ORIGINAL ARTICLE

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Interrelationships between water use and growth traits in biomass-producing willows

Abstract Water use, drought response and growth were examined under controlled conditions in four interbreeding willow species from different geographical origins (two clones of *Salix viminalis* L., one clone of *S. viminalis* × *S. schwerenii* E. Wolf and one clone of *S. purpurea* L.). The levels of soil water depletion that plants could sustain without wilting varied markedly between the clones. The level of drought resistance expressed this way was positively related to resistance to xylem cavitation, negatively related to the maximum stomatal conductance, and positively related to early stomatal closure. The rate of stomatal closure, however, was negatively related to the resistance to xylem cavitation. Prior to drought, there were no significant differences between leaf-specific hydraulic conductances of the clones when whole plants were considered. However, there were differences if the roots and shoots were considered separately. Drought resistance was negatively related to maximum growth yields. This is probably because resources were diverted away from leaf production to the production of denser wood (wood density was positively related to cavitation resistance), and, for one clone, to the growth of a larger root system. In addition, because the level of drought resistance was negatively related to the maximum stomatal conductance, growth may have been adversely affected as a result of reduced photosynthesis. Given its high water extraction ability, one of the clones started to wilt sooner than expected, although only lateral shoots were affected. This appeared to indicate a strategy of sacrificing expendable shoots.

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Introduction

Fast-growing willows (*Salix* spp.) are being used, increasingly, for biomass production on arable land in Europe (Makeschin 1999). Such production is limited by water availability even in the cool and humid climate of northern Europe (Lindroth and Båth 1999). Willows therefore appear to be inherently sensitive to water stress. This may be related to their high transpiration rates (Persson and Lindroth 1994) and their seemingly poor ability to maintain xylem integrity when exposed to water stress (Pockman and Sperry 2000). Ongoing willow breeding programmes should gain impetus from an increased understanding of the physiology of water use in willows, of the associated genetic variation, and of the interrelationships between water use and growth.

Water lost by transpiration from leaves must be replaced by water flowing from the soil, otherwise desiccation will occur. The xylem, itself, can significantly restrict this flow (Sperry et al. 2002). The capacity of the xylem to supply water needs to be assessed in relation to the leaf area supplied (Zimmermann 1983). The leaf-specific conductance represents the rate of flow per unit of water potential gradient and leaf area. Tree species that can be classified as pioneer, mesic or drought-avoiding typically display high levels of leaf-specific, whole-plant conductance. This allows them to maintain high water potentials at least when water is non-limiting (Tyree et al. 1998; Nardini et al. 1999). Populations may also exhibit differences in the leaf-specific conductance, which may explain the variation in water use responses within species (Martínez-Vilalta and Piñol 2002).

When water supply becomes limiting, however, hydraulic conductance can decrease as a consequence of air entering conduits along the pressure gradient, causing them to cavitate (embolise). Although the conduits may be refilled (Holbrook et al. 2001), this ability can be lost with repeated cavitation, causing the blockage to become permanent (Hacke et al. 2001b). Several studies using a range of species have indicated that there is a close relationship between the degree of vulnerability to

cavitation and the minimum xylem water potential attained by each species (e.g. Pockman and Sperry 2000).

Stomata also play a key role under drought conditions, by allowing adjustment of the rate of transpiration. Whether stomata close primarily to prevent leaf dehydration or xylem failure is still a matter of discussion, but in some plants, at least, prevention of xylem failure seems to be the primary aim (Cochard et al. 1996).

Increased drought resistance, however, might be achieved at the expense of growth. For instance, increased hydraulic conductance, either as a result of the presence of more xylem conduits or more roots, will divert resources away from leaf growth, the single most important parameter determining plant growth during establishment (Cornelissen et al. 1996). Likewise, resistance to xylem cavitation appears to require structural reinforcement of the wood, thus diverting resources away from leaf growth (Hacke et al. 2001a). Furthermore, although early stomatal closure restricts water loss and thereby improves water economy, it also restricts carbon uptake, so probably has an adverse effect on growth (Cowan 1982). The degree to which growth is affected, however, may vary as a result of all these factors and may also vary between genotypes.

