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Research paper

Hydraulic patterns and safety margins, from stem to stomata, in three eastern US tree species

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Adequate water transport is necessary to prevent stomatal closure and allow for photosynthesis. Dysfunction in the water transport pathway can result in stomatal closure, and can be deleterious to overall plant health and survival. Although much is known about small branch hydraulics, little is known about the coordination of leaf and stem hydraulic function. Additionally, the daily variations in leaf hydraulic conductance (K_{leaf}), stomatal conductance and water potential (Ψ_L) have only been measured for a few species. The objective of the current study was to characterize stem and leaf vulnerability to hydraulic dysfunction for three eastern US tree species (*Acer rubrum, Liriodendron tulipifera* and *Pinus virginiana*) and to measure in situ daily patterns of K_{leaf}, leaf and stem Ψ, and stomatal conductance in the field. Sap flow measurements were made on two of the three species to compare patterns of whole-plant water use with changes in K_{leaf} and stomatal conductance. Overall, stems were more resistant to hydraulic dysfunction than leaves. Stem P_{50} (Ψ resulting in 50% loss in conductivity) ranged from –3.0 to –4.2 MPa, whereas leaf P_{50} ranged from –0.8 to –1.7 MPa. Field Ψ_L declined over the course of the day, but only *P. virginiana* experienced reductions in K_{leaf} (nearly 100% loss). Stomatal conductance was greatest overall in *P. virginiana*, but peaked midmorning and then declined in all three species. Midday stem Ψ in all three species remained well above the threshold for embolism formation. The daily course of sap flux in *P. virginiana* was bell-shaped, whereas in *A. rubrum* sap flux peaked early in the morning and then declined over the remainder of the day. An analysis of our data and data for 39 other species suggest that there may be at least three distinct trajectories of relationships between maximum K_{leaf} and the % K_{leaf} at Ψ_{min}. In one group of species, a trade-off between maximum K_{leaf} and % K_{leaf} at Ψ_{min} appeared to exist, but no trade-off was evident in the other two trajectories.

Keywords: cavitation, embolism, photosynthesis, transpiration, xylem.

Introduction

Water transport from plant stems into and throughout leaves is critical for maintenance of adequate leaf water status. To prevent stomatal closure, and to permit photosynthetic carbon gain, this water pathway must remain functional. However, during periods of drought stress, dysfunction in this hydraulic pathway may occur. Reductions in leaf and stem hydraulic capacity can result in reduced photosynthesis and even plant mortality via carbon starvation, desiccation or some combination of both (e.g., McDowell et al. 2008). In stems, it appears that the primary source of hydraulic dysfunction is xylem embolism (e.g., Tyree and Sperry 1989). There is some debate in the literature as to the mechanism of this dysfunction in leaves, although most evidence points to leaf xylem embolism as the cause of loss of leaf hydraulic conductance (K_{leaf}; Nardini et al. 2001, 2003, Bucci et al. 2003, Woodruff et al. 2007, Johnson et al. 2009*a*). Partial collapse of leaf xylem has also been proposed as another mechanism responsible for reductions in K_{leaf} during dehydration (Cochard et al. 2004, Brodribb and Cochard 2009, Blackman et al. 2010). Additionally, reductions in extra-xylary

conductance (e.g., membrane permeability and aquaporin expression) could also impact K_{leaf} (Cochard et al. 2007, Kaldenhoff et al. 2008, Voicu et al. 2008, Heinen et al. 2009).

Although much research has been done on the hydraulic parameters of small-diameter stems and roots in various species from a variety of habitats, little is known about how plant hydraulic parameters are coordinated throughout the entire plant hydraulic continuum from root to leaf (Meinzer et al. 2009, 2010). Even the coordination of hydraulic properties at the terminal portion of the pathway, at the level of stem and leaf, is poorly understood, although there has been some previous work dealing with the subject (Salleo et al. 2001, Choat et al. 2005). It has been proposed that rapidly reversible diurnal changes in K_{leaf} may constitute part of an essential hydraulic signal that enables stomata to maintain stem and leaf water potential at set points that ensure the integrity of the stem water transport system upstream (Brodribb and Holbrook 2003, Meinzer et al. 2004, 2008, 2009, Woodruff et al. 2007). Failure of stomata to respond quickly to rapid increases in transpiration could result in sharp increases in stem xylem tension and loss of conductivity from embolism. However, the fact that many species operate so close to the threshold of declining stem hydraulic conductance (Meinzer et al. 2009) suggests that there are mechanisms (stomatal regulation or stem capacitance) that regulate minimum stem water potentials and prevent substantial losses in hydraulic function. Although these are critical processes governing carbon capture and survival in plants, diurnal coordination of leaf and stem water potential, stomatal conductance and K_{leaf} have only been explored in a few species (e.g., Woodruff et al. 2007, Meinzer et al. 2008, Johnson et al. 2009*b*).

The objective of this study was to evaluate leaf and stem vulnerability to embolism and to determine the degree of embolism experienced in situ by leaves and stems of three tree species that occur naturally in the eastern USA. Two of the three species were also selected for sap flow measurements to compare patterns of whole-plant water use with changes in K_{leaf} and stomatal conductance. In addition, the relationship between maximum K_{leaf} and the portion of K_{leaf} remaining at midday was explored because in an earlier survey of 31 species we found that they tended to fall into one of two groups: species that maintained near-maximal K_{leaf} at their minimum daily Ψ_L and species that lost >50% of their maximum K_{leaf} at their minimum daily Ψ_L (Johnson et al. 2009*b*). We hypothesized that there would be a trade-off of maximum leaf hydraulic capacity against the ability to maintain leaf hydraulic capacity throughout the day.

