DOI: 10.1007/s10535-013-0316-2 BIOLOGIA PLANTARUM 57 (3): 531-539, 2013

Impact of phloem girdling on leaf gas exchange and hydraulic conductance in hybrid aspen

A. SELLIN*, A. NIGLAS, E. ÕUNAPUU, and A. KARUSION

Institute of Ecology and Earth Sciences, University of Tartu, Lai 40, 51005 Tartu, Estonia

Abstract

We investigated phloem-xylem interactions in relation to leaf hydraulic capacity in hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) by using phloem girdling method. Removal of bark tissues (phloem girdling) at the branch base resulted in a substantial decline in stomatal conductance (g_S), net photosynthetic rate (P_N), and leaf hydraulic efficiency, and in increase of leaf water potential (Ψ_L). Although g_S declined more than P_N (83 versus 78 %), the ratio of intercellular to ambient CO_2 concentrations (c_i/c_a) increased from 0.67 to 0.87 in three days after girdling. Girdling induced a decrease in leaf hydraulic conductance (K_L) on average by 43 % ($P = 0.006$). The changes in g_S and leaf conductance to water vapour were co-ordinated with K_L only in girdled branches whereas intrinsic water-use efficiency was invariant to K_L. The declines in K_L with girdling were not accompanied by changes in potassium ion concentration ($[K^+]$), electrical conductivity, or pH of xylem sap. The results suggest that phloem girdling at the branch base does not influence the recirculation of ions between the phloem and xylem in hybrid aspen and the decrease of K_L in response to the manipulation is not related to changes in $[K^+]$ and total ionic content of xylem sap.

Additional key words: leaf water potential, net photosynthetic rate, phloem-xylem interactions, potassium, stomatal conductance, water-use efficiency, xylem sap.

Introduction

Vascular bundles of tracheophytes contain xylem and phloem, two principal long-distance liquid transport pathways. Xylem serves as conducting tissue primarily to transfer water and nutrients from the soil to leaves (ascending transport) whereas phloem serves as the pathway for photosynthate movement from leaves or storage organs to regions of active growth (descending or ascending transport). The two pathways are functionally interconnected by parenchyma rays or specialised transfer cells (radial transport) which play a role in nutrient exchange between xylem and phloem (Esau 1977, Van Bel 1990, Pate and Jeschke 1995, Offler *et al.* 2002). However, our knowledge of the mechanism of phloem-xylem exchange is limited. There is water exchange between the two pathways: phloem derives water from xylem and phloem can act as a water reserve for the transpiration stream occurring in xylem (Zweifel *et al.* 2000, Sevanto *et al.* 2011). Although the concentration of several ions (K^+, Na^+, Mg^{2+}, Ca^{2+}, and Cl^-) in the xylem sap is reported to depend on recirculation from the phloem (Jeschke and Pate 1991, Pate and Jeschke 1995, De Boer and Volkov 2003), the exact origin of ions transferred into xylem remains unclear.

Metal cations, K^+ in particular, play a significant role in regulating the hydraulic efficiency of xylem as indicated by the variation of xylem hydraulic conductivity with changes in ion concentration of liquids perfused through the vessels (Van Ieperen *et al.* 2000, Zwieniecki *et al.* 2001, Gasco *et al.* 2006, Nardini *et al.* 2007, Cochard *et al.* 2010) or of xylem sap extracted from stems. The ion-mediated changes in xylem hydraulic efficiency are attributed to the shrinking and swelling the intervessel pit membrane hydrogels (pectins) in response to changes in the osmotic concentration of the perfused solution (Ryden *et al.* 2000, Zwieniecki *et al.* 2001, Van Ieperen 2007). Recently, Lee *et al.* (2012)

Received 11 May 2012, *accepted* 20 November 2012.

Abbreviations: c_i/c_a - ratio of intercellular to ambient CO_2 concentration; E - transpiration rate; g_L - leaf conductance to water vapour; g_S - stomatal conductance to water vapour; IWUE - intrinsic water-use efficiency; $[K^+]$ - potassium ion concentration of xylem sap; K_L - leaf hydraulic conductance; P_N - net photosynthetic rate; T_L - leaf temperature; σ_{sap} - electrical conductivity of xylem sap; Ψ_B - branch xylem water potential; Ψ_L - leaf water potential.

Acknowledgements: This study was supported by the Estonian Science Foundation (Grant No. 8333), by the Estonian Ministry of Education and Research (target financing project SF0180025s12), and by the EU through the European Regional Development Fund (Centre of Excellence in Environmental Adaptation). We are grateful to Robert Szava-Kovats for language revision.

* Corresponding author; fax: (+372) 7376380; e-mail: arne.sellin@ut.ee

presented a support for the notion that bordered pit membranes are composite materials formed from fibrous structure submerged in amorphous gel. Water does not flow through discrete pores, but instead has to pass through gel matrix. The ionic effect may result from changes in thickness of the membrane/gel matrix. The discovery of ion-mediated changes in xylem conductance has generated the idea that plants may modulate their hydraulic conductance with respect to environmental conditions by adjusting sap ionic composition. Experiments performed by Nardini *et al.* (2010) and Sellin *et al.* (2010a) confirm that short-term modulation of stem hydraulic conductance mediated by changes in K^+ concentration in xylem sap depends on irradiance. In addition, a close coupling between aquaporins and K-channel transporters in water uptake of roots has been observed (Kanai *et al.* 2011). The condition of pit membrane hydrogels and thus xylem conductivity may also depend on the pH of the permeating fluid although no significant effect has been observed within the typical pH range (5.8 to 8.0); sap with a lower pH exhibits considerably enhanced hydraulic conductivity of xylem (Sperry and Tyree 1988, Sperry *et al.* 1988, Zwieniecki *et al.* 2001). Acidification of the outlet solutions in permeation experiments attests to cation exchange (*i.e.* substitution of H^+ for metal cations) by non-dissociated carboxyl groups of pectic chains (Gasco *et al.* 2008).

The pectin-swelling hypothesis has been challenged as pectin may not be present in the pit membranes, at least in many species (Van Doorn *et al.* 2011). An alternative hypothesis involves the shrinking of any polyelectrolyte polymers in the pit membrane and changes in volume of the mobile phase in the pit pores as a result of electrostatic events. Some pit membrane polymers are negatively charged and are compensated by cations in the aqueous phase, which form a diffuse double layer. A less mobile and more viscous layer of water in the channel of a charged pit pore can be reduced by cations allowing a higher rate of water flow (Van Doorn *et al.* 2011).