The objectives of this study were threefold: (1) to assess the range of variation in hydraulic properties and stomatal regulation among four interbreeding willow clones with potential for biomass production; (2) to assess the resulting variation in drought responses; and finally (3) to investigate the degree to which drought resistance can be enhanced without adversely affecting growth.

Materials and methods

Plant material

Four clones of *Salix*, belonging to a group of closely related species that have shown promising results in biomass plantations, were obtained from Svalöf-Weibull, Sweden. Clones were coded to indicate their different geographical origins: the N/C (northern/central) hybrid clone (commercial name 'Tora') had parents originating from Siberia (*S. schwerenii*) and S. Sweden (*S. viminalis*); the C (central) clone was from south Sweden (*S. viminalis*, 'Jorr'); the N (northern) clone was from north Sweden (*S. viminalis*, '93/40'); and the S (southern) clone was from Greece (*S. purpurea*).

Thirty plants of each clone were raised from 15-cm cuttings, in large pots (5 dm^3 for the slow-growing S clone but otherwise 10 dm^3), using the substrates and nutrients described by Ögren (1999). Only one bud per cutting was allowed to develop into a shoot. Two growth rooms were used. The day and night temperatures averaged 14.7°C and 5.5°C in one and 14.9°C and 5.8°C in the other. Temperature readings were taken using thermocouples (0.05 mm in diameter). The irradiance, provided by metal halogen lamps (HQI-TS 400 W; Osram, Berlin, Germany), averaged 506 µmol m^{-2} s^{-1} in the first growth room and 546 µmol m^{-2} s^{-1} in the second (19-h photoperiod). This was measured using quantum sensors (Li-189; Li-Cor, Lincoln, Neb., USA) placed at half plant height, and adjacent to the plants. The water vapour pressure deficit of the air, during the photoperiod, averaged 1.1 kPa and 1.3 kPa in the first and second growth room, respectively, and was measured using humidity probes (50Y; Campbell Scientific, Loughborough, UK). All sensor readings were logged at 5 s intervals (CR10X;

Campbell Scientific, Logan, Utah, USA). One of the growth rooms had extra fans, but neither this difference nor the differences in environmental conditions described above resulted in any significant difference in plant characteristics. This was confirmed by ANOVA analyses (data not shown). After 14–19 weeks of growth, when the plants had reached a height of 1.3–2.1 m, random sets of plants were characterised for growth and water use traits. Other sets of plants were subjected to drought treatment.

Hydraulic conductance

The hydraulic conductance of roots and shoots was assessed using a high pressure flowmeter (HPFM; Dynamax, Houston, Tex., USA). In general, the method followed that described by Tyree et al. (1995). Early in the photoperiod, and about 1 h before analysis, the plant was enclosed in a black plastic bag to stimulate the stomata to close, in order to reduce xylem tension and avoid dehydration during subsequent handling. The shoot stem was cut about 5 cm above the ground and then re-cut under water before it was inserted into the compression seal. It was perfused with distilled and degassed water at a pressure of 0.15 MPa until water began dripping out of the stomata. Two transient measurements were then made, the second to ensure consistent results. This procedure was repeated for the roots, except there was no pre-pressurisation. Up to four transient measurements were required to obtain consistent results.

Following analysis, the total leaf area was assessed. Representative sub-samples were used to establish the area-to-dry weight relationship and the resulting ratio was used to estimate leaf area from total leaf dry weight. Shoot (K_S) and root (K_R) conductance were expressed per unit of leaf area present. Whole-plant conductance (K_P) was calculated as $1/(1/K_S+1/K_R)$.

