Intraspecific differences in drought tolerance and acclimation in hydraulics of *Ligustrum vulgare* and *Viburnum lantana*

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**Summary** An adequate general drought tolerance and the ability to acclimate to changing hydraulic conditions are important features for long-lived woody plants. In this study, we compared hydraulic safety (water potential at 50% loss of conductivity, \( \Psi_{50} \)), hydraulic efficiency (specific conductivity, \( k_s \)), xylem anatomy (mean tracheid diameter, \( d_{mean} \)), conduit wall thickness, \( t \), conduit wall reinforcement, \((t/b)_h^2 \)) and stomatal conductance, \( g_s \), of forest plants as well as irrigated and drought-treated garden plants of *Ligustrum vulgare* L. and *Viburnum lantana* L. Forest plants of *L. vulgare* and *V. lantana* were significantly less resistant to drought-induced cavitation (\( \Psi_{50} \) at \(-2.82 \pm 0.13 \) MPa and \(-2.79 \pm 0.17 \) MPa) than drought-treated garden plants (\(-4.58 \pm 0.26 \) MPa and \(-3.57 \pm 0.15 \) MPa). When previously irrigated garden plants were subjected to drought, a significant decrease in \( d_{mean} \) and \( d_h \), and an increase in \( t \) and \((t/b)_h^2 \) were observed in *L. vulgare*. In contrast, in *V. lantana* conduit diameters increased significantly but no change in \( t \) and \((t/b)_h^2 \) was found. Stomatal closure occurred at similar water potentials (\( \Psi_{sc} \)) in forest plants and drought-treated garden plants, leading to higher safety margins (\( \Psi_{sc} - \Psi_{50} \) ) of the latter (*L. vulgare* 1.63 MPa and *V. lantana* 0.43 MPa). These plants also showed higher \( g_s \) at moderate \( \Psi \), more abrupt stomatal closure and lower cuticular conductivity. Data indicate that the development of drought-tolerant xylem as well as stomatal regulation play an important role in drought acclimation, whereby structural and physiological responses to drought are species-specific and depend on the plant’s hydraulic strategy.

**Keywords:** embolism, hydraulic efficiency, hydraulic safety, phenotypic plasticity, stomatal conductance, vulnerability, xylem anatomy.

**Introduction**

Environments are highly heterogeneous in both space and time (Valladares et al. 2007). Thus, survival and distribution of sessile organisms such as plants depend strongly on their ability to adjust to environmental variation. This may be of particular importance for long-lived woody plants which cannot survive adverse periods in the form of desiccation-tolerant seed stages (e.g., Larcher 1995, Magnani et al. 2002). Adaptation enables plants to optimize their life processes to prevailing environmental conditions at an evolutionary scale. In contrast, acclimation (i.e., positive response to new conditions) occurs within a plant’s lifetime and without altering the genetic constitution (Batanouny 2000, Giennapp et al. 2008). Acclimation processes (structural or physiological) occur in the range of phenotypic plasticity (Sultan 1995, 2000). They are the long-term responses taking place over the course of weeks to years, and thus differ from the short-term physiological regulations.

Drought is an important stress factor and can lead to several physiological and structural responses to maintain balanced water relations (e.g., Jones and Sutherland 1991, Larcher 1995, Maherali and Delucia 2000, Froux et al. 2005, Breda et al. 2006, Poyatos et al. 2007, McDowell et al. 2008). Stomatal closure is by far the most efficient reaction to daily and seasonal water shortage (Froux et al. 2005) as it occurs within a few minutes, and it is dynamic and reversible (modulative response; Jones and Sutherland 1991, Larcher 1995, Breda et al. 2006). By closing their stomata, plants prevent a critical decrease in water potential (\( \Psi \); Tyree and Sperry 1988, Saliendra et al. 1995, Cochard et al. 2002, Sperry et al. 2002), though this goes at the expense of reduced CO\(_2\) assimilation (Jones and Sutherland 1991, Tyree and Ewers 1991, Cochard et al. 1996, 2002, Breda et al. 2006). In the long term, increased biomass allocation to roots versus leaves and leaf shedding are additional possibilities to reduce water losses. Furthermore, changes in conductivity related to ion concentrations, water storage and refilling processes might play an important role (see Larcher 1963, Gasco et al. 2007, Nardini et al. 2007) in avoiding critical \( \Psi \).