Zwieniecki *et al.* (2004) demonstrated that phloem can influence the hydraulic performance of xylem on scales of minutes to hours. Therefore phloem-girdling, *i.e.* removal of secondary phloem, has been used to assess phloem-xylem interactions (Zwieniecki *et al.* 2000, 2004, Salleo *et al.* 2004). Disruption of phloem transport could disturb lateral ion transfer to xylem. A subsequent decrease in ion concentration in xylem sap could, in turn, induce a decline in xylem hydraulic conductance. Decline of stem hydraulic conductance (Cernusak and Marshall 2001, Zwieniecki *et al.* 2004) or inhibition of recovery from xylem embolism in response to phloem girdling (Zwieniecki *et al.* 2000, Salleo *et al.* 1996, 2004, Christman *et al.* 2012) has been witnessed in several previous studies. Only one paper (Domec and Pruyn 2008) has indicated the influence of phloem girdling on leaf hydraulic conductance. Stem girdling also reduces content of K^+ and nitrate in xylem sap (Fumuro 1998), and content of N, P, K, Ca and Mg in leaf tissues (Cernusak and Marshall 2001, Di Vaio *et al.* 2001, Urban and Alphonsout 2007).

Stem girdling has also a substantial effect on leaf gas exchange and water relations. Common physiological responses to experimentally reduced sink demand, including phloem girdling, comprise accumulation of soluble sugars and starch in leaves and consequent down-regulation of photosynthetic capacity and reduction in quantum yield efficiency of photosystem II through feedback loops (Jang *et al.* 1997, Myers *et al.* 1999, Rivas *et al.* 2007, Urban and Alphonsout 2007, Murakami *et al.* 2008). Stomatal closure and a concurrent decline in water use or photosynthesis in girdled plants may also result from accumulation of abscisic acid in leaves (Setter *et al.* 1980, Williams *et al.* 2000). Nevertheless, plant responses to stem girdling vary largely among taxa and with treatment duration and design. Williams *et al.* (2000) demonstrated that girdling reduced midday net photosynthetic rate (P_N) and stomatal conductance (g_S) in *Vitis vinifera* by approximately 50 % compared to ungirdled vines. Midday leaf water potential was less negative for girdled plants but leaf content of non-structural saccharides did not differ (Williams *et al.* 2000). In horticulture, girdling is used to block assimilate transport out of the shoots to promote floral induction, reduce fruitlet abscission, and increase fruit size (Williams *et al.* 2000, Di Vaio *et al.* 2001, Williams and Ayars 2005, Rivas *et al.* 2007, Urban and Alphonsout 2007). Phloem girdling has been practiced in forestry as a pre-harvest treatment to alter wood properties (Noel 1970) by manipulating annual ring width, wood density, duration of cambial activity, and latewood production (Wilson and Gartner 2002, Domec and Pruyn 2008, Maunoury-Danger *et al.* 2010).

Because hydraulic capacity of different segments of the soil-to-leaf hydraulic pathway (root system, stem, and leaves) is regulated co-ordinately, we hypothesize that leaf hydraulic conductance will also decrease as a consequence of phloem severing. The primary aim of this study was to test this hypothesis experimentally. Secondly, we wanted to test the involvement of potassium ions in this putative decline of leaf hydraulic conductance, as on average over 50 % of leaf liquid-phase resistance is located in veins consisting of dead xylem conduits (Sack *et al.* 2005, Sack and Holbrook 2006). In addition to potassium ion concentration, the xylem sap was also tested for changes in electrical conductivity (a proxy of the total ionic content) and pH in order to assess possible effect of these characteristics on hydraulic conductance.

Materials and methods

The study site, located at Rõka village (58° 14'N, 27° 17'E; 40 - 48 m above see level), eastern Estonia, experiences a northern temperate climate (boreo-nemoral zone). Experiments were conducted on hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) saplings growing in an experimental forest plantation at the *FAHM* site with the stand density of 0.25 trees m^{-2} (Kupper *et al.* 2011). The long-term average annual precipitation in the region is 650 mm and the average temperature is 17.0 °C in July and -6.7 °C in January. The growing season usually lasts 175 - 180 d from mid-April to October. The soil is a fertile endogenic mollic planosol (WRB) with an A-horizon thickness of 27 cm. Total nitrogen content is 0.11 - 0.14 %, C/N ratio is 11.4, and pH is 5.7 - 6.3. In August 2010, we sampled ten 5-year-old aspen trees (4.0 ± 0.09 m).

Two neighbouring branches (mean height above the ground 129 ± 3.6 cm and mean length 147 ± 4.4 cm) of equal size were chosen from each tree for gasometric and hydraulic measurements: on one branch, phloem was girdled at the branch base, the second branch was left untreated (as a control). Two 1-cm strips of bark (cortex and phloem) were removed with a razor blade without damaging the xylem: one at the basal end of the branch, the second ~15 cm above the first. The girdled regions were immediately covered with silicon grease and wrapped tightly in adhesive plastic tape to prevent desiccation. The girdling treatment was intended to block phloem transport out of the branch, which was expected to result in assimilate accumulation in the foliage and induction of stomatal closure through a feedback loop (Myers *et al.* 1999, Cernusak and Marshall 2001). The initial measurements were taken immediately before phloem girdling, with subsequent measurements 1, 2, and 3 d thereafter. A brief pilot study indicated that there were no reliably detectable physiological responses within 6 h following phloem girdling.

Both the girdled and control branches were measured simultaneously in the midday period (~10-14 h) using two instruments. Net photosynthetic rate (P_N), stomatal conductance to water vapour (g_S), and ratio of intercellular to ambient CO_2 concentrations (c_i/c_a) were measured with a *LCpro+* portable photosynthesis system (*ADC BioScientific*, Great Amwell, UK) on four leaves at saturating photosynthetic photon flux density of 1196 $\mu mol\ m^{-2}\ s^{-1}$, c_a of 360 $\mu mol\ mol^{-1}$, water vapour pressure of 1.5 kPa and temperature of 25 ºC. Whole leaf conductance to water vapour (g_L), transpiration rate (E), and leaf temperature (T_L) were measured on eight leaves with a *LI-1600M* steady-state diffusion porometer (*Li-Cor*, Lincoln, NE, USA) at ambient conditions. Intrinsic water-use efficiency (IWUE) was calculated as the P_N/g_s ratio. Bulk leaf water potential (Ψ_L) was determined in four detached leaves by a Scholander-type pressure chamber simultaneously with gas-exchange measurements. Xylem water potential of the branches (Ψ_B) was estimated by applying the bagged leaves method (Nardini *et al.* 2001, Brodribb and Holbrook 2003), sampling two leaves per branch, prepared the previous evening. Water potential of the non-transpiring (bagged) leaves, presumed to have equilibrated with the xylem water potential of the branch proximal to the petiole, was taken as an estimate of Ψ_B. The leaves for gas exchange and water potential measurements were chosen randomly along the branch, all above the girdled region in severed branches. Two additional bagged leaves per tree situated near the main stem at the crown base (60 - 80 cm above the ground) were sampled just before dawn and the corresponding readings were used as a proxy of soil water potential (Ψ_S). To prevent overnight water loss and promote full equilibrium between soil and plant water potentials by dawn (Sellin 1999), we decided to use bagged leaves.