Vulnerability to xylem cavitation

Immediately after assessing the shoot conductance, the vulnerability of the xylem to cavitation was determined using, essentially, the procedure described by Sperry and Saliendra (1994). The 40 cm segment from the centre of the main axis was cut off under water. The bark, petioles and any lateral shoots present were removed, then eight notches, 1 mm deep, were cut into the xylem at regular intervals around the central 6-cm section that was to be inserted into the cavitation chamber. The stem base was connected to a supply of distilled and degassed water that had been adjusted to pH 2 using HCl; a filter (0.2 µm) was mounted between the water supply and the stem. The stem was flushed at a pressure of 0.15 MPa for 10 min to remove any pre-existing embolisms. The pressure was then reduced to 0.01 MPa, as defined by the height of the water column. After first equilibrating the system for 4 min, effluents from the free stem end were collected in pre-weighed vials filled with cotton wool over 1-min intervals. The average effluent over four intervals, assessed by weighing, was taken as a relative measure of conductance. The water supply was then disconnected and the central section was enclosed in the cavitation chamber (PMS Instrument, Corvallis, Ore., USA). The chamber was pressurised in stages, each lasting 10 min, pilot studies having demonstrated that this was long enough to achieve a steady level of cavitation. After each stage, conductance was measured as described above. Loss of conductance was related to the conductance at the outset.

The vulnerability of the xylem to cavitation was also assessed by treating intact plants to drought. The central section of the main axis was harvested and its conductance was determined, as described above, except that the first reading was taken without pre-flushing. This reading was related to a second reading obtained after flushing the stem at 1.5 MPa for 30 min to remove the embolisms that had been induced by drought. The loss of conductance was related to the stem water potential assessed by analysing enclosed leaves prior to harvest (see below).

Growth analyses

The plants used for the determination of hydraulic conductance and cavitation vulnerability were also used for growth analysis. Shoots were separated into wood, main leaves on main shoots, and 'auxiliary' leaves on lateral shoots (leaves that were smaller and more short-lived than the main leaves as discussed below). The material was oven-dried at 70°C to constant weight.

Rates of biomass increase for shoots were obtained by dividing their dry weights by time since planting, thus assuming a linear rate of growth. This was justified because soon after planting, canopy closure occurred, which is known to cause a transition from exponential to pseudo-linear growth (Beadle 1993). It is widely accepted that plant growth rate is determined largely by the leaf area ratio (LAR), i.e. the amount of leaf area per unit plant weight, rather than its other component, the net assimilation rate (NAR), i.e. the rate of increase in plant weight per unit leaf area. The LAR, in turn, is the product of the specific leaf area (SLA), i.e. the amount of leaf area per unit leaf weight, and the leaf weight ratio (LWR), i.e. the fraction of the plant weight allocated to leaves (Lambers et al. 1998). Because of difficulties associated with extracting the entire root system, plant weight was approximated by shoot weight.

The wood density was determined by dividing the dry weight of the main axis by its volume. The stem was assumed to be approximately conical, with a volume of $1/3\pi r^2 h$, where r and h represent the radius at the base and the height, respectively. The diameter at the base was measured using a digital calliper, and an average of several readings utilised.

Stomatal conductance and water potential

Stomatal conductance of the main leaves, located about two-thirds of the way up the plant, was assessed using a portable porometer (LCA–3; ADC BioScientific, Hoddesdon, UK).

Stem water potential was assessed indirectly by determining the water potentials of attached leaves, adjacent to those used to assess stomatal conductance. The leaves being investigated were wrapped in aluminium foil to neutralise the water potential gradient between stem and leaf. For analysis, the leaf was unwrapped and a disk punched out halfway between the margin and the mid-vein, using an 8 mm dermal puncher (Miltex Instruments, N.Y., USA). Within 6 s, the disk was enclosed in one of ten psychrometer chambers connected via a switchbox to the microvoltmeter (HR-33T, C-52 chambers; Wescor, Logan, Utah, USA). After 2 h of equilibration at a constant temperature, the water potential was recorded with the instrument set to its dew point mode. The calibration of the chambers was checked at least daily. Two to five readings, from different leaves, were taken 1–3 times a day from each plant during the drought cycle.

Drought treatment

Water was withheld from seven to eight plants of each clone. Soil water levels were assessed by weighing the plants in their pots, using a set of load cells (PW2GC3 15 kg; Hottinger Baldwin Messtechnik, Darmstadt, Germany) and were logged at 5-s intervals (see above). The difference in weight after watering to field capacity and the final weight after drying to constant weight at 70°C was taken to represent the absolute water content, corresponding to 100% relative water content. The relative soil water content at which leaves started to wilt was taken as a measure of the drought resistance.