However, due to cuticular transpiration, \( \Psi \) can continue to decrease even after complete stomatal closure (e.g., Baig...
and Tranquillini 1980, Larcher 1995, Mayr et al. 2003, Froux et al. 2005) leading to xylem embolism when air enters the pits and breaks the water columns (cavitation; e.g., Sperry and Tyree 1990, Tyree and Ewers 1991, Sperry and Sullivan 1992, Tyree and Zimmermann 2002). Normally, the surface tension at the porous pit membrane (angiosperms) or the pit apparatus itself (conifers) prevents this ‘air-seeding’ (Sperry and Tyree 1990, Tyree et al. 1994, Tyree and Zimmermann 2002), but when ψ decreases below specific thresholds, these mechanisms fail. Therefore, a sufficient safety margin (i.e., range of ψ between the time of stomatal closure and cavitation) is required to protect the xylem from embolism (see Hacke and Sauter 1996, Pockman and Sperry 2000, Froux et al. 2005, Breda et al. 2006). As the hydraulic safety (i.e., the ability of the xylem to prevent embolism) and the hydraulic efficiency (i.e., hydraulic conductivity) correlate with several anatomical parameters (e.g., Sperry and Tyree 1990, Sperry and Sullivan 1992, Sperry and Saliendra 1994, Sperry et al. 1994, 2007, Tyree et al. 1994, Hacke and Sperry 2001, Hacke et al. 2001, Tyree and Zimmermann 2002), structural modifications of the xylem enable an increase in its resistance to drought-induced embolism.

Many authors demonstrated the impacts of drought on hydraulic parameters of conifers (e.g., Maherali and Delucia 2000, Froux et al. 2005, Breda et al. 2006, Ladjal et al. 2007) and tropical, Mediterranean or desert angiosperms (e.g., Kolb and Davis 1994, Fotelli et al. 2000, Hacke et al. 2000, Brodrribb et al. 2003, Manes et al. 2006, Poggi et al. 2007). Only few studies focused on temperate and boreal angiosperms (e.g., Saliendra et al. 1995, Alder et al. 1996, Vogt 2001, Cochard et al. 2002, Tissier et al. 2004, Breda et al. 2006), although acclimation to drought may be particularly important in these predominantly humid regions with sporadically occurring drought periods. In these regions, an appropriate phenotypic plasticity in hydraulic traits may become even more important when frequency and intensity of drought periods increase due to climate change (Alcamo et al. 2007). There is also little knowledge on structural changes and intraspecific response in hydraulic upon changing environmental conditions (e.g., Mencuccini 2003, Maseda and Fernandez 2006).

In this study, we analysed intraspecific differences in hydraulic safety, xylem anatomy and stomatal conductance (gs) of Ligustrum vulgare L. and Viburnum lantana L., subjected to different soil moisture conditions. Both species are woody shrubs and often occur associated in the understorey of temperate forests, but can also be found at drier, even rocky sites. Thus, we hypothesized that they do not develop a high cavitation resistance a priori, but are able to acclimate within a broad range when subjected to drought. Our study should also reveal whether these two species, growing under identical environmental conditions, might differ in their strategies regarding transpiration and hydraulic acclimation to avoid drought stress.

Materials

Plant material

Measurements were made on L. vulgare (privet) and V. lantana (snowball bush). Both species are up to 4 m high, deciduous, semi-ring-porous shrubs (Grosser 1977); L. vulgare has small (4–6 cm long and 1–2 cm broad), slightly leathery leaves, whereas V. lantana has broader leaves (10–20 cm long and 4–9 cm broad; see Kollmann and Grubb 2002, Fischer et al. 2005). Both species are characterized as ‘European temperate’ (Preston and Hill 1997, Kollmann and Grubb 2002) and show a similar range of distribution (native to Europe, the temperate zone of East Asia and North Africa; Kollmann and Grubb 2002). In Austria (Central European Alps), L. vulgare and V. lantana often occur associated in colline and submontane dry-warm forests (e.g., Erico-Pinion, Quercion pubescenti-petraeae) on calcareous soils (Fischer et al. 2005).

Study sites and water conditions

The study was carried out in a natural pine stand (Erico-Pinion sylvestris) in Telfs (Tyrol, Austria; 630 m a.s.l.; 47°18′N, 11°54′E) and in the Innsbruck botanical garden (600 m a.s.l.; 47°16′2″N and 11°23′34″ E). Precipitation (monthly sum) and temperature (monthly mean) did not differ significantly between both sites (Central Institute for Meteorology and Geodynamics, Regional Office for Tyrol and Vorarlberg). The same can be assumed for light conditions, as on both sites plants grew in the shadow of taller trees. Nevertheless, due to soil and irrigation treatments, plants grew on a gradient from optimal to suboptimal moisture conditions (irrigated garden plants > forest plants > drought-treated garden plants) as described in the following: (i) Plants in the botanical garden originated from the cuttings of native individual plants and grew on a loamy sand (<12% clay; FAO 2006). They were irrigated daily so that despite the low field capacity (~25%), it can be assumed that the soil water potential (Ψsoil) remained constantly high and that the plants grew under optimal water conditions. (ii) After vulnerability analysis in August 2005, the irrigation was stopped. In the following growing seasons (2006–2008), sufficient soil humidity was reached only for a few days after rainfalls, while most of the time the soil dried out and the plants grew under suboptimal conditions. Measurements of Ψsoil in the rooting area of the same species on similar sites revealed a decrease below –1.4 MPa within 4 days. In 2006, climatic conditions were also overall drier than in 2005; e.g., monthly sum of precipitation in July 2006 was 109 mm less compared to that in 2005. The longest drought period was observed in June 2006 with nine successive days without precipitation. (iii) Plants in the natural pine stand grew on a humous, humid forest soil (Rendzina). Accompanying, several indicator species for mesic conditions (e.g., Molinia caerulea Mill. and Frangula alnus (L.) Moench) were found.
Experimental design