Materials and methods

Field sites and species

The field site used for this study was a common garden plot planted in 1996 near State College, PA, USA (40.79 N, 77.86 W). All plants measured were within 20 m of each other. All measurements were carried out during July of 2010, with the addition of measurements of stem and leaf water potentials, and hydraulic vulnerability on a subset of *Pinus virginiana* and *Liriodendron tulipifera* stems carried out in July of 2009 (see below). In order to represent different plant functional groups, we selected two deciduous broadleaf species (*Acer rubrum* L. and *L. tulipifera* L.) and one evergreen conifer (*P. virginiana* Mill.). Diameters at breast height for the three species were 5.6 (±0.3 cm), 7.7 (±0.4 cm) and 7.7 (±0.2 cm) for *A. rubrum*, *L. tulipifera* and *P. virginiana*, respectively. Individual tree heights ranged from 8.1 to 12.4 m.

Stem hydraulic conductivity and vulnerability

Branches ~50 cm long were collected in the field, bagged and transported back to the lab (~30 min in transit). Segments of branches (~20 cm in length and 5.5–7 mm in diameter with bark removed) were cut under water and were flushed with filtered, distilled water at pH 2 before hydraulic measurements. Embolisms were removed by submerging the stem segments in filtered, distilled (pH 2) water in a vacuum chamber overnight. To measure maximum hydraulic conductivity, a hydrostatic pressure head (~70 cm) was used to induce flow through the segments. The resulting volume flow rate was measured by timing the intervals for water to reach successive gradations on a pipette attached with tubing to the distal end of the segment. Hydraulic conductivity (k_h) was calculated by dividing the volume flow rate of water flowing through the stem by the hydrostatic pressure gradient along the stem.

Vulnerability curves were constructed using the air injection method (Sperry and Saliendra 1994). Briefly, after measurement of maximum hydraulic conductivity ($k_{h\ max}$), stems were placed in a pressure sleeve, and were pressurized to 1 MPa for 2 min. The stem was then removed from the pressure sleeve and k_h was measured using the same method used for maximum conductivity. This process was repeated at 1 MPa increments of increasing pressure until k_h had fallen to <10% of its maximum value. The percentage loss in hydraulic conductivity (PLC) was calculated as

$$PLC = 100 \times \left(1 - \left(\frac{k_h}{k_{hmax}}\right)\right)$$

Leaf hydraulic conductance and vulnerability

Leaf hydraulic conductance (mmol m^{-2} s^{-1} MPa^{-1}) was determined using a timed rehydration method described in Brodribb and Holbrook (2003), which is based on an analogy between rehydrating a leaf and recharging a capacitor:

$$K_{leaf} = C \ln(\Psi_o / \Psi_f) / t$$

where C = capacitance, Ψ_o = leaf water potential prior to partial rehydration, Ψ_f = leaf water potential after partial rehydration and t = duration of rehydration. Branches ~30–50 cm long were collected from trees early in the morning prior to significant transpirational water loss and were transported to the lab, re-cut under water and allowed to rehydrate for at least 4 h. Shoots were dried on the bench top for varying lengths of time, placed in a plastic bag and sealed and then kept in the dark for at least 1 h to equilibrate. Measurements of leaf rehydration kinetics were conducted over the next 3 days (shoots kept in the dark at 4 °C, unless measured on the same day as they were dehydrated) on excised leaves/fascicles for initial values (Ψ_o) and for final values after a period of rehydration of t seconds (Ψ_f), which was between 60 and 120 s. Adjacent or paired leaves/fascicles were used for each K_{leaf} measurement and a total of 34–63 leaf/fascicle pairs were used to construct each K_{leaf} vulnerability curve. Distilled water was used for rehydration of K_{leaf} samples and water temperature was maintained between 21 and 23 °C.

Values of C were estimated from pressure–volume curves (Scholander et al. 1965, Tyree and Hammel 1972) using the methods described by Brodribb and Holbrook (2003). Briefly, the Ψ_L corresponding to turgor loss was estimated as the inflection point of the graph of Ψ_L vs. relative water content (RWC). The slope of the curve prior to, and following, turgor loss provided C in terms of RWC (C_{rwc}) for pre-turgor loss and post-turgor loss, respectively. Five to six leaves of each species were used to construct pressure–volume curves and estimate C.

Pressure–volume curves were conducted on individual leaves for the broadleaf species and on fascicles of two needles for *P. virginiana*. Branch samples of ~30–50 cm, from the same individuals that were used for rehydration and measurement of K_{leaf}, were excised early in the morning and re-cut under water in the lab. Branches were allowed to rehydrate for at least 4 h before pressure–volume analyses were performed. Pressure–volume curves were created by plotting the inverse of Ψ_L against RWC with alternate determinations of fresh mass and Ψ_L repeated during slow dehydration of the twig on the laboratory bench until values of Ψ_L exceeded the measuring range of the pressure chamber (–4.0 MPa). Leaf water potential was measured using a pressure chamber (PMS Instrument Company, Albany, OR, USA). For normalizing C on a leaf area basis, leaf areas for the broadleaf species were obtained with a scanner and ImageJ version 1.27 image analysis software (Abramoff et al. 2004, National Institute of Mental Health, Bethesda, MD, USA) and needle areas for *Pinus* were determined by multiplying mean needle lengths and circumferences (n = 6 leaves/needles per species).