Statistical analysis

The significance of the differences between the clones was tested using ANOVA: two-way for the cavitation vulnerability curves but otherwise one-way. The significance of the differences in soil water levels that initiated wilting (Fig. 2) was tested using a Gehan-Wilcoxon test for survival distributions. All tests were carried out using the Statistix 7.0 package (Analytical Software, Tallahassee, Fla., USA).

Results

Hydraulic and stomatal conductance

Most of the variation in hydraulic conductance between the four willow clones was accounted for by differences between the northern and southern clones. The N clone had a K_S that was 2.3 times higher than the S clone (Fig. 1a). This difference, however, was more than offset by the K_R of the S clone being 4.5 times greater than that of the N clone (Fig. 1b). As a result, there were no significant differences in K_P between clones (Fig. 1c).

Maximum stomatal conductance of the main leaves, g_{st}, varied 2.3-fold between the clones, and was independent of the variation in hydraulic conductance (Fig. 1c, d). The highest and lowest values were exhibited by the N/C and S clones, respectively.

Drought response

Figure 2 shows how stem water potential, ψ_{stem}, for the different clones, declined as the relative soil water content (RSW) decreased when watering was discontinued. The dashed lines indicate the average ψ_{stem} and RSW values at which wilting commenced. Wilting was initiated at widely different RSW values for the different clones: 44%, 32%, 31% and 24% for the N/C, N, C and S clones, respectively (values extracted from Fig. 2, also shown in Fig. 1e with significance levels indicated). The ψ_{stem} values at which wilting was initiated also varied: –0.90, –1.26, –1.33 and –1.40 MPa for the N/C, S, C and N clones, respectively. Thus, the N/C clone was most susceptible to wilting with respect to both RSW and ψ_{stem} values.

Figure 3 shows how the stomata closed as ψ_{stem} declined when watering ceased. The timing of stomatal closure relative to the timing of the appearance of drought stress symptoms varied between the clones. At the points of initial wilting (dashed line) and of 70% cavitation-induced loss of stem conductance (dotted line, values presented below), the stomata had already closed in the C clone, nearly closed in the N and S clones, but had barely started to close in the N/C clone. The rate of stomatal closure, with respect to the rate of decline in ψ_{stem} normalised for the differences in g_{st} at the start, i.e. ($\Delta g_{st}/g_{st, start}$)/$\Delta\psi_{st}$, also varied between the clones. This was, however, largely independent of the variation in the timing of its onset, with values of 0.53, 0.69, 0.97 and
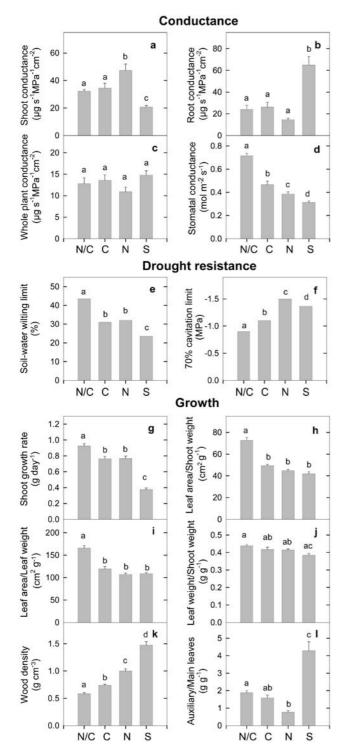
Conductance