Vulnerability analyses and conductivity measurements were made on up to 2-m-tall forest plants and irrigated plants in 2005, and on drought-treated garden plants in 2006. Measurements were done in August, thus plants were subjected to respective conditions for the main growing season. For measurements, whole main shoots were cut, wrapped in dark plastic bags and transported to the laboratory. All individual plants were recut twice (about 5 cm) under water and saturated for 24 h. In 2007, further vulnerability measurements on forest plants and drought-treated garden plants were made to account for possible changes in vulnerability to drought-induced embolism. At the same time, \( g_s \) of forest plants and drought-treated garden plants was measured. Anatomical analyses were done on samples that were previously used for vulnerability analyses.

Methods

Vulnerability analysis and conductivity measurements

Vulnerability curves were obtained by dehydrating samples to differing extents and plotting the fractional (%) loss of conductivity versus the \( \Psi \). Curves were fitted using the exponential sigmoidal equation given in Pammenter and Vander Willigen (1998):

\[
\text{PLC} = 100 \left( 1 + \exp \left( a (\Psi - \Psi_0) \right) \right),
\]

where per cent loss of hydraulic conductivity (PLC) is the per cent loss of conductivity, \( a \) is related to the slope of the curve, \( \Psi \) is the corresponding water potential (MPa) and \( \Psi_0 \) is the \( \Psi \) value corresponding to 50% loss of conductivity.

The \( \Psi \) was measured using a pressure chamber (Model 1000 Pressure Chamber, PMS Instrument Co., Corvallis, OR). Measurements were made on up to 10-cm-long end segments of side-twigs \( (L. \text{vulgare}) \) or on leaves \( (V. \text{lantana}) \); PLC was calculated by measuring the loss of specific hydraulic conductivity \( (k_s) \) after dehydration of the samples and \( k_s \) was measured using a modified Sperry apparatus \( (\text{Sperry et al. 1988, Chiu and Ewers 1993, Vogt 2001}) \). In both species, PLC was quantified using the paired-segment-method \( (\text{Sperry and Tyree 1990, Sperry and Sullivan 1992}) \), as flushing was not possible due to the soft and highly vulnerable pith. Therefore, conductivities of two consecutive segments within a main shoot were compared: these samples were taken when the branch was saturated \( (k_{sat}) \) and after dehydration \( (k_{sd}) \). Between two segments, at least 3 cm was discarded to avoid embolism induced by cutting. Previous experiments proved that this distance is sufficient for paired segment measurements in the species under study (data not shown). All cuttings were done under water. To avoid an underestimation of drought-induced embolism due to native embolism, dye stainings were carried out on saturated samples with Phloxine B \( (\text{Sigma Chemical Co., St. Louis, MO; 2\% (w/v)}; \text{see Mayr and Cochard 2003}) \) to prove full conductivity at the beginning of dehydration experiments. The PLC was calculated, assuming that \( k_{sat} \) corresponded to 0% loss of conductivity, according to

\[
\text{PLC} = \left( 1 - \frac{k_{sd}}{k_{sat}} \right) \times 100. \tag{2}
\]

For measurements, samples, free of side branches and up to 6 cm long (mean vessel length was about 5 cm), were immersed in distilled water, decorticated, recut with a sharp wood-carving knife and sealed into the silicone tubes of the apparatus \( (\text{see Mayr et al. 2002}) \). Measurement pressure was set to 4 kPa, and the flow rate was determined using a PC-connected balance \( (\text{Sartorius BP615, 0.1 mg precision, Sartorius AG, Göttlingen, Germany}) \) by mass registration every 10 s. Flow rates were calculated by linear regression over 200 s. For measurements, distilled, filtered \( (0.22 \mu m) \) and degassed water containing 0.005% \( (v/v) \) ‘Micropur Forte MF 1000F’ \( (\text{Katadyn Products Inc., Wallisellen, Switzerland}) \) a mixture containing \( \text{Ag}^+ \) and \( \text{Na}^+ \) hypochlorite sold for water sterilization and preservation) was used to prevent microbial growth \( (\text{Sperry et al. 1988, Mayr et al. 2006}) \). The specific hydraulic conductivity \( (k_s) \) was calculated as follows:

\[
k_s = \frac{Q l}{A_x \Delta P}, \tag{3}
\]

where \( Q \) is the volume flow rate \( (m^3 \text{ s}^{-1}) \), \( l \) is the segment length \( (m) \), \( A_x \) is the xylem cross-sectional area \( (\text{sapwood less heartwood}) \, m^2 \) and \( \Delta P \) is the pressure difference between the segment ends \( (\text{Pa}) \). Calculations were corrected to 20 °C to account for changes in the viscosity of water with temperature.