For measurement of K_{leaf} in the field, branches (~10–20 cm in length) were collected from trees, and leaves were then excised for determination of Ψ_o, with no equilibration time (Ψ for leaves on the same shoot typically varied by <0.1 MPa). Leaf samples from the same branch were then rehydrated for a period of t seconds and Ψ_f was measured. Distilled water was used for rehydration of K_{leaf} samples and all measurements took place in the shade. These measurements (both field and lab) were performed on individual leaves of *Acer* and *Liriodendron* and fascicles (two needles each) of *Pinus*.

Field measurements of K_{leaf} along with corresponding measurements of Ψ_L (predawn and midday), stem water potential and stomatal conductance were performed over 4 days in July of 2010 (20, 22, 24 and 25 July). Additionally, predawn and midday (stem and leaf) water potentials for *Pinus* and *Liriodendron* were measured on 12 and 13 July 2009 and were not significantly different from those measured in 2010 (although 2009 *Liriodendron* predawn values were slightly more negative than 2010 values, by ~0.04 MPa). All measurements were made on three to six leaves from five preselected individuals approximately every 120 min from 530–600 h (predawn) until 1600–1630 h Eastern Daylight Time. All individuals were in open areas and fully sunlit branches/leaves were chosen for measurement (with the exception of predawn measurements).

Leaf and stem water potentials and stomatal conductance

Stomatal conductance (g_s) was measured with a steady-state porometer (LI-1600; Li-Cor, Lincoln, NE, USA) and leaf temperatures were measured concomitantly with a fine-wire thermocouple (located in the LI-1600 chamber). One-sided leaf areas of foliage from the porometer measurements were obtained with a scanner and ImageJ. Leaf water potential was measured using a pressure chamber on individual leaves of *Acer* and *Liriodendron* and fasicles (two needles each) of *Pinus*. Measurements of g_s and Ψ_L were conducted on the same dates and over the same time intervals as K_{leaf} measurements, and on three to five leaves of each species (per time interval). Additionally, measurements of stem water potential were performed in order to estimate the amount of embolism that occurred in stems of the measured trees. Large disequilibria can exist between stem and leaf water potentials, especially at midday (Bucci et al. 2004). Therefore, it was necessary to bag and cover shoots (with a sealable plastic bag covered in aluminum foil) before dawn and then measure the midday water potential of bagged leaves to get an estimate of stem water potential.

Sap flow

Heat dissipation sap flow probes with heated and reference sensors 20 mm in length (Granier 1985) were used to determine sap flux in *A. rubrum* and *P. virginiana*. For probe installation, two holes separated axially by 10 cm were drilled into the sapwood (2 cm depth) and the heated sensor installed above the reference sensor. The sensors were coated with thermally conductive silicone heat sink compound prior to

insertion. All probes were protected from ambient radiation by reflective insulation. Signals from the sap flow probes were scanned every minute and 10-min means were recorded by a data logger (CR10X; Campbell Scientific Corp., Logan, UT, USA) equipped with a 32-channel multiplexer (AM416; Campbell Scientific). Differential voltage measurements between the heated and reference sensors were converted to a temperature difference (ΔT), which was converted to sap flux (v; g m^{-2} s^{-1}) using the empirical calibration of Granier (1985):

$$v = 119\ k^{1.231}$$

where $k = (\Delta T_m - \Delta T)/\Delta T$, and where ΔT_m is the temperature difference when sap flux is assumed to be zero. Sap flux values were averaged over five clear days for three individuals of each species.

Our primary interest was in the temporal dynamics of sap flow as opposed to actually quantifying total water use, or the spatial variability of sap flow, in these trees. Although there is likely to be sap flow inwards of 2 cm on these trees, the majority of water use in these stems should be captured by the 2 cm probes due to the fact that the outermost regions of sapwood typically represent the area where sap flow is highest and due to the fact that these stems were relatively small in diameter.

Comparison of maximum K_{leaf} and remaining K_{leaf} at midday

Absolute maximum values of K_{leaf} were obtained from multiple sources, including the current study, previous studies by our research group and published data from other researchers (see Table 2). Values of K_{leaf} were converted to relative values by dividing each species' maximum value by the overall maximum K_{leaf} out of all species in the study (*Myrsine guianensis*, maximum K_{leaf} = 75.5 mmol m^{-2} s^{-1} MPa^{-1}). Then, the percentage of maximum K_{leaf} remaining at that species' lowest water potential (typically midday) was calculated, based on published vulnerability curves and minimum leaf water potential, or reported as measured (as in the current study). The relative maximum K_{leaf} and the percentage of maximum K_{leaf} remaining at midday were then compared to determine whether there was a trade-off between maximum conductance and vulnerability to hydraulic dysfunction. To ensure that there was no bias due to differences in measurement techniques, data obtained by using bulk leaf capacitance in combination with rehydration kinetics were plotted as a comparison.

Results

Precipitation during the month of July 2010 was only 8.2 cm, below the average of 10.9 cm. The daily maximum temperature for the four measurement days was 31.8 °C, which was much greater than the historical mean maximum July temperature of 27.1 °C.