Hydraulic conductance of leaves (K_L) was estimated by the evaporative flux method (Wullschleger *et al.* 1998, Brodribb and Holbrook 2003, Sellin *et al.* 2008) under steady-state conditions and was calculated according to the Ohm's law analogy: $K_L = E/(\Psi_B - \Psi_L)$, where E is expressed per unit leaf area and so the values of K_L have been scaled by leaf area. K_L was standardized for the dynamic viscosity of water at 25 °C.

After the final gasometric and water potential measurements (on the third day after girdling), all leaves were removed immediately from the sample branches to end transpiration, the branches were excised from the tree and xylem sap was extracted according to Stark *et al.* (1985). The sap was extracted from branch segments at a pressure of 2.0 MPa generated by a pressure chamber (*Model 1000*; *PMS Instrument Company*, Albany, USA) (see Sellin *et al.* 2011 for details). In order to investigate the possible role of potassium ions in determining changes in K_L, the potassium ion concentration ($[K^+]$) of the sap samples collected in 1.5-cm^3 Eppendorf tubes was measured immediately using a *Cardy C-131* potassium ion meter (*Horiba*, Kyoto, Japan). Sap electrical conductivity (σ_{sap}) and acidity were determined using an electric conductometer (*Twin Cond B-173*; *Horiba*) and pH meter (*Twin B-212*; *Horiba*), respectively.

Statistical data analysis was carried out using *Statistica*, v. 7.1 (*StatSoft Inc.*, Tulsa, OK, USA). The effect of phloem girdling (treatment) on leaf gas exchange and hydraulic characteristics was analysed by applying a two-way *ANOVA*. Normality and homogeneity of variances were checked using the Kolmogorov-Smirnov D-statistics and the Levene test, respectively (Sokal and Rohlf 1995). When appropriate, logarithmic or complex transformations were applied to the data. Post hoc mean comparisons were conducted using the Tukey HSD test. Bivariate relationships between the studied characteristics and independent variables were assessed by using simple linear or non-linear least squares regression.

Results

Stomatal conductance, net photosynthetic rate, and c_i/c_a ratio varied significantly ($P < 0.001$) both with girdling treatment and time after girdling. As a consequence of phloem girdling, mean g_S decreased from 0.457 to 0.078 mol m^{-2} s^{-1} (by 83 %) over three days (Fig. 1*A*). Although P_N declined less (by 78 %; Fig. 1*B*) across the whole range of variation, mean c_i/c_a increased from 0.67 to 0.87 ($P < 0.001$; Fig. 1*C*). Even if g_S and g_L were measured on different leaves and under different conditions (controlled *versus* ambient conditions, respectively), the variables were highly correlated ($R^2 = 0.781$, $P < 0.001$). Although the mean values of intrinsic water-use efficiency (IWUE) were lower in girdled than in control branches (30.7 *versus* 38.0 µmol mol^{-1} 3 d after girdling), the treatment effect proved to be statistically insignificant ($P > 0.05$). An inverse relationship between c_i/c_a and IWUE was observed both for the control and girdled branches (Fig. 2). P_N was strongly related to g_S for both treatments combined (Fig. 3*A*). Nevertheless, at first g_S and P_N responded co-ordinately to an obvious accumulation of sugars in leaves, but later (g_S below 0.2 mol m^{-2} s^{-1}) P_N decreased faster inducing a decline in IWUE (Fig. 3*B*).

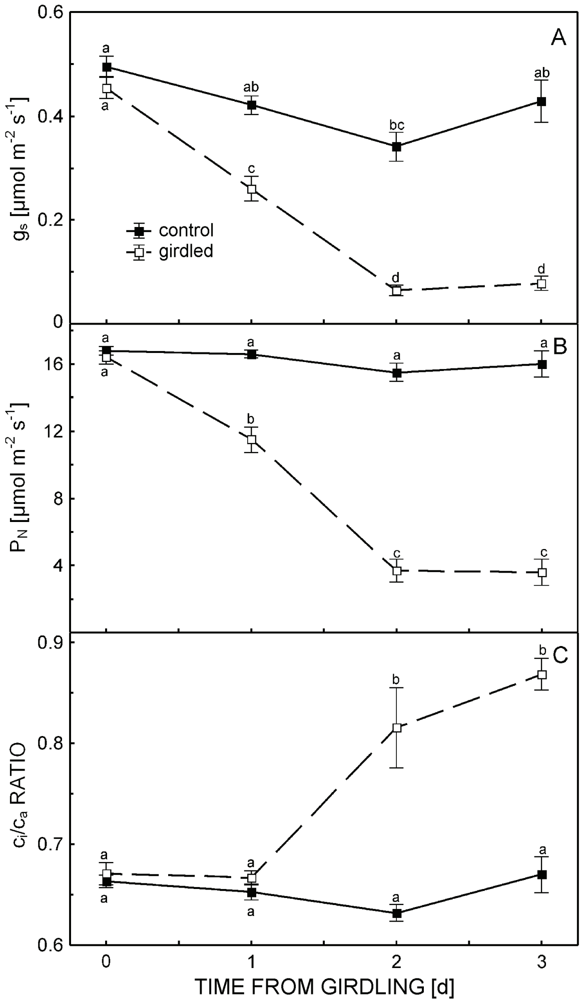

Fig. 1. Changes in stomatal conductance to water vapour (g_S; *A*), net photosynthetic rate (P_N; *B*), and ratio of intercellular to ambient CO_2 concentrations (c_i/c_a; *C*) in response to phloem girdling. Day 0 - measured just before girdling. *Error bars* represent ± SE; different letters denote statistically significant ($P < 0.05$) differences between means.