Anatomical measurements

For anatomical measurements, samples were soaked in an ethanol–glycerol–water solution \( (1:1:1, \text{v/v/v}) \) for 2 weeks. Cross sections were cut using a microtome \( (\text{Schlittenmikrotom OME, Reichert, Vienna, Austria}) \) and stained with phloroglucinol–HCl \( (\text{stains lignin brightly red}) \). Anatomical parameters were analysed with a light microscope \( (\text{Olympus BX 41, System Microscope, Olympus Austria, Vienna, Austria}) \) interfaced with a digital camera \( (\text{Sony, Cyber-shot, DSC-W17, Vienna, Austria}) \) and image analysis software \( (\text{ImageJ, 1.37, National Institutes of Health (NIH), Bethesda, USA, public domain}) \). Anatomical measurements were made in radial sectors of the youngest annual ring. Radial sectors were located opposite to compression wood, which is hydraulically of minor importance \( (\text{see also Mayr and Cochard 2003}) \). Individual conduit areas \( (i.e., \text{lumen}, A) \) were measured directly and the respective diameters were calculated from \( A \) assuming that the conduits had a circular shape:

\[
A = \pi r^2 = \pi \left( \frac{2A}{\pi} \right)^2
\]

\[
\text{PLC} = \left( 1 - \frac{k_{sd}}{k_{sat}} \right) \times 100, \tag{2}
\]

\[
k_s = \frac{Q l}{A_x \Delta P}, \tag{3}
\]

\[
A = \pi r^2 = \pi \left( \frac{2A}{\pi} \right)^2
\]
\[ d = 2 \times \sqrt{\frac{A}{\pi}} \] (4)

The mean hydraulic conduit diameter \( (d_h) \) was calculated from the individual diameters according to (Sperry and Hacke 2004):

\[ d_h = \frac{\sum d^3}{\sum d}. \] (5)

To characterize the conduit wall reinforcement, we measured the ‘thickness to span ratio’ \( (d/b) \) (Hacke et al. 2001). Therefore, the wall thickness between the conduits \( (t) \) and the conduit wall span \( (b) \) were measured directly on conduit pairs that averaged within \( d_h \pm 10 \mu m \). The percentage of conducting area per total xylem area \( (CA) \) was calculated from the lumen area and the total xylem area of the annual ring.

Stomatal conductance

Stomatal conductance was measured on sunny days using an AP4 Porometer (Delta-T Devices Ltd., Cambridge, UK). In the previous evening and in the morning of measurement days, respectively, all plants were saturated by watering. When stomata were fully open, the main shoots were cut and \( \Psi \) and \( g_s \) were measured at intervals until stomatal closure. Thereby, cut shoots were further exposed to conditions at the study site. As stomatal closure occurred relatively fast (within 1 h), it can be assumed that during dehydration neither light and CO2 concentration nor temperature or vapour pressure deficit changed considerably, and stomatal closure thus occurred in response to decreasing \( \Psi \) (see Larcher 1995, Hubbard et al. 2001). The \( \Psi \) at 10% \( g_s \) was defined as \( \Psi \) at stomatal closure \( (\Psi_{sc}) \). It was calculated by plotting the per cent stomatal conductance (PSC) versus \( \Psi \). Curves were fitted according to Eq. (1), whereby PLC was substituted by PSC and \( \Psi_{sc} \) corresponded to \( \Psi \) at 50% \( g_s \). The safety margin was defined as the difference in \( \Psi \) between \( \Psi_{sc} \) and \( \Psi_{50} \) (also see Brodribb and Hill 1999) and was calculated as follows:

\[ \text{Safety margin} = \Psi_{sc} - \Psi_{50}. \] (6)

Number of samples and statistics

Vulnerability analyses and conductivity measurements were made on up to 30 individuals per species, site and treatment. For anatomical measurements, 7–10 samples per species, site and treatment were analysed. Mean diameter \( (d_{\text{mean}}) \) and mean hydraulic diameter \( (d_h) \) were calculated of a total of 194–1096 conduits per annual ring. The conduit wall thickness \( (t) \) and the ‘wall thickness to span ratio’ \( (t/b) \) were determined for 12–98 conduit pairs per annual ring. Diurnal courses of \( g_s \) and desiccation experiments were made on five to six individuals per species and site, respectively. For each run, three side-twigs per individual plant were taken for \( \Psi \) measurements and 10 leaves per individual plant for measuring \( g_s \).

The differences between sites and treatments within a species were tested with the Mann–Whitney U test \( (d_{\text{mean}}, t \) and \( (t/b) \) \( \), the Welch-test \( (d_h, \text{vulnerability thresholds and } \Psi_{sc}) \) or Student’s \( t \) test \( (k_c, g_s \) and \( CA) \). All tests were made at a probability level of 5% (except the Welch test) after testing for normal distribution and for variance of the data. Correlation coefficients were tested with Pearson’s product–moment coefficient.