Overall, stems were much less vulnerable to embolism than leaves (Figure 1). *Acer* stems showed a 50% loss in conductivity (P_{50}) at –3.9 MPa, whereas leaves from the same species had a P_{50} of –1.7 MPa. *Liriodendron* had stem and leaf P_{50}s of –3.0 and –1.2 MPa, respectively, and *Pinus* had a stem P_{50} of –4.2 MPa and a leaf P_{50} of –0.8 MPa. The largest difference in stem and leaf P_{50} was 3.4 MPa, in *Pinus*. *Pinus* and *Acer* had high maximum K_{leaf} values (Table 1; 32.8 and 29.2 mmol m^{-2} s^{-1} MPa^{-1},

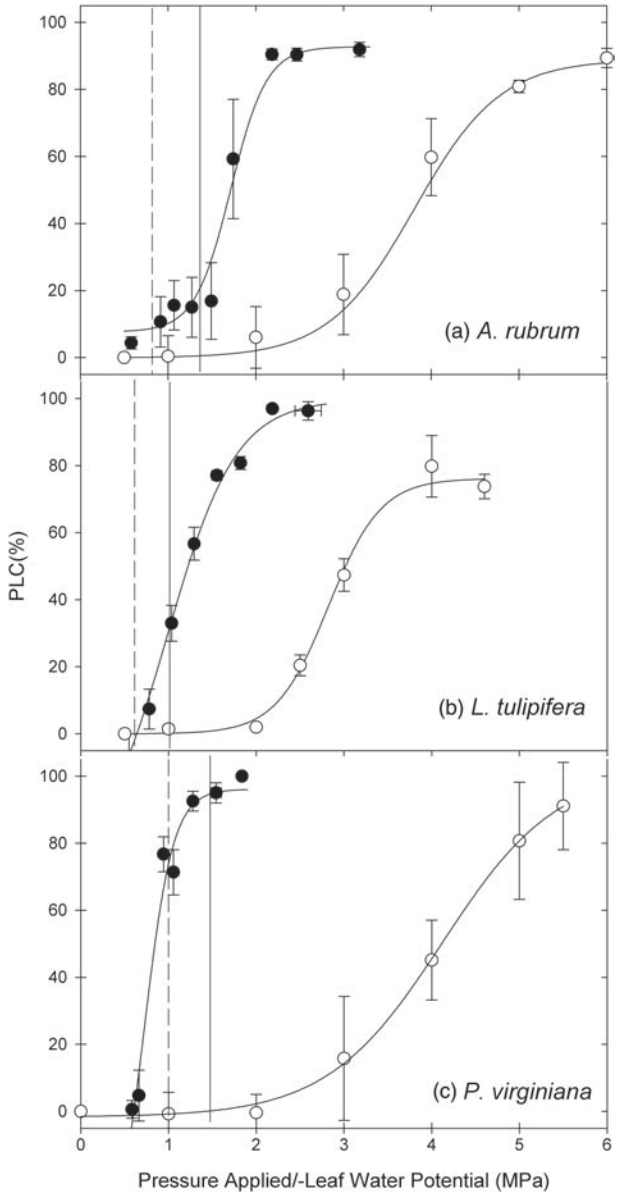

Figure 1. PLC of leaf hydraulic conductance (closed circles) and stem hydraulic conductivity (open circles) across a range of water potentials for (a) *A. rubrum*, (b) *L. tulipifera* and (c) *P. virginiana*. Midday minimum leaf water potentials and stem water potentials are indicated by vertical solid and dashed lines, respectively. Error bars represent standard error.

Table 1. Leaf turgor loss point (TLP), pre- and post-turgor loss point capacitance (C) and maximum leaf hydraulic conductance (K_{leaf}). TLP and C values from pressure–volume analyses. Numbers in parentheses are standard errors.

Species	TLP (MPa)	Pre-TLP C (mol m^{-2} MPa^{-1})	Post-TLP C (mol m^{-2} MPa^{-1})	Maximum K_{leaf} (mmol m^{-2} s^{-1} MPa^{-1})
A. rubrum	–1.59 (0.03)	0.87 (0.15)	3.06 (0.54)	29.2 (0.7)
L. tulipifera	–1.13 (0.08)	0.35 (0.05)	1.31 (0.08)	9.8 (0.5)
P. virginiana	–1.98 (0.18)	1.38 (0.31)	2.50 (0.97)	32.8 (1.2)

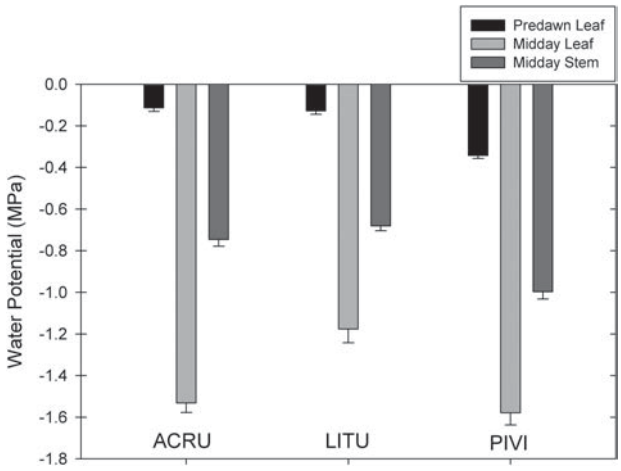

Figure 2. Measured water potential of leaves predawn, midday and stem water potential measured midday. ACRU is *A. rubrum*, LITU is *L. tulipifera* and PIVI is *P. virginiana*. Vertical error bars represent standard error.

respectively) whereas *Liriodendron* had a much lower maximum K_{leaf} (9.8 mmol m^{-2} s^{-1} MPa^{-1}).