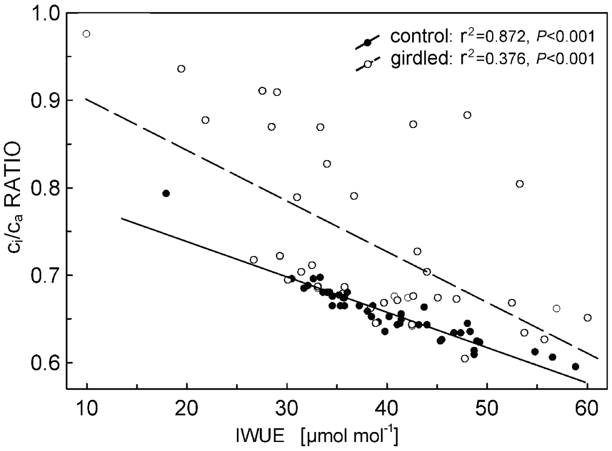

Fig. 2. Inverse relationship between intrinsic water-use efficiency (IWUE) and ratio of intercellular to ambient CO_2 concentrations (c_i/c_a) in control and girdled branches.

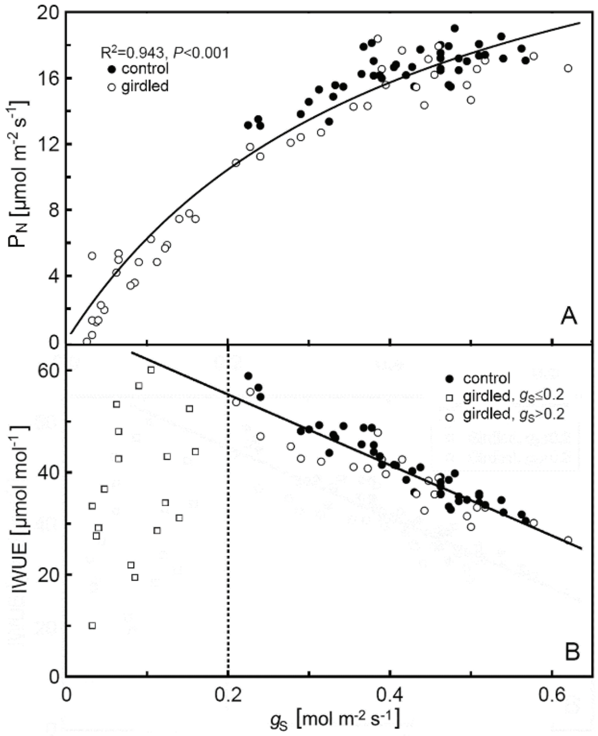

Fig. 3. *A* - Stomatal conductance (g_S) *versus* net photosynthetic rate (P_N) across control and girdled branches. *B* - g_S *versus* intrinsic water-use efficiency (IWUE) across control and girdled branches; $R^2 = 0.848$, $P < 0.001$ at $g_S \geq 0.2$ mol m^{-2} s^{-1} and $R^2 = 0.126$, $P = 0.147$ at $g_S < 0.2$ mol m^{-2} s^{-1}.

Phloem girdling induced a considerable increase in leaf water potential (Ψ_L; Fig. 4), owing to decreasing transpirational water loss (Fig. 5*A*) due to the drastic decline in g_S (Fig. 1*A*). The girdled branches demonstrated higher Ψ_L than the control branches at all measurement times after phloem girdling; the differences were highly significant on the second and third days ($P < 0.001$ and $P = 0.002$, respectively). We observed no differences in Ψ_B ($P = 0.539$) and T_L ($P = 0.587$) between the treatments.

Leaf hydraulic conductance responded significantly ($P = 0.006$) to girdling: mean K_L decreased from 6.64 to 3.78 mmol m^{-2} s^{-1} MPa^{-1} (by 43 %) during 3 d after the treatment (Fig. 5*C*). The variation of K_L in the control branches was more governed by changes in driving forces ($\Delta\Psi$) than by evaporative flux (E) − the daily means varied by factor of 1.5 *versus* 1.3, respectively. In the girdled branches, on the contrary, the dynamics of K_L was primarily determined by decreasing E, as the mean values varied by factor of 7.6 *versus* 3.4 for E and $\Delta\Psi$, respectively (Fig. 5*A,B*). The partial correlation coefficients were 0.85 and -0.75, respectively. K_L was invariant to Ψ_L ($P > 0.05$). The gas exchange parameters (g_S, g_L, P_N, and c_i/c_a) were co-ordinated with leaf hydraulic efficiency ($y = f(K_L)$; $R^2 = 0.295 - 0.441$, $P < 0.001$) only in the girdled branches. IWUE depended on K_L neither in control ($R^2 = 0.001$, $P = 0.899$) nor girdled branches ($R^2 = 0.037$, $P = 0.237$).

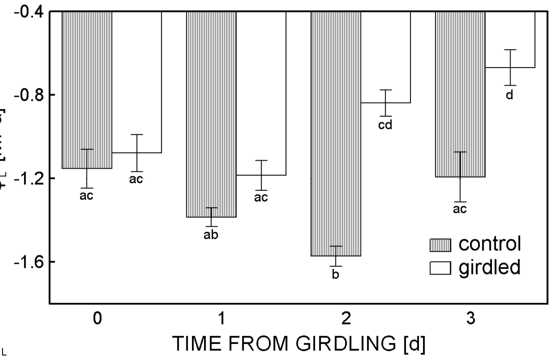

Fig. 4. Response of leaf bulk water potential (Ψ_L) to phloem girdling. Day 0 - measured just before girdling. *Error bars* represent ± SE; different letters denote statistically significant ($P < 0.05$) differences between means.

Phloem girdling had no impact on the measured physico-chemical properties of xylem sap. The mean potassium ion concentration was 0.44 and 0.65 mM ($P = 0.180$) in the control and girdled branches, respectively. Similarly, the respective shifts in mean σ_{sap} (0.59 → 0.64 mS cm^{-1}; $P = 0.557$) and pH (6.3 → 6.5; $P = 0.185$) proved to be non-significant. Variation in K_L was not related ($P > 0.05$) to [K$^+$], σ_{sap} or pH of xylem sap. We observed a near-perfect correlation ($R^2 = 0.907$, $P < 0.001$) between [K$^+$] and σ_{sap} across both the treatments combined.

