondo, F. and A. Nardini. 2001. Vulnerability to cavitation of leaf minor veins; any impact on leaf gas exchange? *Plant, Cell and Environment* 24:851-859
- Sennerby-Forsse, L. and L. Zsuffa. 1995. Bud structure and resprouting in coppiced stools of *Salix viminalis* L., *S. eriocephala* Michx., and *S. amygdaloidea* Anders. *Trees*. 9:224-234
- Saliendra, N.Z., Sperry, J.S. and J.P. Comstock. 1995. Influence on leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula Occidentalis*. *Journal of Experimental Botany*. 196:357-366
- Shiklomanov, I.A., Shiklomanov, R.B., Lammers, B.J.Peterson and C.J. Vörösmarty. 2000. In the fresh water budget of the Arctic Ocean. E.L. Lewis Ed. (Kluwer Academic, Dordrecht, Netherlands, pp.281-296
- Shinozaki, K., Yoda, K., Hozumi, K. and T. Kira. 1964. A quantitative analysis of plant form- the pipe model theory: I, Basic analysis, *Jpn. J.Ecol.* 14:97-105
- Sperry, J.S. 2000. Hydraulic constraints on plant gas exchange. *Agricultural and Forest Metrology*. 104:13-23
- Sperry, J.S. and N.Z. Saliendra. 1994. Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant Cell Environ.* 17:1233-1241
- Sperry, J.S., Donnelly, J.R. and M.T. Tyree. 1988. A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell and Environment* 11, 35-40

- Sperry, J.S., Saliendra, N.Z., Pockman, W.T., Cochard, H., Cruiziat, P., Davis, S.D., Ewers, F.W. and M.T. Tyree. 1996. New evidence for large negative xylem pressures and their measurement by the pressure chamber method. *Plant, Cell and Environment*. 19:427-436
- Sperry J.S., Hacke U.G., Oren R and J.P. Comstock. 2002. Water deficits and hydraulic limits to leaf water supply. *Plant, Cell and Environment* 25:251-263
- Steudle, E. 2001. The Choeshion -Tension Mechanism and the Acquisition of Water by Plant Roots. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.* 52:847-875
- Steudel, E. and C.A. Peterson. 1998. How does water get through roots? *Journal of Experimental Botany*. 49:775-588
- Tardieu, F., Zang, J., Katerji, N. Béthenod, O., Palmer, S. and W.J. Davies. 1992. Xylem ABA controls the stomatal conductance of field-grown maize subjected to soil compaction or drying. *Plant, Cell and Environment*. 15:193-197
- Tsuda, M. and M. T. Tyree. 1997. Whole-plant hydraulic resistance and vulnerability segmentation in *Acer saccharinum*. *Tree Physiology*. 17:351-357
- Tuskan, G.A. and M.E. Walsh. 2001. Short-rotation and woody crop systems, atmospheric carbon dioxide and carbon management: A U.S. case study. *Forest Chronicle* 77:259-264
- Tyree, M.T. and J.S. Sperry. 1989a. Vulnerability of xylem to cavitation and embolism. *Annu. Rev Plant Physiology Molecular Biology*. 40:19-38
- Tyree, M.T. and J.S. Sperry. 1989b. Characterization and propagation of acoustic emission signals in woody plants: towards an improved acoustic emission counter. *Plant, Cell and Environment*. 12:371-382
- Tyree, M.T., Fiscus, E.L., Wullscheger, S.D. and M.A. Dixon. 1986. Detection of xylem cavitation in corn under field conditions. *Plant Physiology*. 82:597-599
- Tyree, M.T., Alexander, J. and J.-L. Machado. 1992. Loss of hydraulic conductivity due to water stress in intact juveniles of *Quercus rubra* and *Populus deltoids*. *Tree Physiology*. 10:411-415
- Tyree, M.T., Kolb, K.J., Rood, S.B. and S. Patiño. 1994. Vulnerability to drought-induced cavitation of riparian cottonwoods in Alberta: a possible factor in the decline of the ecosystem? *Tree Physiology*. 14:455-466
- Tyree, M.T., Patiño, S., Bennink, J. and J. Alexander. 1995. Dynamic measurements of root hydraulic conductance using a high-pressure flowmeter in the laboratory and field. *Journal of Experimental Botany*. 46:83-94