Drought resistance

Growth

Fig. 1a–l Comparisons of water use and growth traits of clones. Hydraulic conductance of **a** shoots, **b** roots and **c** whole plants, expressed per unit of leaf area present and measured prior to drought; **d** stomatal conductance prior to drought; **e** soil-water wilting limit, i.e. the relative soil water content causing initial wilting (from Fig. 2); **f** cavitation limit, arbitrarily set at 70% loss of stem conductance (from Fig. 4b); **g** shoot growth rate; **h** leaf area per shoot weight; **i** leaf area per leaf weight; **j** leaf weight per shoot weight; **k** wood density; **l** ratio of weights of 'auxiliary' leaves on lateral shoots to that of main leaves on the main axis. The mean ± SE values are shown for 10–14 replicates except in **e** and **f** where the mean values are for 5–8 replicates. Different letters represent significant differences between clones (*P*<0.05); in **f** this refers to the whole vulnerability curve
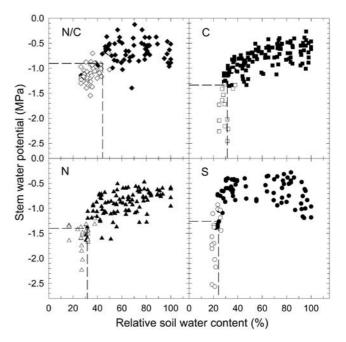

Fig. 2 Stem water potential of the different clones, plotted as a function of relative soil water content as it declined during drought. *Open symbols* indicate plants where wilting had started. The *dashed lines* indicate average limits of initial wilting, with respect to *y* and *x*. Pooled data of 7–8 replicates are shown

1.0 MPa^{-1} for the S, N, N/C and C clones, respectively (regression over the whole decline phase; Fig. 3). Thus, the inability of the N/C clone to close its stomata before wilting occurred was not due to the stomata responding slowly but because they began to close later.

The resistance of stem xylem to cavitation was assessed by exposing detached stems to stepwise, increasing ambient air pressure (Fig. 4a) and by exposing intact plants to drought (Fig. 4b). The relative ranking of clones with respect to cavitation resistance was consistent between the two methods, but the curves were shifted towards higher ψ_{stem} values (closer to atmospheric pressure) when cavitation was induced by drought instead of air pressurisation. Cavitation resistance increased in the sequence: N/C, C, S and N. For example, during drought, cavitation corresponding to a 70% loss of conductance occurred at a ψ_{stem} value that was about 0.6 MPa less negative for the N/C clone, which was the most sensitive, than for the N clone, which was the most resistant (Fig. 1f, values extracted from Fig. 4b using methods of interpolation and, for the N clone, extrapolation).

Growth and morphological traits

The maximum rate of shoot growth varied between clones, with the N/C clone having the highest rate: it was 1.2 times higher than that of the C and N clones and 2.7 times higher than that of the S clone (Fig. 1g). This variation in growth was largely the result of a variation in the relative investment in leaf area, LAR (Fig. 1h). LAR
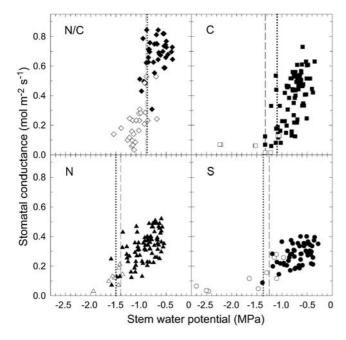
Fig. 3 Stomatal conductance of the different clones, plotted as a function of stem water potential as it declined during drought. *Open symbols* indicate plants where wilting had started. The *dashed line* indicates the average limit of initial wilting (from Fig. 2) and the *dotted line* indicates the 70% cavitation-induced loss of stem conductance (from Fig. 4b). Pooled data of 7–8 replicates are shown