Results

Hydraulic safety and hydraulic efficiency

In \( L. \) vulgare and \( V. \) lantana, \( \Psi \) at 10, 50 and 90% loss of conductivity \( (\Psi_{10}, \Psi_{50} \) and \( \Psi_{90} \) (also see Mayr et al. 2002). Significant differences in specific hydraulic conductivity \( (k_c) \) were only found for irrigated versus drought-treated garden plants of \( L. \) vulgare (Table 1).

Anatomy

In both species, conduit wall thickness \( (t) \) increased and, accordingly, percentage of conducting area \( (CA) \) decreased after drought treatment. For mean diameter \( (d_{\text{mean}}) \), mean hydraulic diameter \( (d_h) \) and conduit ‘wall thickness to span ratio’ \( (t/b) \), different responses to the drought treatment were observed: while \( d_{\text{mean}} \) and \( d_h \) decreased and \( (t/b) \) increased in \( L. \) vulgare, the opposite was found in \( V. \) lantana (Table 2; Figure 3). The cross-species analysis revealed an increase in \( t \) and \( (t/b) \), and a decrease in \( CA \) with decreasing \( \Psi_{50} \), though correlations were not statistically significant (Figure 2).

Stomatal conductance

Stomatal conductance differed significantly between forest plants and drought-treated garden plants: in drought-treated garden plants, \( g_s \) at moderate \( \Psi \) was higher, stomata closed more abruptly and cuticular conductivity (stomatal conductivity after stomatal closure) was lower compared to forest plants (Figure 4). The peak in \( g_s \) at \(-3 \) MPa of \( L. \) vulgare forest plants is statistically not significant; \( g_s \) at
Table 1. Water potential at 10, 50 and 90% loss of conductivity ($\Psi_{10}$, $\Psi_{50}$ and $\Psi_{90}$), parameter $a$ (slope of the vulnerability curve), specific hydraulic conductivity ($k_s$), $\Psi$ at stomatal closure ($\Psi_{sc}$) and safety margin ($\Psi_{sc} - \Psi_{50}$) of irrigated and drought-treated garden plants as well as forest plants of *L. vulgare* and *V. lantana*. Circles and asterisks indicate significant intraspecific differences ($P < 0.05$) of drought-treated from irrigated garden plants and forest plants, respectively. Mean ± SE.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Garden plants</th>
<th>Forest plants</th>
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<tbody>
<tr>
<td></td>
<td>Irrigated</td>
<td>Drought-treated</td>
</tr>
<tr>
<td><em>L. vulgare</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Psi_{10}$ (MPa)</td>
<td>$-0.66 \pm 0.64$</td>
<td>$-0.90 \pm 1.05$</td>
</tr>
<tr>
<td>$\Psi_{50}$ (MPa)</td>
<td>$-3.11 \pm 0.15^*$</td>
<td>$-4.58 \pm 0.26$</td>
</tr>
<tr>
<td>$\Psi_{90}$ (MPa)</td>
<td>$-5.55 \pm 0.34^*$</td>
<td>$-8.27 \pm 0.54$</td>
</tr>
<tr>
<td>Parameter $a$</td>
<td>$0.90 \pm 0.17$</td>
<td>$0.60 \pm 0.12$</td>
</tr>
<tr>
<td>$k_s$ (m$^2$ s$^{-1}$ MPa$^{-1}$ 10$^{-4}$)</td>
<td>$13.99 \pm 0.53^*$</td>
<td>$6.57 \pm 0.29$</td>
</tr>
<tr>
<td>$\Psi_{sc}$ (MPa)</td>
<td>$-2.95 \pm 0.10$</td>
<td>$2.48 \pm 0.26$</td>
</tr>
<tr>
<td>Safety margin ($\Psi_{sc} - \Psi_{50}$)</td>
<td>$1.63$</td>
<td>$0.34$</td>
</tr>
<tr>
<td><em>V. lantana</em></td>
<td></td>
<td></td>
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<tr>
<td>$\Psi_{10}$ (MPa)</td>
<td>$0.47 \pm 0.45^*$</td>
<td>$-1.49 \pm 0.58$</td>
</tr>
<tr>
<td>$\Psi_{50}$ (MPa)</td>
<td>$-1.70 \pm 0.14^*$</td>
<td>$-3.57 \pm 0.15$</td>
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<td>$\Psi_{90}$ (MPa)</td>
<td>$-3.87 \pm 0.16^*$</td>
<td>$-5.65 \pm 0.29$</td>
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<td>Parameter $a$</td>
<td>$1.01 \pm 0.14$</td>
<td>$1.06 \pm 0.21$</td>
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<tr>
<td>$k_s$ (m$^2$ s$^{-1}$ MPa$^{-1}$ 10$^{-4}$)</td>
<td>$12.86 \pm 0.88$</td>
<td>$11.07 \pm 0.93$</td>
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<tr>
<td>$\Psi_{sc}$ (MPa)</td>
<td>$-3.14 \pm 0.27$</td>
<td>$3.32 \pm 0.40$</td>
</tr>
<tr>
<td>Safety margin ($\Psi_{sc} - \Psi_{50}$)</td>
<td>$0.43$</td>
<td>$-0.53$</td>
</tr>
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</table>