Discussion

Phloem girdling at the branch base of hybrid aspens induced a sharp decrease in g_S and P_N over the following 3 d (Fig. 1). Similar reductions in both P_N and g_S have been observed in *Mangifera indica* (by 77 and 71 %, respectively), *Pinus monticola* (by factor of ~2 and ~3, respectively), and *Vitis vinifera* (both by 50 %) in response to girdling (Williams *et al.* 2000, Cernusak and Marshall 2001, Urban and Alphonsout 2007). Phloem girdling represents an experimental manipulation of source-sink relationships leading to a disturbance of saccharides allocation within plants. Disruption of the basipetal movement of assimilates through phloem results in an accumulation of non-structural saccharides in leaves and stems above the girdle and a consequent decline in P_N through end-product inhibition (Jang *et al.* 1997, Myers *et al.* 1999, Murakami *et al.* 2008). The decline in P_N is attributable to a decrease in the rate of photosynthetic electron transport and quantum yield efficiency of photosystem II (Urban and Alphonsout 2007, Rivas *et al.* 2007). Declining P_N, in turn, causes increase in instantaneous values of c_i/c_a (Fig. 1*C*) and stomatal narrowing (Fig. 1*A*).

Estimates of IWUE in hybrid aspen revealed no differences among the treatments. The ratio c_i/c_a reflects the proportion of supply and demand for CO_2 within leaf chloroplasts (Farquhar *et al.* 1989). The increase in c_i/c_a (Fig. 1*C*) can be explained by nonlinear (asymptotic) nature of the relationship between g_S and P_N – at high stomatal conductance the net photosynthetic rate has been saturated by CO_2 supply (Fig. 3*A*). Thus, the initial decrease in g_S in response to the girdling was not accompanied by the P_N decline; across the linear part of the $P_N = f(g_S)$ relationship the net photosynthesis decreased considerably faster than stomatal conductance. Similar results obtained on mango plants were attributed to the depressing effect of girdling primarily on photosynthesis (decreased electron transport rate) but not to changes in g_S (Urban and Alphonsout 2007). Nevertheless, due to good co-ordination between P_N and g_S in aspen saplings, c_i/c_a demonstrated moderate alteration (from 0.67 to 0.87) despite substantial changes in g_S and K_L (Figs. 1*A* and 5). This response agrees with that published for *P. monticola* (Cernusak and Marshall 2001). Girdling had no effect on the intercellular concentration of CO_2 in leaves of mango (Urban and Alphonsout 2007). Cernusak and Marshall (2001) suggested that correlated reductions in g_S and P_N are a general response to reduced hydraulic conductance. The present results obtained in hybrid aspen seem to support this conclusion (Fig. 3*A*).

Low g_S as a consequence of girdling reduced transpirational water loss from foliage (Fig. 5*A*) resulting in considerably less negative Ψ_L for the girdled branches than for the control branches (-0.67 versus -1.19 MPa, respectively, on the third day after girdling; Fig. 4). Thus, phloem girdling had a significant impact on leaf water status of aspen trees in contrast to data published for *Diospyros kaki*, where a negligible effect of girdling on leaf and stem water potential was observed (Fumuro 1998), and *Acer saccharum*, where no effect was observed in distal leaves (Murakami *et al.* 2008). However, reduction in g_S due to girdling resulted in lower E, and therefore more favourable leaf water status in grape vine (Williams *et al.* 2000).

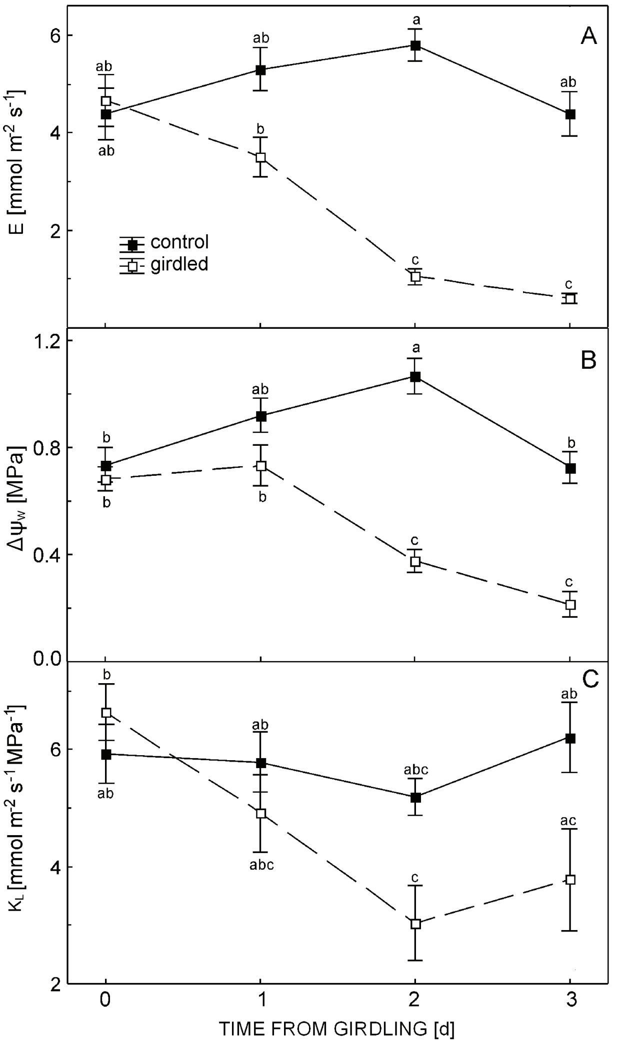

Fig. 5. Changes in transpiration rate (E; *A*), water potential drop along the leaf ($\Delta\Psi_w$; *B*), and leaf hydraulic conductance (K_L; *C*) in response to phloem girdling. Day 0 - measured just before girdling. Error bars represent ± SE, different letters denote statistically significant ($P < 0.05$) differences between means.