Predawn leaf water potentials were between –0.1 and –0.4 MPa in all three species and declined to minima of –1.2 to –1.8 MPa by midday (Figure 2). Midday stem water potentials varied from –0.6 to –1.0 MPa. *Pinus* had the lowest predawn and midday leaf and stem water potentials. No daily reductions in K_{leaf} were observed in either *Acer* or *Liriodendron* (Figure 3). However, *Pinus* had a complete loss of K_{leaf} by late afternoon but recovered slightly by the end of the measurement period (1600). Based on the stem vulnerability curves and midday stem water potentials, no loss in stem hydraulic conductivity was predicted for any of the three species.

Stomatal conductance was greater in *Pinus* than either *Acer* or *Liriodendron* and declined by midday for all species (Figure 4). Although *Pinus* lost the greatest percentage (~65% reduction) of its maximum measured stomatal conductance, it still remained higher than either *Acer* or *Liriodendron* throughout most of the day. On the other hand, *Acer* and *Liriodendron* had lower absolute maximum stomatal conductance, but their percentage reduction in stomatal conductance (52% and 35% for *Acer* and *Liriodendron*, respectively) was less than for *Pinus*.

Diurnal courses of sap flux differed in *Acer* and *Pinus* (Figure 5). In *A. rubrum* sap flow peaked early in the day (by

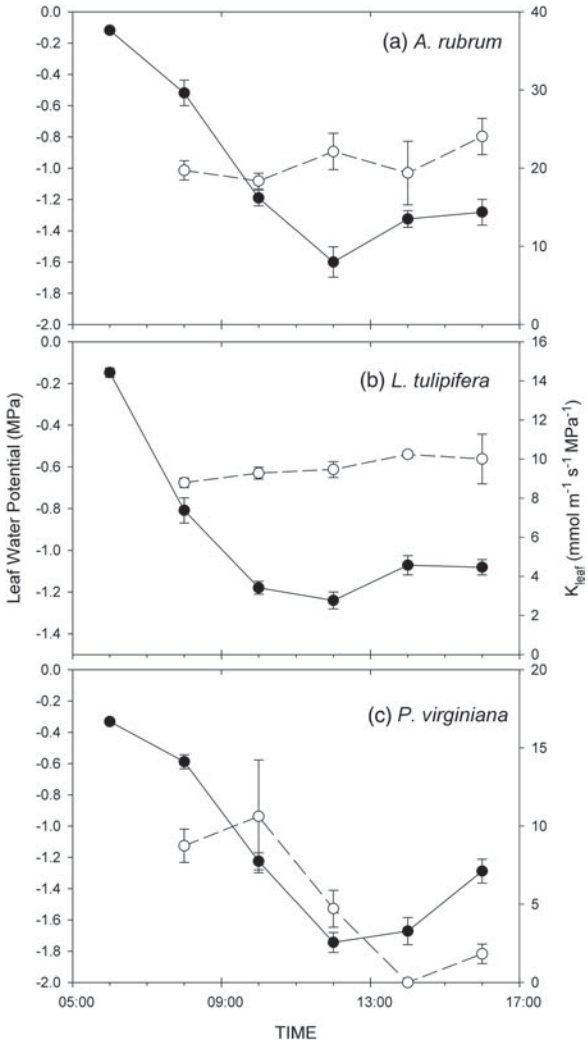

Figure 3. Leaf water potential (Ψ_{leaf}, closed circles) and leaf hydraulic conductance (K_{leaf}, open circles) measured in the field for (a) *A. rubrum*, (b) *L. tulipifera* and (c) *P. virginiana*.

0800 h), was reduced by an average of 38% by mid-afternoon, and then fell to near zero at the end of the day. In *P. virginiana* sap flux followed a more bell-shaped trajectory, reaching a maximum between 1000 and 1300 h. Daily courses of sap flux for these two species were consistent with their daily courses of stomatal conductance (cf. Figure 4). Mean maximum values of sap flux were somewhat greater for *Pinus* (31 g m^{-2} s^{-1}) than for *Acer* (23 g m^{-2} s^{-1}), although this difference was not significant ($P = 0.18$).

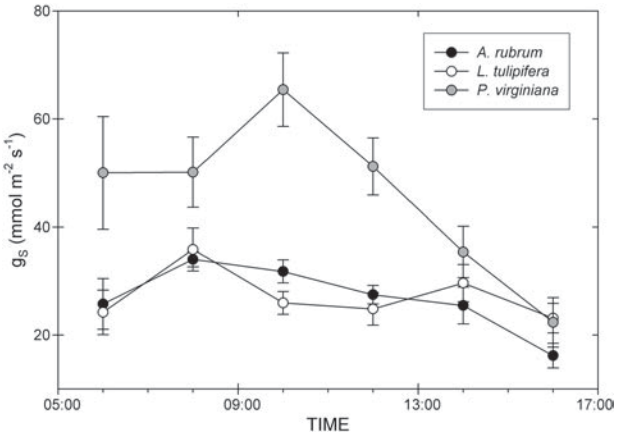

Figure 4. Stomatal conductance (g_s) measured in the field for *A. rubrum*, *L. tulipifera* and *P. virginiana*. Vertical error bars represent standard error.