Figure 1. The per cent loss of conductivity (closed circles and solid curve lines) and per cent $g_s$ (open circles and dashed curve lines) versus $\Psi$ of irrigated and drought-treated garden plants as well as forest plants of *L. vulgare* and *V. lantana*. Vertical lines show $\Psi$ at 50% loss of xylem conductivity ($\Psi_{50}$, solid lines) and $\Psi_{sc}$ (dashed lines), respectively. Diamonds show data points measured in 2007. Asterisks indicate statistically significant ($P < 0.05$) regressions.
Maximum $g_s$ of drought-treated garden plants was significantly higher in *V. lantana*, while in *L. vulgare* the difference was less pronounced. Stomatal closure occurred at similar $W$ in forest plants and drought-treated garden plants (Table 1; Figure 1), although $W_{50}$ of the latter was significantly lower (Table 1; Figure 4). This led to a higher safety margin in drought-treated garden plants (Table 1).

**Discussion**

A key component in drought tolerance of woody plants is the ability to avoid the formation of embolism (Maherali et al. 2004). This can be achieved by the avoidance of critical $\Psi$ or by building up a xylem tissue which is resistant to low $\Psi$ (e.g., Jones and Sutherland 1991, Tyree and Ewers 1991, Tyree et al. 1994, Sperry 2004). The xylem’s vulnerability to drought-induced embolism was found to be highly plastic in *L. vulgare* and *V. lantana*. In both species, $\Psi$ at 50% loss of conductivity ($\Psi_{50}$) significantly decreased after drought treatment (Table 1). This demonstrates that both species exhibited a considerable acclimation potential (Table 1; Figure 1). It supports our hypothesis that *L. vulgare* and *V. lantana* do not develop a high resistance to drought-induced embolism a priori, but are able to acclimate when extended drought periods occur. We assume that this might be similar in other temperate woody plants, as the development of generally drought-tolerant tissues may be even maladaptive: it is costly (building costs, reduction in net carbon assimilation; Magnani et al. 2000, Pockman and Sperry 2000) and thus can lower a plant’s competitive potential. In any case, the acclimation potential seems to be highly species-specific even in species of the same ecosystem. In this study, *V. lantana* was less drought tolerant than *L. vulgare*, but at the same time it was able to acclimate to altered soil moisture conditions within a broader range (Table 1). Intraspecific differences in vulnerability to embolism were reported in several studies (e.g., Cochard et al. 1992, Alder et al. 1996, Pockman and Sperry 2000). Even organs within a plant (e.g., shaded versus sun-exposed branches, Cochard et al. 1999) or leaf rachides within a canopy (Cochard et al. 1997) were demonstrated to differ considerably in drought tolerance, and Kolb and Sperry (1999) showed that vulnerability to drought-induced embolism can also change seasonally. These and our findings support the hypothesis of Cochard et al. (1997) that xylem vulnerability is not only species- or organ-specific, but can also depend on the acclimation of the plants to environmental conditions (see also Alder et al. 1996, Brodribb and Hill 1999, Maherali and Delucia 2000, Cornwell et al. 2007). Maherali et al. (2004) reported significant phylogenetically independent correlation between $\Psi_{50}$ and annual precipitation for conifers and evergreen angiosperms but not for deciduous angiosperms. The authors stated that relatively vulnerable temperate species may have arisen from more resistant ancestors and that high resistance to cavitation may be costly in mesic environments.

Cavitation resistance depends mainly on pit properties (e.g., size, permeability and stability of the torus) and conduit wall reinforcement (Sperry and Tyree 1990, Sperry and Sullivan 1992, Sperry and Saliendra 1994, Sperry et al. 1994, Tyree et al. 1994, Hacke and Sperry 2001, Hacke et al. 2001, Tyree and Zimmermann 2002, Pittermann et al. 2006). In *L. vulgare*, cell wall thickness ($t$) and ‘cell wall thickness to span ratio’ ($t/b_h$) increased significantly.

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Figure 2. (A) Conduit wall thickness ($t$), (B) conduit ‘wall thickness to span ratio’ ($t/b_h$) and (C) percentage conducting area per total area (CA; C) versus $\Psi$ at 50% loss of conductivity ($\Psi_{50}$) for *L. vulgare* and *V. lantana*. Values are shown for irrigated (L+ and V+) and drought-treated garden plants (L− and V−) and forest plants (L and V). For forest plants of *V. lantana* (V), $t$ and ($t/b_h$) could not be calculated as not enough tracheid pairs were available ($P < 0.05$).
Table 2. Mean diameter ($d_{\text{mean}}$), mean hydraulic diameter ($d_h$), conduit wall thickness ($t$), conduit ‘wall thickness to span ratio’ ($t/b_h^2$) and percentage of conducting area to total area (CA) of irrigated and drought-treated garden plants of $L$. vulgare and $V$. lantana. Asterisks indicate significant intraspecific differences ($P < 0.05$). Mean ± SE.