is ideally calculated as leaf area per plant weight, but since the roots could not be completely extracted, LAR was approximated by the leaf area per shoot weight. The variation in LAR defined this way did not, however, entirely explain the variation in growth, since the S clone displayed a lower rate of growth than would have been predicted from its LAR. However, this clone showed a very high K_R relative to its K_S value (Fig. 1b), which indicates a large root relative to canopy size. Thus, the practice of excluding roots from analyses caused an overestimation of the true LAR of the S clone. The variation in LAR (Fig. 1h) was largely linked to a variation in the SLA (leaf area per leaf weight; Fig. 1i), although a variation in the LWR (leaf weight per shoot weight, ideally plant weight) also seems to have contributed (Fig. 1j). Interestingly, wood density varied 2.5-fold between the clones (Fig. 1k), and was negatively correlated with shoot growth rate (Fig. 1g) and LWR (Fig. 1j). A reciprocal relationship between wood density and shoot growth is not unexpected: investment in wood density is likely to be at the expense of leaf growth and, hence, shoot growth. The relative investment in auxiliary leaves on short-lived lateral shoots also varied: the S clone had the largest relative fraction of such leaves (Fig. 1l). This had a bearing on the responses of the S clone during drought, because the auxiliary leaves were more drought sensitive than the main leaves (see below).
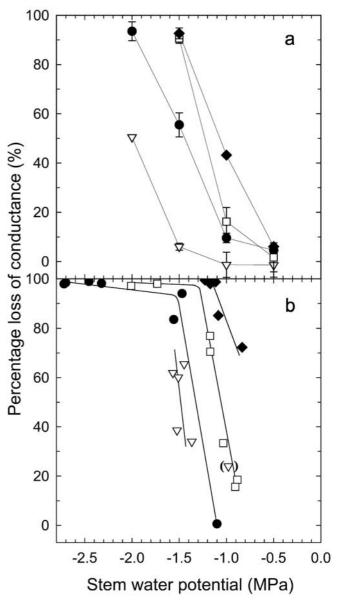

Fig. 4 Cavitation vulnerability curves for stems assessed by **a** perfusing stem segments with pressurised air, and **b** exposing intact plants to drought: *diamonds* N/C; *squares* C; *circles* S; *inverted triangles* N. The mean ± SE values for 10–14 replicates are shown in **a**, and single values for 5–7 plants in **b**

Discussion

The level of soil water depletion that plants could withstand without wilting varied considerably between the four willow clones (Fig. 1e). This suggests that clones had different abilities to balance their rates of transpiration with those of water uptake/transport. Prior to drought, however, clones had similar capacities for water uptake/transport, K_P (Fig. 1c). Although two of the clones differed with respect to the partial components, K_R and K_S (Fig. 1a, b), these differences were essentially neutralised when combined to assess K_P. As drought increased, however, the differences in K_P also became more

pronounced, because clones displayed different sensitivities to xylem cavitation. The relative ranking of the clones was essentially the same with respect to cavitation resistance and drought resistance (cf. Fig. 1e, f; low soil-water wilting limit = high drought resistance). This is consistent with observations that cavitation resistance plays a key role in conferring drought resistance to plants (Sperry et al. 2002).

Xylem cavitation was assessed for stems only. The stem xylem may be more vulnerable than the root xylem in some species (Tsuda and Tyree 1997; Cochard et al. 2002) but typically is less vulnerable (e.g. Alder et al. 1996). Still, analysis of stems might provide rough estimates of the general resistance levels of plants when comparing closely related species. Even in the unlikely event that only stems suffered from cavitation, the ca. 70% loss of conductance at the point of initial wilting (Fig. 3) seems sufficiently large an effect per se to have reduced K_P (Meinzer 2002).

In addition, differences in transpiration rate seem to have contributed to the variations in drought resistance, since drought resistance was negatively correlated with g_{st} (cf. Fig. 1d, e; low soil-water wilting limit = high drought resistance). Stomata must not have closed too early if the g_{st} assessed prior to drought is to have any influence during the drought period. Indeed, stomata closed particularly late in the N/C clone, which had the highest g_{st} (see Results). Thus, a high initial g_{st}, late stomatal closure and a high vulnerability to cavitation combined to sensitise the N/C clone to drought (Fig. 1d, f). It is not known whether clones also differed in their abilities to maintain leaf turgor by osmotic regulation, but the possibility cannot be excluded since willows are certainly capable of such regulation (Ögren and Öquist 1985).

Clones' abilities to withstand drought seem to have compromised their ability to grow under favourable conditions (cf. Fig. 1e, g; low soil-water wilting limit = high drought resistance). Different trade-offs can be assumed to have contributed to this. First, a functional trade-off between growth and drought resistance mediated by stomatal conductance (an initial high g_{st} and late stomatal closure) was negatively associated with drought resistance (see above). This should have benefited photosynthesis, and thus growth, before drought became severe (Cowan 1982). In addition, two trade-offs with respect to investment strategies can be assumed. Investment in leaf area is by far the most important factor in promoting growth during establishment (Cornelissen et al. 1996), but it is at the expense of investment in other structures, for example wood density and roots. Indeed, wood density (Fig. 1k) was negatively correlated with growth (Fig. 1g), but positively correlated with drought resistance (Fig. 1e; low soil-water wilting limit = high drought resistance) and cavitation resistance (Fig. 1f). This agrees with previous observations that wood density promotes drought resistance by increasing resistance to cavitation (Hacke et al. 2001a).