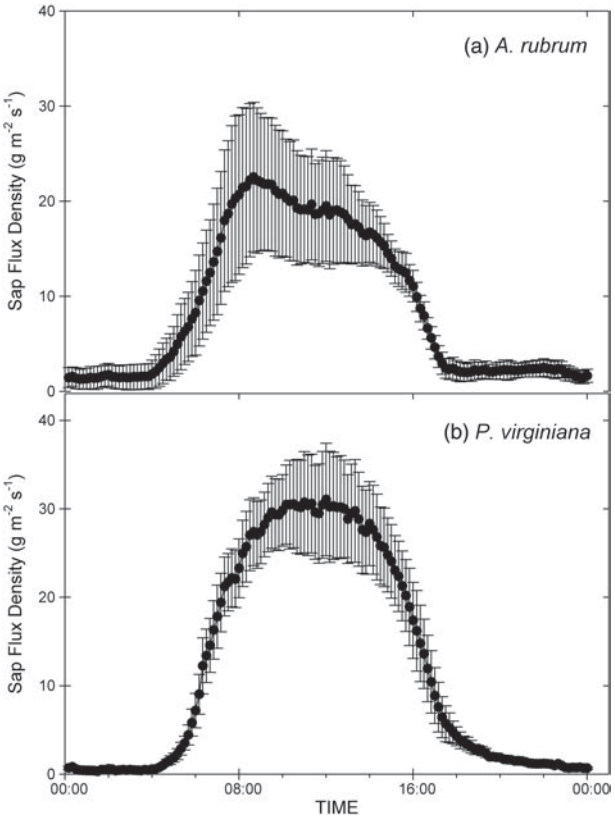

Figure 5. Sap flux measurements for three individuals of each (a) *A. rubrum* and (b) *P. virginiana*. Each plot is an average of five sunny days during the measurement period and vertical bars represent standard error.

The 42 species assessed for the relationship between relative maximum K_{leaf} and the percentage of maximum K_{leaf} remaining at midday appeared to fall into three groups based on their trajectories of this relationship (Figure 6, see Table 2 for groupings). Species in Group A showed relatively small losses of K_{leaf} at midday, and the trajectory of a linear fit through these data

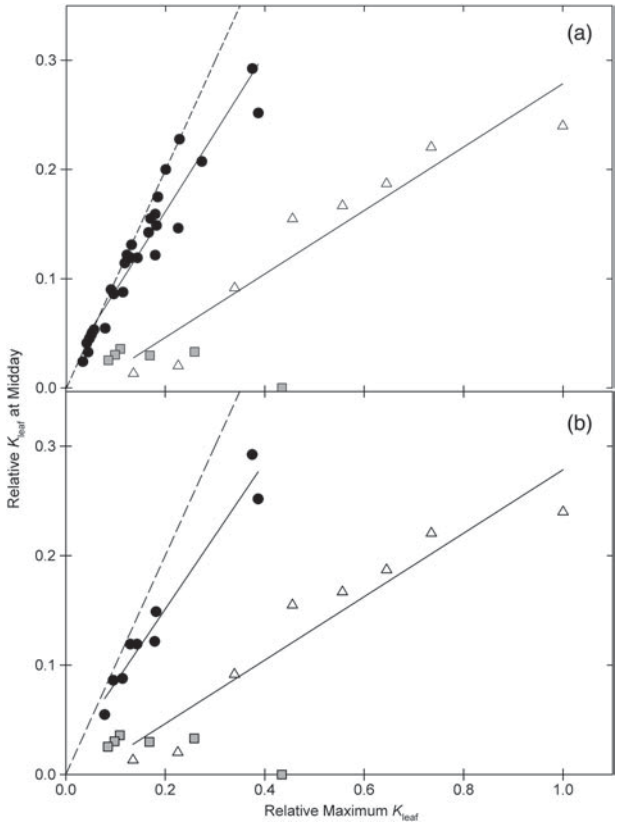

Figure 6. Relative maximum K_{leaf} (as compared with maximum values found in the literature; see the text for references) and the relative remaining K_{leaf} at midday. The 1:1 line (dashed line) indicates where a leaf would have lost no leaf hydraulic conductance at midday. Closed circles represent Group A, open triangles represent Group B and gray squares represent Group C (see Table 2 for groupings). Solid lines represent best-fit linear regressions through Groups A and B. For regression parameters, see the text. Panel (a) includes all data from Table 2 and panel (b) includes only data from the rehydration kinetics method using bulk leaf capacitance.

($R^2 = 0.92$, $P < 0.0001$) did not differ significantly from that of a 1:1 relationship even though its slope was 0.72. In contrast, species in Group B tended to have greater relative maximum K_{leaf} and experienced greater loss of K_{leaf} at midday (linear fit: slope = 0.29, $R^2 = 0.91$, $P = 0.0002$). Finally, species in Group C (Pinaceae and Cupressaceae) had overall low values of maximum K_{leaf} and all six species in this group lost the majority of their K_{leaf} at midday (linear fit through data not significant; $R^2 = 0.64$, $P = 0.0545$, slope = –0.08).

Discussion

Coordination of leaf and stem vulnerabilities

Although considerable research has been performed on stem and leaf hydraulics, few studies have addressed either the coordination of stem and leaf hydraulic vulnerability or the daily variation of leaf hydraulics in situ. In the current study, stems of each species were more resistant to embolism than were

Table 2. Species maximum K_{leaf} and percent of maximum K_{leaf} at daily Ψ_{min}. Species were grouped based on the percentage of K_{leaf} remaining at midday and whether the species was a gymnosperm or an angiosperm.