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<thead>
<tr>
<th>Parameter</th>
<th>$L$. vulgare</th>
<th>$V$. lantana</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Irrigated</td>
<td>Drought-treated</td>
</tr>
<tr>
<td>$d_{\text{mean}}$ (µm)</td>
<td>19.22 ± 0.21</td>
<td>17.44 ± 0.21$^*$</td>
</tr>
<tr>
<td>$d_h$ (µm)</td>
<td>26.46 ± 0.00</td>
<td>25.55 ± 0.00$^*$</td>
</tr>
<tr>
<td>$t$ (µm)</td>
<td>2.94 ± 0.13</td>
<td>3.81 ± 0.15$^*$</td>
</tr>
<tr>
<td>$(t/b_h)^2$</td>
<td>0.016 ± 0.001</td>
<td>0.024 ± 0.002$^*$</td>
</tr>
<tr>
<td>CA (%)</td>
<td>12.31 ± 0.84</td>
<td>10.52 ± 1.03</td>
</tr>
</tbody>
</table>

Figure 3. Transverse section of the xylem of $L$. vulgare (A and B) and $V$. lantana (C and D) before (A and C) and after (B and D) drought treatment.

upon drought treatment, whereas in $V$. lantana no clear trend was observed (Table 2). In the cross-species analysis, an increase in $t$ and $(t/b)^2$ with decreasing $\Psi_{50}$, and a decrease in the percentage conducting area per total area (CA) was observed, but correlations were not significant (Figure 2; see also Hacke et al. 2001, Jacobsen et al. 2007). Recent studies also confirmed an indirect impact of conduit diameter on cavitation resistance as larger conduits have more pits, and a greater total pit area increases by chance the size of the single largest pit membrane pore, which offers the weakest capillary barrier to air-seeding (pit area hypothesis; Wheeler et al. 2005, Hacke et al. 2006, Sperry et al. 2007). Under dry conditions, plants may develop smaller pit pores and thicker walls to increase drought tolerance, but they do not necessarily have to reduce conduit diameter. Accordingly, Sperry and Hacke (2004) found that species with high cavitation resistance and thus low-conductive pits do not automatically have a low overall conductivity, if conduit width and length increase to overcome the added pit resistance. In $L$. vulgare, mean diameter ($d_{\text{mean}}$) and mean hydraulic diameter ($d_h$) decreased significantly after drought treatment (Table 2; Figure 3) and, accordingly, a significant decrease in $k_s$ was observed (Table 1). In contrast, in $V$. lantana no difference in $k_s$ was observed after drought treatment although conduit diameters increased (Tables 1 and 2; Figure 3). We conclude that $V$. lantana developed wider conduits to compensate for a higher resistance at the pits, which was probably related to smaller pit pores less vulnerable to air-seeding. Anatomical adjustments to increase drought resistance are obviously species-specific and based on concerted changes of several structures (Table 3).

Despite the potentially higher risk for embolism (according to the pit area hypothesis), maintaining or even increasing $k_s$ by the formation of larger conduits after water limitation may be advantageous; it can optimize water
transport and consequently carbon fixation during periods of sufficient water availability and can facilitate higher transpiration at less negative $\Psi$ (e.g., Breda et al. 2006, Cornwell et al. 2007). This is also consistent with the findings of Maherali et al. (2004), who observed an increase in $k_s$ with decreasing rainfall in deciduous angiosperms and concluded that the evolution of increased $k_s$ may be an important adaptation to water limitation in this functional group. Recent studies have shown that also short-term physiological regulations of ion concentrations may alter $k_s$ (see Gasco et al. 2007, Nardini et al. 2007). Adjustments of $k_s$ may therefore play an important role in avoiding critical $\Psi$ and thus embolism (see Tyree et al. 1994), and support transpiration control as the main mechanism to maintain moderate $\Psi$.