Our experiment confirmed the hypothesis that phloem severing induces changes in K_L similarly as demonstrated for stem hydraulic conductance (Cernusak and Marshall 2001, Zwieniecki *et al.* 2004). Phloem girdling caused a substantial decline in K_L of leaves attached to the treated branches (Fig. 5*C*). Domec and Pruyn (2008) proposed that the decline in K_L of *Pinus ponderosa* in response to girdling ensued from changes in leaf symplastic compartment. At first glance, our results seem to support this idea taking into account the fact that the changes in K_L were unrelated to $[K^+]$ in the xylem sap. Experiments on model plant systems also seem to support this idea because changes in transpirational flux induce coordinated up/down-regulation of many aquaporin genes in leaves (Levin *et al.* 2007, Kuwagata *et al.* 2012). On the other hand, one cannot completely discard potential consequences on leaf vein network, as mechanical injury (phloem disruption) may induce the occlusion of vessels by pectin-like polysaccharides (Tyree and Zimmermann 2002), lipid substances (Nemec 1975) or water-soluble proteins (Neumann *et al.* 2010). Thus, the mechanism by which girdling affects leaf hydraulic conductance remains unclear and needs further investigation.

Phloem severance at the branch base had no impact on physico-chemical properties of the xylem sap during the following three days, and thus the variation in K_L was unrelated to $[K^+]$, σ_{sap}, or pH of xylem sap. Consequently, girdling did not affect ion recycling from phloem to xylem (radial transport of K^+ included) in the aspen trees. Our results contrast to those obtained in two *Acer* species (Zwieniecki *et al.* 2004) where the phloem girdling induced a decrease in the osmotic potential of xylem sap suggesting that ion redistribution from the phloem was likely responsible for the hydraulic decline. Likewise, Domec and Pruyn (2008) did not rule out the possibility that changes of K_L in ponderosa pine in response to girdling were caused by a decrease in the concentration of ions in the xylem sap. Stem girdling also induced a decline of $[K^+]$ in xylem sap of *Diospyros kaki* (Fumuro 1998). The strong relationship ($R^2 = 0.907$, $P < 0.001$) between $[K^+]$ and σ_{sap} revealed in this study confirmed that potassium ions constituted a major component of the xylem sap in hybrid aspen, as reported for hybrid poplar (*Populus tremula* × *P. alba*) where K^+ was the most abundant cation in the xylem exudate representing nearly 50 % of total inorganic ions (Siebrecht *et al.* 2003).

The experiment with hybrid aspen revealed a relationship between leaf hydraulic capacity and gas exchange parameters – g_S, g_L, c_i/c_a, and P_N – only for the girdled branches. The results suggest that the water transport system of hybrid aspen features a functional redundancy under normal conditions and gas exchange is not constrained by leaf hydraulic efficiency. Stomatal conductance and/or photosynthetic capacity depend first of all on hydraulic conductance of the pathway from soil to leaves as shown in several works (Hubbard *et al.* 2001, Santiago *et al.* 2004, Taylor and Eamus 2008, Chen *et al.* 2009, Zhang and Cao 2009). However, co-ordination of liquid and gaseous phase conductances becomes evident under conditions of diminishing water flux (Fig. 5*A*), whereas the corresponding mechanism should have been unconnected to leaf water status. Firstly, K_L was completely independent of leaf water status within the Ψ_L range observed in the present experiment; secondly, Ψ_L increased (Fig. 4) in manipulated branches in spite of decreasing water fluxes (Fig. 5*A*). The hydraulic redundancy probably confers a fundamental advantage in fluctuating environments for a fast-growing pioneer species, such as aspen, over late-successional tree species. Both parent species, *P. tremula* and *P. tremuloide,* are fast-growing species with broad ecological amplitude over a widespread area including extreme conditions (high altitudes, dry, or cold climates) in Eurasia and North America, respectively (Dickmann and Kuzovkina 2008).

In our study, in which girdling effects were evaluated over a short time scale (3 d), IWUE was affected by changes of K_L neither in the control nor girdled branches. This contrasts to works analysing plant responses during longer periods (months or developmental time scale) in which a trade-off has been observed between hydraulic capacity and water-use efficiency at whole-tree or branch levels (Cernusak and Marshall 2001, Santiago *et al.* 2004, Taylor and Eamus 2008, Chen *et al.* 2009, Sellin *et al.* 2010b). It is probable that converse relationship between hydraulic conductance and water-use efficiency (long-term or intrinsic WUE) can develop during a long period associated with acclimation to specific environmental conditions but not as a short-term response to mechanical interference.

In conclusion, our results support the hypothesis that phloem girdling induces a decrease in leaf hydraulic conductance. Disruption of phloem transport by girdling at branch base did not disturb lateral ion transfer to xylem in hybrid aspen, and the decrease of leaf hydraulic conductance in response to girdling did not result from changes in [K^+] and total ionic content of the xylem sap.

References

Brodribb, T.J., Holbrook, N.M.: Changes in leaf hydraulic conductance during leaf shedding in seasonally dry tropical forest. - New Phytol. **158**: 295-303, 2003.

Cernusak, L.A., Marshall, J.D.: Responses of foliar δ^{13}C, gas exchange and leaf morphology to reduced hydraulic conductivity in *Pinus monticola* branches. - Tree Physiol. **21**: 1215-1222, 2001.

Chen, J.-W., Zhang, Q., Cao, K.-F.: Inter-species variation of photosynthetic and xylem hydraulic traits in the deciduous and evergreen Euphorbiaceae tree species from a seasonally tropical forest in south-western China. - Ecol. Res. **24**: 65-73, 2009.

Christman, M.A., Sperry, J.S., Smith, D.D.: Rare pits, large vessels and extreme vulnerability to cavitation in a ring-porous tree species. - New Phytol. **193**: 713-720, 2012.

Cochard, H., Herbette, S., Hernández, E., Hölttä, T., Mencuccini, M.: The effects of sap ionic composition on xylem vulnerability to cavitation. - J. exp. Bot. **61**: 275–285, 2010.

De Boer, A.H., Volkov, V.: Logistics of water and salt transport through the plant: structure and functioning of the xylem. - Plant Cell Environ. **26**: 87-101, 2003.

Dickmann, D.I., Kuzovkina, J.: Poplars and Willows of the World, with Emphasis on Silviculturally Important Species. - FAO, Rome 2008.

Di Vaio, C., Petito, A., Buccheri, M.: Effect of girdling on gas exchanges and leaf mineral content in the 'Independence' nectarine. - J. Plant Nutr. **24**: 1047-1060, 2001.

Domec, J.-C., Pruyn, M.L.: Bole girdling affects metabolic properties and root, trunk and branch hydraulics of young ponderosa pine trees. - Tree Physiol. **28**: 1493-1504, 2008.

Esau, K.: Anatomy of Seed Plants. - John Wiley & Sons, New York 1977.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T.: Carbon isotope discrimination and photosynthesis. - Annu. Rev. Plant Physiol. Plant mol. Biol. **40**: 503-537, 1989.