Species	K_{leaf} max mmol m^{-2} s^{-1} MPa^{-1}	% remaining at Ψ_{min}	Reference
Group A			
Vochysia ferruginea	28.3	78	FCM, unpublished
Protium panamense	13.5	68	Johnson et al. (2009*b*)
Q. garryana	8.6	77	Johnson et al. (2009*b*)
Arbutus menziesii	13.7	82	Johnson et al. (2009*b*)
Alnus rubra	5.9	70	Johnson et al. (2009*b*)
A. rubrum	29.2	65	Current study
L. tulipifera	9.8	92	Current study
Byrsonima crassifolia	17.2	100	Brodribb and Holbrook (2006)
Rehedra trinervis	20.6	76	Brodribb and Holbrook (2006)
Genipa americana	17.0	65	Brodribb and Holbrook (2006)
Cercis siliquastrum	12.5	86	Nardini et al. (2003)
Anacardium excelsum	10.8	83	DMJ and KAM, unpublished
Miconia argentia	7.2	90	DMJ and KAM, unpublished
Orites diversifolus	9.9	100	Blackman et al. (2010)
Gaultheria hispida	6.8	100	Blackman et al. (2010)
Richea scoparia	3.8	97	Blackman et al. (2010)
Atherospermum moschatum	3.1	100	Blackman et al. (2010)
Nothofagus gunnii	13.5	89	Blackman et al. (2010)
Tasmannia lanceolata	3.5	98	Blackman et al. (2010)
Lomatia polymorpha	4.2	96	Blackman et al. (2010)
Telopea truncata	9.2	100	Blackman et al. (2010)
Nothofagus cunninghamii	3.9	98	Blackman et al. (2010)
Olearia pinifolia	2.5	73	Blackman et al. (2010)
Pittosporum bicolor	3.3	75	Blackman et al. (2010)
Coprosma nitida	12.8	92	Blackman et al. (2010)
Eucalyptus coccifera	8.9	97	Blackman et al. (2010)
Hakea lissosperma	13.9	95	Blackman et al. (2010)
Hakea microcarpa	15.1	100	Blackman et al. (2010)
Group B			
Simarouba glauca	42.0	30	Brodribb and Holbrook (2004)
Hymenea stignocarpa	55.5	30	Hao et al. (2008)
Aegiphila lhotzkiana	34.4	34	Hao et al. (2008)
Myrsine guianensis	75.5	24	Hao et al. (2008)
S. ferrugineus	48.7	29	Hao et al. (2008)
Tapirira guianensis	17.0	9	Hao et al. (2008)
Tachigalia versicolor	25.6	27	Johnson et al. (2009*b*)
Quercus rubra	10.2	10	DMJ and KAM, unpublished
Group C			
P. virginiana	32.8	0	Current study
P. ponderosa	8.2	33	Johnson et al. (2009*b*)
P. menziesii	7.4	31	Johnson et al. (2009*b*)
P. taeda	6.4	30	Domec et al. (2009)
Tsuga heterophylla	19.5	13	DMJ and KAM, unpublished
Thuja plicata	12.7	18	DMJ and KAM, unpublished

leaves, consistent with the findings of Hao et al. (2008) for 10 forest and savanna tree species and Chen et al. (2010) in *Hevea brasiliensis*. Interestingly, Chen et al. (2009) found that leaves were significantly more vulnerable to embolism than stems in three evergreen members of the Euphorbiaceae, but that leaf and stem vulnerabilities were not different in three deciduous members of the same family. It was hypothesized by Chen et al. (2010) that leaf embolism may serve as a 'safety valve' to isolate and protect the upstream hydraulic pathway, although this may not be the case in those species where stem and leaf vulnerability was similar. It would seem reasonable that emboli in leaf xylem, due to its proximity to living tissue, would be more easily refilled than air-filled conduits in stems (e.g., Zwieniecki and Holbrook 2009). Additionally, leaves should also be more 'disposable' due to their lower construction cost as compared with branches so sacrificing one or more

leaves may protect the stem (and other associated leaves) from runaway xylem embolism and possible stem dieback. This is consistent with earlier studies that have proposed that leaf xylem embolism may serve as a trigger to induce stomatal closure (e.g., Sperry 1986) to protect branches and other upstream components from embolism.

Daily patterns of K_{leaf}, water potential and stomatal conductance

Although several studies have reported declines and partial or complete recovery of K_{leaf} over the course of a day (Bucci et al. 2003, Brodribb and Holbrook 2004, Meinzer et al. 2004, Johnson et al. 2009*b*), it is becoming apparent that this is not the case for all species (Blackman et al. 2010). In species that experience declines in K_{leaf} on a daily basis, there seems to be one or more mechanisms for repair of the dysfunction by the next day even when water in adjacent functional conduits is under considerable tension (e.g., Bucci et al. 2003, Nardini et al. 2008, Johnson et al. 2009*b*, Zwieniecki and Holbrook 2009).

In the current study, leaves of *P. virginiana* lost nearly 100% of their K_{leaf} but began to recover late in the day, while water potentials were still more negative than –1.0 MPa. Leaves of *Pinus ponderosa* and *Pseudotsuga menziesii* also lost large percentages of K_{leaf} but began to recover in the late afternoon while leaf water potentials were still highly negative (Johnson et al. 2009*b*). However, *Acer* and *Liriodendron* (from the current study) lost none or very little of their K_{leaf} as previously observed in *Quercus garryana* and *Arbutus menziesii* (Johnson et al. 2009*b*) and 16 other species from Tasmania (Blackman and Brodribb 2010). In fact, there was a slight increase in K_{leaf} in both *Acer* and *Liriodendron* between early morning and noon. Increases in K_{leaf} with increasing temperature and light have been previously reported for several species (Sack et al. 2004, Scoffoni et al. 2008, Sellin et al. 2008, Voicu et al. 2008).