Adequate stomatal regulations can stave off significant drought effects in the short term. As stated by Sperry (2004) ‘Walking the tightrope between avoiding hydraulic failure and maximizing gas exchange requires rapid and well-regulated stomatal responses to these factors’. A strong relationship of stomatal regulation and avoidance of xylem cavitation was also observed by other authors (e.g., Cochard et al. 2002, Sperry et al. 2002, Brodribb et al. 2003, Breda et al. 2006). We found forest plants of L. vulgare and V. lantana to potentially exhibit high embolism rates before stomatal closure was evident. In V. lantana, $\Psi$ at stomatal closure ($\Psi_{sc}$) was at $-3.32$ MPa. At this $\Psi$, vulnerability analysis already indicated about 65% loss of conductivity (Table 1; Figure 1). This might be related to relatively humid conditions at the sample site (see the Materials section), where drought probably is rare and, in consequence, the risk of embolism low. In drought-treated garden plants, $\Psi_{sc}$ was in the same range as in forest plants (Table 1). Also Martínez-Vilalta et al. (2004) reported that different species of pines, growing in habitats of different water availability, closed their stomata within only a small range of needle $\Psi$. Little plasticity in $\Psi_{sc}$ was also found in Fagus, Acer and Rhododendron species (Beikircher, unpublished). Due to the acclimation in cavitation resistance ($\Psi_{sc}$; see above) and constantly high $\Psi_{sc}$, safety margins ($\Psi_{sc} - \Psi_{so}$) increased upon drought treatment in L. vulgare and V. lantana (Table 1; Figure 1). There are several examples for species with large safety margins, including species periodically experiencing severe drought in their habitat (Pockman and Sperry 2000, Breda et al. 2006), species with low vulnerability thresholds (Froux et al. 2005) or species which have no mechanisms of embolism reversal by refilling (Tyree and Sperry 1988).

During severe drought periods, stomatal closure may not be sufficient to avoid critical water losses. In this case, structural and physiological long-term acclimation processes to reduce transpiration are required (see Introduction section). Reducing $g_s$ can also be achieved by reducing the number and size of stomata or by altering stomatal sensitivity to environmental conditions (see Jones and Sutherland 1991, Larcher 1995). In L. vulgare and V. lantana, the course of $g_s$ differed considerably between forest plants and drought-treated garden plants: in the latter, $g_s$ remained high at moderate $\Psi$, stomata closed more abruptly and cuticular conductivity was lower (Figure 4). This was particularly evident in V. lantana, where maximum $g_s$ was also significantly higher in drought-treated plants compared to

Table 3. Overview of general changes in hydraulic and anatomical parameters upon drought treatment of L. vulgare and V. lantana. Arrows in parentheses indicate nonsignificant intraspecific differences between irrigated and drought-treated plants. Different trends between species are marked by solid arrows.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>L. vulgare</th>
<th>V. lantana</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Psi_{so}$ (MPa)</td>
<td>$\langle \delta \rangle$</td>
<td>$\delta$</td>
</tr>
<tr>
<td>$\Psi_{so}$ (MPa)</td>
<td>$\delta$</td>
<td>$\delta$</td>
</tr>
<tr>
<td>$k_s$</td>
<td>$\langle \delta \rangle$</td>
<td>$\delta$</td>
</tr>
<tr>
<td>$d_{mean}$ (µm)</td>
<td>$\downarrow$</td>
<td>$\uparrow$</td>
</tr>
<tr>
<td>$d_h$ (µm)</td>
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<td>$t$ (µm)</td>
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</tr>
</tbody>
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forest plants. This may enable drought-treated garden plants to maintain sufficient CO₂ uptake at moderate Ψ and reduce water losses at critical Ψ. The remarkable changes in stomatal regulation of V. lantana indicate that this species changed from an isohydric (forest plants) to an anisohydric strategy (drought-treated plants; see Tardieu and Sonnanteau 1998, Hubbard et al. 2001, McDowell et al. 2008). Isohydric plants reduce gs to maintain a constant Ψ under dry conditions, whereas anisohydric plants allow Ψ to decline with drought. McDowell et al. (2008) stated that anisohydric species tend to occupy more drought-prone habitats compared to isohydric species, although this is not a general feature. Similar to our study, West et al. (2008) also found both strategies within one vegetation type. Overall, the shift to an anisohydric behaviour and observed changes in xylem anatomy (see above) indicate that V. lantana tried to facilitate transpiration under humid conditions. Despite the resultant higher risk for hydraulic failure, this strategy may be advantageous for woody species in temperate zones where drought periods are not likely to occur for a long time.

Our measurements on L. vulgare and V. lantana demonstrate that different hydraulic strategies can be successful in the same ecosystem and that these strategies may even be altered within a species due to the changing conditions. Adaptation, acclimation and short-term physiological regulations enable plants to balance trade-offs between avoidance of hydraulic failure and maintenance of photosynthetic activity.

Acknowledgments

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References


Appendix

Abbreviations

PLC per cent loss of hydraulic conductivity
Ψ water potential
Ψsoil soil water potential
Ψ10, Ψ50 and Ψ90 water potential at 10, 50 and 90% loss of conductivity
Ψsc water potential at stomatal closure, corresponding to a water potential at 10% stomatal conductance
gs stomatal conductance
ks specific hydraulic conductivity of the xylem
ksat specific hydraulic conductivity of the xylem at saturation
ksd specific hydraulic conductivity of the xylem after dehydration
A lumen area
dmean mean conduit diameter
dh mean hydraulic diameter
t conduit wall thickness
b conduit wall span (side of a square with equal area to the conduit)
((t/b)² conduit ‘wall thickness to span ratio’
CA percentage conducting area per total xylem area


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