- Tyree, M.T., Salleo, S., Nardini, A., Lo Gullo, M.A. and R. Mosca. 1999. Refilling of embolized vessels in young stems of laurel. Do we need a new paradigm? *Plant. Physiol.* 120:11-21
- Troughton, J. and L.A. Donaldsson. 1972. *Probing Plant Structures*. Chapman and Hall, London. pp.85
- Uemura, A., Ishida, A., Tobias, D.J., Koike, N. and Y. Matsumoto. 2004. Linkage between gas exchange and hydraulic acclimation in the top canopy leaves of *Fagus* trees in a mesic forest in Japan. *Trees*. 18:452-459
- Vandeleur, R., Niemietz, C., Tilbrook, J. and S.D. Tyerman. 2005. Roles of aquaporines in root responses to irrigation. *Plant and Soil*. 274:141-161
- Verwijst, T. and D.Z. Wen. 1996. Leaf allometry of *Salix viminalis* during first growing season. *Tree Physiol.* 16:655-660
- Weih, M. 2001. Evidence for increased sensitivity to nutrient and water stress in a fast-growing hybrid willow compared with a natural willow clone. *Tree Physiology*. 21:1141-1148
- Weih, M. and N.E. Nordh. 2002. Characterizing willows for biomass and phytoremediation: growth, nitrogen and water use of 14 willow clones under different irrigation and fertilization regimes. *Biomass and Bioenergy*. 23:397-413
- West, G.B., Brown, J.H. and B.J. Enquist. 1999. A general model for the structure and allometry of the plant vascular system. *Nature*. 400:664-667
- Wheeler, J.K., Sperry, J.S., Hacke, U.G. and N. Hoang. 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 and Environment*. 28:800-812
- Wei, C., Steudle, E. and M.T. Tyree. 1999a. Water ascent in plants: do ongoing controversies have a sound basis? *Trends Plant Sci.* 4:372-375

- Wei, C., Tyree, M.T. and E. Steudle. 1999b. Direct measurement of xylem pressure in leaves of intact maize plants: a test of the cohesion-tension theory taking hydraulic architecture into consideration. *Plant Physiol.* 121:1191-1205
- Wikberg, J. and E. Ögren. 2004. Interrelationships between water use and growth traits in biomass-producing willows. *Trees* 18:70-76, paper I
- Wikberg, J. and E. Ögren. 2006. Genotypic variation in drought resistance, drought acclimation and water conservation in biomass-producing willows. Manuscript, Paper II
- Wikberg, J. and E. Ögren. 2006. Acoustic detection for the quantitative analysis of xylem cavitation in willows. Submitted to *Journal of Experimental Botany*, paper III
- Wilson J.B. 1988. A Review of Evidence on the Control of Shoot: Root Ratio, in Relation to Models. *Annals of Botany*. 61:433-449
- Wookey, P.A., Robinson, C.H., Parsons, A.N., Welker, J.M., Press, M.C., Callaghan, T.V. and J.A. Lee. 1995. Environmental constraints on the growth, photosynthesis and reproductive development of *Dryas octopetala* at a high Arctic polar semidesert, Svalbard. *Oecologia*. 102:478-489
- Yin, X. 2002. Response of leaf nitrogen concentration and specific leaf area to atmospheric CO₂ enrichment; a retrospective synthesis across 62 species. *Global Change Biol.* 8:631-642
- Zimmermann, M.H. and C.L. Brown. 1971. *Trees. Structure and Function*. Springer, New York
- Zimmermann, H.M. 1978. Hydraulic architecture of some diffuse porous trees. *Can. J. Bot.* 56:2286- 2295
- Zimmermann, M.H. 1983. *Xylem structure and the ascent of sap*. Springer Berlin Heidelberg New York
- Zimmermann, M.H. and A.A. Jeje. 1981. Vessel-length distribution of some American woody plants. *Canadian Journal of Botany* 59, 1882-1892
- Zwieniecki, M.A. and N.M. Holbrook. 2000. Bordered Pit Structure and Vessel Wall Surface Properties. Implications for Embolism Repair. *Plant Physiology*. 123:1015-1020
- Zwieniecki, M.A., Melcher P.J. and N.M. Holbrook. 2001. Hydraulic control of Xylem Hydraulic Resistance in Plants. *Science*. 291:1025-1062