A resource trade-off involving root growth can also be assumed. The S clone had a lower than expected rate of shoot growth (Fig. 1g), relative to its investment in leaves (Fig. 1j) and its g_{st} (Fig. 1d). However, it had a very high K_R relative to K_S value (Fig. 1a, b), indicating a high root-to-shoot ratio. This is a typical feature of drought resistant plants with a low growth rate (Passioura 1982). Thus, a functional trade-off involving stomata, and two resource trade-offs involving wood density and root growth, can be assumed to have contributed to the overall trade-off between growth and water use. Interestingly, the superior performance of the N/C clone under favourable conditions, and its inferior performance under unfavourable conditions, compared with standard clones, has also been demonstrated in the field (Weih 2001). The trade-off relations discussed above could also be interpreted in terms of developmental control, since several of the traits that promote drought resistance and impede growth are associated with an increased abscisic acid level (Aasamaa et al. 2002).

The stem xylem was more sensitive to cavitation when the stems were still attached to the plant and exposed to drought than when detached and exposed to air pressurisation (cf. Fig. 4a, b). This seems to contradict the findings of Sperry and Saliendra (1994) that stem xylem is equally sensitive to bench dehydration and air pressurisation. However, during drought, but not during bench dehydration, the xylem may undergo several cycles of cavitation and refilling, thus weakening the cell walls and increasing the cavitation vulnerability. Populus species that otherwise behave similarly to willows have certainly been demonstrated to exhibit such cavitation 'fatigue' following repeated cavitation-refilling cycling (Hacke et al. 2001b).

It is believed that stomata close, at least in part, to avoid xylem failure (Cochard et al. 2002; Lemoine et al. 2002). A link between stomata and xylem functions was also apparent here: stomata closed more rapidly with a drop in ψ_{stem} in the more cavitation-vulnerable N/C and C clones than in the more resistant N and S clones (see text). However, stomata of the N/C clone did not close rapidly enough to prevent extensive xylem cavitation. This was the result of a particularly vulnerable xylem, compared with the highest vulnerabilities so far reported for woody plants (Tyree et al. 1994; Tsuda and Tyree 1997).

Trees of some Populus species sacrifice their peripheral shoots—the result of irreparable xylem cavitation—during periods of drought, in order to improve their water economy (Rood et al. 2000). It is possible that the willows studied here rely on this strategy, since they typically shed their lateral shoots over the summer (Sennerby-Forsse and Zsuffa 1995). Furthermore, these shoots are low-cost constructions (Verwijst and Wen 1996), which supports the hypothesis of a branch-sacrificing strategy (Zimmermann 1983). The S clone, in particular, appeared to rely on this strategy because it started to wilt at an unexpectedly high ψ_{stem} value considering its ability to extract water (Fig. 2). However, only leaves on the lateral shoots wilted (data not shown); these leaves accounted for a much larger proportion of the total leaf mass than in the other clones (Fig. 1l).

In conclusion, there does seem to be considerable genetic variation in water use and growth traits between interbreeding willows, suggesting that there is much potential for improving the drought resistance of cultivars. The data presented here, however, do not offer much hope for any immediate improvement without adversely affecting growth. To determine whether resistance is possible without reducing growth, more extensive genetic screening is required, a formidable task given the number of traits involved.