Fumuro, M.: Effects of trunk girdling during early shoot elongation period on tree growth, mineral absorption, water stress, and root respiration in Japanese persimmon (*Diospyros kaki* L.) cv. Nishimurawase. - J. jap. Soc. hort. Sci. **67**: 219-227, 1998.

Gascó, A., Gortan, E., Salleo, S., Nardini, A.: Changes of pH of solutions during perfusion through stem segments: further evidence for hydrogel regulation of xylem hydraulic properties? - Biol. Plant. **52**: 502-506, 2008.

Gascó, A., Nardini, A., Gortan, E., Salleo, S.: Ion-mediated increase in the xylem hydraulic conductivity: role of pits and consequences for the impact of cavitation on water transport in plants. - Plant Cell Environ. **29**: 1946-1955, 2006.

Hubbard, R.M., Ryan, M.G., Stiller, V., Sperry, J.S.: Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. - Plant Cell Environ. **24**: 113-121, 2001.

Jang, J.-C., Leon, P., Zhou, L., Sheen, J.: Hexokinase as a sugar sensor in higher plants. - Plant Cell **9**: 5-19, 1997.

Jeschke, D.W., Pate, J.S.: Cation and chloride partitioning through xylem and phloem within the whole plant of *Ricinus communis* L. under conditions of salt stress. - J. exp. Bot. **42**: 1105-1116, 1991.

Kanai, S., Moghaieb, R.E., El-Shemy, H.A., Panigrahi, R., Mohapatra, P.K., Ito, J., Nguyen, N.T., Saneoka, H., Fujita, K.: Potassium deficiency affects water status and photosynthetic rate of the vegetative sink in green house tomato prior to its effects on source activity. - Plant Sci. **180**: 368-374, 2011.

Kupper, P., Sõber, J., Sellin, A., Lõhmus, K., Tullus, A., Räim, O., Lubenets, K., Tulva, I., Uri, V., Zobel, M., Kull, O., Sõber, A.: An experimental facility for Free Air Humidity Manipulation (FAHM) can alter water flux through deciduous tree canopy. - Environ. exp. Bot. **72**: 432-438, 2011.

Kuwagata, T., Ishikawa-Sakurai, J., Hayashi, H., Nagasuga, K., Fukushi, K., Ahamed, A., Takasugi, K., Katsuhara, M., Murai-Hatano, M.: Influence of low air humidity and low root temperature on water uptake, growth and aquaporin expression in rice plants. - Plant Cell Physiol. **53**: 1418-1431, 2012.

Lee, J., Holbrook, N.M., Zwieniecki, M.A. Ion induced changes in the structure of bordered pit membranes. - Front. Plant Sci. **3**: 55. doi: 10.3389/fpls.2012.00055, 2012.

Levin, M., Lemcoff, J.H., Cohen, S., Kapulnik, Y.: Low air humidity increases leaf-specific hydraulic conductance of *Arabidopsis thaliana* (L.) Heynh (Brassicaceae). - J. exp. Bot. **58**: 3711-3718, 2007.

Maunoury-Danger, F., Fresneau, C., Eglin, T., Berveiller, D., François, C., Lelarge-Trouverie, C., Damesin, C.: Impact of carbohydrate supply on stem growth, wood and respired CO_2 $\delta^{13}C$: assessment by experimental girdling. - Tree Physiol. **30**: 818-830, 2010.

Murakami, P.F., Schaberg, P.G., Shane, J.B.: Stem girdling manipulates leaf sugar concentrations and anthocyanin expression in sugar maple trees during autumn. - Tree Physiol. **28**: 1467-1473, 2008.

Myers, D.A., Thomas, R.B., DeLucia, E.H.: Photosynthetic responses of loblolly pine (*Pinus taeda*) needles to experimental reduction in sink demand. - Tree Physiol. **19**: 235-242, 1999.

Nardini, A., Gasco, A., Trifilo, P., Lo Gullo, M.A., Salleo, S.: Ion-mediated enhancement of xylem hydraulic conductivity is not always suppressed by the presence of Ca^{2+} in the sap. - J. exp. Bot. **58**: 2609-2615, 2007.

Nardini, A., Grego, F., Trifilò, P., Salleo, S.: Changes of xylem sap ionic content and stem hydraulics in response to irradiance in *Laurus nobilis*. - Tree Physiol. **30**: 628-635, 2010.

Nardini, A., Tyree, M.T., Salleo, S.: Xylem cavitation in the leaf of *Prunus laurocerasus* and its impact on leaf hydraulics. - Plant Physiol. **125**: 1700-1709, 2001.

Nemec, S.: Vessel blockage by myelin forms in citrus with and without rough-lemon decline symptoms. - Can. J. Bot. **53**: 102-108, 1975.

Neumann, P.M, Weissman, R., Stefano, G., Mancuso, S.: Accumulation of xylem transported protein at pit membranes and associated reductions in hydraulic conductance. - J. exp. Bot. **61**: 1711-1717, 2010.

Noel, A.R.A.: The girdled tree. - Bot. Rev. **36**: 162-195, 1970.

Offler, C.E., McCurdy, D.W., Patrick, J.W., Talbot, M.J.: Transfer cells: cells specialized for a special purpose. - Annu. Rev. Plant Biol. **54**: 431-454, 2002.

Pate, J.S., Jeschke, W.D.: Role of stems in transport, storage, and circulation of ions and metabolites by the whole plant. - In: Gartner, B.L. (ed.): Plant Stems. Physiology and Functional Morphology. Pp. 177-204. Academic Press, San Diego 1995.

Rivas, F., Gravina, A., Agustí, M.: Girdling effects on fruit set and quantum yield efficiency of PSII in two *Citrus* cultivars. - Tree Physiol. **27**: 527-535, 2007.

Ryden, P., MacDougall, A.J., Tibbits, C.W., Ring, S.G.: Hydration of pectic polysaccharides. - Biopolymers **54**: 398-405, 2000.

Sack, L., Holbrook, N.M.: Leaf hydraulics. - Annu. Rev. Plant Biol. **57**: 361-381, 2006.

Sack, L., Tyree, M.T., Holbrook, N.M.: Leaf hydraulic architecture correlates with regeneration irradiance in tropical rainforest trees. - New Phytol. **167**: 403-413, 2005.

Salleo, S., Lo Gullo, M.A., De Paoli, D., Zippo, M.: Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism. - New Phytol. **132**: 47-56, 1996.