In the current study, overall values of stomatal conductance were low, which is likely due to lower than average July rainfall (~25% less than average). In addition, the data from the current study were in contrast to earlier work showing a close relationship between maximum K_{leaf} and maximum stomatal conductance (Brodribb et al. 2005). The most plausible explanation for this is that the data in the current study were not representative of maximum stomatal conductance due to higher than normal temperatures or lower than average rainfall.

Acer and *Liriodendron* exhibited much more conservative behavior than did *Pinus* in that their stomatal conductance was low early in the day and then gradually decreased, avoiding low values of Ψ_L that would have provoked substantial embolism. In contrast, *Pinus* had high stomatal conductance until it began to show loss of K_{leaf}, at which point stomatal conductance began to decline, although only by ~60% (cf. Figures 4 and 5).

These two different approaches are mirrored in the sap flow profiles for *Acer* and *Pinus* (Figure 5), where *Acer* displayed a reduction in sap flow early in the morning and *Pinus* sap flow did not slow until late afternoon. *Pinus ponderosa* exhibited behavior similar to that of *P. virginiana*: K_{leaf} was reduced to ~40% of its maximum value, but stomatal conductance only decreased by ~40% and only after initial decreases in K_{leaf} (Johnson et al. 2009*b*). This behavior is in contrast to a hypothesis put forth by Zwieniecki et al. (2007) which predicts that needle-leaved species like *Pinus* may close their stomata early (before reaching the water potential resulting in embolism), to protect the mesophyll cells, since they are not well irrigated by the xylem.

Potential trade-offs between maximum K_{leaf} and K_{leaf} at Ψ_{min}

Although there is evidence for a hydraulic safety vs. efficiency trade-off in other plant organs (Sperry and Saliendra 1994, Domec and Gartner 2003, Wheeler et al. 2005, Domec et al. 2006, Hacke et al. 2006), such as stems and roots, this is a topic of ongoing debate (e.g., Meinzer et al. 2010). To our knowledge, no trade-off between leaf hydraulic vulnerability and maximum K_{leaf} has been previously described. It should be noted that absolute maximum K_{leaf} values can vary based on the method used (e.g., Blackman and Brodribb 2011), and although multiple methods were used in measuring the maximum K_{leaf} values in Figure 6, this should not change the overall results or groupings in the three trajectories. In fact, using only the data from the rehydration kinetics method and bulk leaf capacitance resulted in the same groupings (see Figure 6b). Although using bulk leaf capacitance for the rehydration kinetics method tends to overestimate K_{leaf} as compared with other methods, it does so in a systematic nature across a wide range of leaf types. For example, Blackman and Brodribb (2011), when using bulk leaf capacitance as compared with flow-based estimates of capacitance, overestimated K_{leaf} by 59% on average (SE = 10.2% when one outlier, greater than four standard deviations from the mean was removed; 76% overestimation with outlier not removed and SE = 13.5%).

In a recent study by Blackman et al. (2010), none of the 16 species measured lost even 30% of K_{leaf} at the minimum seasonal leaf water potential, which would place them in Group A (Table 2, Figure 6) of the current study. Of the six conifers represented in Figure 6, all lose the majority of their K_{leaf} at midday. For example, Domec et al. (2009) observed 70–77% losses of K_{leaf} at midday for *Pinus taeda*, depending on the treatment (e.g., elevated carbon dioxide or fertilization). The reason for the observed large daily declines and recovery of K_{leaf} in conifer species may be related to the limited hydraulic connections between different tissues inside conifer leaves (Zwieniecki et al. 2007) or the observed delay in *P. taeda*, *P. ponderosa*, *P. virginiana* and *P. menziesii* stomatal conductance

reductions in response to losses of K_{leaf} (Domec et al. 2009, Johnson et al. 2009*b* and the current study). It is also feasible that xylem embolism in small, needle-type leaves may be easier or less costly to refill than that in larger broadleaves. This may also be related to the occurrence of transfusion tissue in the Pinaceae, and its ability to store solutes that could be released into adjacent tracheids, prompting refilling (Canny 1993, Zwieniecki and Holbrook 2009, Liesche et al. 2011).

An explanation of the differences in trajectory of Groups A and B in Figure 6 may be related to differences in leaf anatomy or stomatal responsiveness to changes in leaf water status. For example, *Styrax ferrugineus* (Group B) leaf water potential dropped to –1.7 MPa (corresponding to ~70% reduction in K_{leaf}) before stomata began to close (Bucci et al. 2004). Even when stomata began to close, the resulting decline in stomatal conductance was only ~30%. In *Simarouba glauca* (Group B, Brodribb and Holbrook 2004), reductions in stomatal conductance occurred only after K_{leaf} declined during the wet season, and although stomatal conductance decreased earlier in the day in the dry season, it still did not prevent massive (~65%) losses of K_{leaf}. The fact that there are many evergreen and sclerophyllous species in Group A (little loss of K_{leaf}) may reflect the investment in those tissues and the need for a more conservative strategy as opposed to a less conservative strategy where (i) large losses of K_{leaf} could lead to leaf death or (ii) large losses in K_{leaf} must be repaired by what is likely an energetically expensive process (e.g., Bucci et al. 2003, Zwieniecki and Holbrook 2009).

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