References

Aasamaa K, Sõber A, Hartung W, Niinemets Ü (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

Alder NN, Sperry JS, Pockman WT (1996) Root and stem xylem embolism, stomatal conductance, and leaf turgor in *Acer grandidentatum* populations along a moisture gradient. Oecologia 105:293–301

Beadle CL (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

Cochard H, Bréda N, Granier A (1996) Whole tree hydraulic conductance and water loss regulation in *Quercus* during drought: evidence for stomatal control of embolism? Ann Sci For 53:197–206

Cochard H, Coll L, Le Roux X, Améglio T (2002) Unravelling the effects of plants hydraulics on stomatal closure during water stress in walnut. Plant Physiol 128:282–290

Cornelissen JHC, Diez PC, Hunt R (1996) Seedling growth, allocation and leaf attributes in a wide range of woody plant species and types. J Ecol 84:755–765

Cowan IR (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

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

Hacke UG, Stiller V, Sperry JS, Pittermann J, McCulloh KA (2001b) Cavitation fatigue. Embolism and refilling cycles can weaken the cavitation resistance of xylem. Plant Physiol 125:779–786

Holbrook NM, Ahrens ET, Burns MJ, Zwieniecki MA (2001) In vivo observation of cavitation and embolism repair using magnetic resonance imaging. Plant Physiol 126:27–31

Lambers H, Chapin FS, Pons TL (1998) Plant physiological ecology. Springer, Berlin Heidelberg New York

Lemoine D, Cochard H, Granier A (2002) Within crown variation in hydraulic architecture in beech (*Fagus sylvatica* L): evidence for a stomatal control of xylem embolism. Ann For Sci 59:19–27

Lindroth A, Båth A (1999) Assessment of regional willow coppice yield in Sweden on basis of water availability. For Ecol Manage 121:57–65

Makeschin F (1999) Short rotation forestry in Central and Northern Europe—introduction and conclusions. For Ecol Manage 121:1–7

Martínez-Vilalta J, Piñol J (2002) Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian peninsula. For Ecol Manage 161:247–256

Meinzer FC (2002) Co-ordination of vapour and liquid phase water transport properties in plants. Plant Cell Environ 25:265–274

Nardini A, Lo Gullo MA, Salleo S (1999) Competitive strategies for water availability in two Mediterranean *Quercus* species. Plant Cell Environ 22:109–116

Ögren E (1999) Fall frost resistance in willows used for biomass production. I. Characterization of seasonal and environmental variation. Tree Physiol 19:749–754

Ögren E, Öquist G (1985) Effects of drought on photosynthesis, chlorophyll fluorescence and photoinhibition susceptibility in intact willow leaves. Planta 166:380–388

Passioura JB (1982) Water in the soil-plant-atmosphere continuum. 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 5–33

Persson G, Lindroth A (1994) Simulating evaporation from short-rotation forest - variations within and between seasons. J Hydrol 156:21–45

Pockman WT, Sperry JS (2000) Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. Am J Bot 87:1287–1299

Rood SB, Patiño S, Coombs K, Tyree MT (2000) Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. Trees 14:248–257

Sennerby-Forsse L, Zsuffa L (1995) Bud structure and resprouting in coppiced stools of *Salix viminalis* L., *S. eriocephala* Michx., and *S. amygdaloides* Anders. Trees 9:224–234

Sperry JS, Saliendra NZ (1994) Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. Plant Cell Environ 17:1233–1241

Sperry JC, Hacke UG, Oren R, Comstock JP (2002) Water deficits and hydraulic limits to leaf water supply. Plant Cell Environ 25:251–263

Tsuda M, Tyree MT (1997) Whole-plant hydraulic resistance and vulnerability segmentation in *Acer saccharinum*. Tree Physiol 17:351–357

Tyree MT, Kolb KJ, Rood SB, Patiño S (1994) Vulnerability to drought-induced cavitation of riparian cottonwoods in Alberta: a possible factor in the decline of the ecosystem? Tree Physiol 14:455–466

Tyree MT, Patiño S, Bennink J, Alexander J (1995) Dynamic measurements of root hydraulic conductance using a high-pressure flowmeter in the laboratory and field. J Exp Bot 46:83–94

Tyree MT, Velez V, Dalling JW (1998) Growth dynamics and shoot hydraulic conductance in seedlings of five neotropical tree species: scaling to show possible adaptation to differing light regimes. Oecologia 114:293–298

Verwijst T, Wen D-Z (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 Physiol 21:1141–1148

Zimmermann MH (1983) Xylem structure and the ascent of sap. Springer, Berlin Heidelberg New York