Salleo, S., Lo Gullo, M.A., Trifilò, P., Nardini, A.: New evidence for a role of vessel-associated cells and phloem in the rapid xylem refilling of cavitated stems of *Laurus nobilis* L. - Plant Cell Environ. **27**: 1065-1076, 2004.

Santiago, L.S., Goldstein, G., Meinzer, F.C., Fisher, J.B., Machado, K., Woodruff, D., Jones, T.: Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. - Oecologia **140**: 543-550, 2004.

Sellin, A.: Does pre-dawn water potential reflect conditions of equilibrium in plant and soil water status? - Acta oecol. **20**: 51-59, 1999.

Sellin, A., Eensalu, E., Niglas, A.: Is distribution of hydraulic constraints within tree crowns reflected in photosynthetic water-use efficiency? An example of *Betula pendula*. - Ecol. Res. **25**: 173-183, 2010b.

Sellin, A., Õunapuu, E., Karusion, A.: Experimental evidence supporting the concept of light-mediated modulation of stem hydraulic conductance. - Tree Physiol. **30**: 1528-1535, 2010a.

Sellin, A., Õunapuu, E., Kupper, P.: Effects of light intensity and duration on leaf hydraulic conductance and distribution of resistance in shoots of silver birch (*Betula pendul*a). - Physiol. Plant. **134**: 412-420, 2008.

Sellin, A., Sack, L., Õunapuu, E., Karusion, A.: Impact of light quality on leaf and shoot hydraulic properties: a case study in silver birch (*Betula pendula*). - Plant Cell Environ. **34**: 1079-1087, 2011.

Setter, T.L., Brun, W.A., Brenner, M.L.: Effect of obstructed translocation of leaf abscisic acid on associated stomatal closure and photosynthesis decline. - Plant Physiol. **65**: 1111-1115, 1980.

Sevanto, S., Hölttä, T., Holbrook, N.M.: Effects of the hydraulic coupling between xylem and phloem on diurnal phloem diameter variation. - Plant Cell Environ. **34**: 690-703, 2011.

Siebrecht, S., Herdel, K., Schurr, U., Tischner, R.: Nutrient translocation in the xylem of poplar: diurnal variations and spatial distribution along the shoot axis. - Planta **217**: 783-793, 2003.

Sokal, R.R., Rohlf, F.J.: Biometry: the Principles and Practice of Statistics in Biological Research. - W. H. Freeman and Co, New York 1995.

Sperry, J.S., Tyree, M.T.: Mechanism of water stress-induced xylem embolism. - Plant Physiol. **88**: 581-587, 1988.

Sperry, J.S., Donnelly, J.R., Tyree, M.T.: A method for measuring hydraulic conductivity and embolism in xylem. - Plant Cell Environ. **11**: 35-40, 1988.

Stark, N., Spitzner, C., Essig, D.: Xylem sap analysis for determining the nutritional status of trees: *Pseudotsuga menziesii*. - Can. J. Forest. Res. **15**: 429-437, 1985.

Taylor, D., Eamus, D.: Coordinating leaf functional traits with branch hydraulic conductivity: resource substitution and implications for carbon gain. - Tree Physiol. **28**: 1169-1177, 2008.

Tyree, M.T., Zimmermann, M.H.: Xylem Structure and the Ascent of Sap. - Springer-Verlag, Berlin 2002.

Urban, L., Alphonsout, L.: Girdling decreases photosynthetic electron fluxes and induces sustained photoprotection in mango leaves. - Tree Physiol. **27**: 345-352, 2007.

Van Bel, A.J.E.: Xylem–phloem exchange via the rays: the undervalued route of transport. - J. exp. Bot. **41**: 631-644, 1990.

Van Doorn, W.G., Hiemstra, T., Fanourakis, D.: Hydrogel regulation of xylem water flow: an alternative hypothesis. - Plant Physiol. **157**: 1642-1649, 2011.

Van Ieperen, W.: Ion-mediated changes of xylem hydraulic resistance in planta: fact or fiction? -Trends Plant Sci. **12**: 137-142, 2007.

Van Ieperen, W., Van Meeteren, U., Van Gelder, H.: Fluid ionic composition influences hydraulic conductance of xylem conduits. - J. exp. Bot. **51**: 769-776, 2000.

Wilson, B.F., Gartner, B.L.: Effects of phloem girdling in conifers on apical control of branches, growth allocation and air in wood. - Tree Physiol. **22**: 347-353, 2002.

Williams, L.E., Ayars, J.E.: Water use of Thompson Seedless grapevines as affected by the application of gibberellic acid (GA_3) and trunk girdling – practices to increase berry size. - Agr. Forest. Meteorol. **129**: 85-94, 2005.

Williams, L.E., Retzlaff, W.A., Yang, W.G., Biscay, P.J., Ebisuda, N.: Effect of girdling on leaf gas exchange, water status, and non-structural carbohydrates of field-grown *Vitis vinifera* L. (cv. Flame Seedless). - Amer. J. Enol. Viticult. **51**: 49-54, 2000.

Wullschleger, S.D., Meinzer, F.C., Vertessy, R.A.: A review of whole-plant water use studies in trees. - Tree Physiol. **18**: 499-512, 1998.

Zhang, J.-L., Cao, K.-F.: Stem hydraulics mediates leaf water status, carbon gain, nutrient use efficiencies and plant growth rates across dipterocarp species. - Funct. Ecol. **23**: 658-667, 2009.

Zweifel, R., Item, H., Häsler, R.: Stem radius changes and their relation to stored water in stems of young Norway spruce trees. - Trees **15**: 50-57, 2000.

Zwieniecki, M.A., Hutyra, L., Thompson, M.V., Holbrook, N.M.: Dynamic changes in petiole specific conductivity in red maple (*Acer rubrum* L.), tulip tree (*Liriodendron tulipifera* L.) and northern fox grape (*Vitis labrusca* L.). - Plant Cell Environ. **23**: 407-414, 2000.

Zwieniecki, M.A., Melcher, P.J., Feild, T.S., Holbrook, N.M.: A potential role for xylem–phloem interactions in the hydraulic architecture of trees: effects of phloem girdling on xylem hydraulic conductance. - Tree Physiol. **24**: 911-917, 2004.

Zwieniecki, M.A., Melcher, P.J., Holbrook, N.M.: Hydrogel control of xylem hydraulic resistance in plants. - Science **291**: 1059-1062, 2001.