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Dynamic changes in petiole specific conductivity in red maple (*Acer rubrum* L.), tulip tree (*Liriodendron tulipifera* L.) and northern fox grape (*Vitis labrusca* L.)

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ABSTRACT

Diurnal variation in petiole specific hydraulic conductivity and simultaneous measurements of leaf water potential were recorded in red maple, tulip tree and fox grape. Petiole specific conductivity was determined from *in situ* measurements of water flow into the distal (leaf-bearing) end of an attached petiole as a function of applied hydrostatic pressure and petiole dimensions. The hydraulic properties of the petiole dominated the measurements, indicating that this technique can be used for rapid estimates of petiole hydraulic conductivity. There was a significant decrease in petiole specific conductivity associated with increasingly more negative leaf water potentials in maple and tulip tree, but not in grape. Petiole specific conductivity increased during the afternoon while the plant was actively transpiring and the xylem sap was under tension. The recovery of petiole conductivity during the afternoon suggests that hydraulic conductivity reflects a dynamic balance between a loss of hydraulic conductivity with increasing water stress, and its restoration as tension within the xylem decreases. Three experimental manipulations were applied to red maple and tulip tree to examine the sensitivity of diurnal changes in petiole conductivity to various physiological perturbations. Both phloem girdling and application of $HgCl_2$ to the transpiration stream resulted in a marked decrease in the degree to which petiole specific conductivity recovered as xylem tension relaxed during the afternoon. Delivery of a surfactant to the xylem, however, did not significantly alter the relation between leaf water potential and petiole hydraulic conductivity.

Key-words: *Acer rubrum*; *Liriodendron tulipifera*; *Vitis labrusca*; cavitation; embolism; refilling; specific conductivity; xylem.

INTRODUCTION

The formation of air embolism in the xylem of transpiring plants appears to be a common, if not inevitable, phenomenon that is functionally related to the existence of tension within the water column (Milburn 1993; Holbrook *et al*. 1995; Pockman *et al*. 1995; Milburn 1996). It has been generally assumed that the reverse process (embolism repair) cannot take place unless positive (above vacuum) pressures are present in the xylem (Tyree & Yang 1992; Yang & Tyree 1992; Lewis *et al*. 1994). This leads to the conclusion that water stress places the xylem at risk of substantial, and over the short-term, irreversible losses in hydraulic conductivity (Tyree & Sperry 1989; Sperry & Pockman 1993). Recent studies, however, suggest the existence of short-term, dynamic changes in xylem hydraulic conductivity (Salleo *et al*. 1996; Zwieniecki & Holbrook 1998; Tyree *et al*. 1999). Hydraulic conductivity was observed either diurnally (overnight; Zwieniecki & Holbrook 1998) or over a period of several hours after transpiration was prevented (Salleo *et al*. 1996; Tyree *et al*. 1999). In both cases, hydraulic conductivity increased despite the absence of positive xylem pressures (based on pressure bomb measurements of non-transpiring leaves). Recent cryogenic scanning electron microscopy (cryo-SEM) observations of decreases in the number of embolized vessels provide further support for embolism repair (Canny 1997; McCully *et al*. 1998; Melcher 1999; Tyree *et al*. 1999). These latter studies are particularly interesting as they indicate that embolism repair can occur concurrently with transpiration.

Dynamic changes in the number of functional conduits raises the question of how embolized conduits could be refilled while the majority of the xylem remains under tension. Although a mechanism for embolism repair that is consistent with the cohesion theory has recently been proposed (Holbrook & Zwieniecki 1999), additional studies with increased temporal resolution, as well as experimental manipulations, are needed. The primary goal of this study was to document changes in hydraulic conductivity in relation to diurnal variation in leaf water potential. The need to make repeated measurements without removing a substantial portion of the total leaf area influenced our decision to focus on the hydraulic conductivity of petioles. We developed an *in situ* technique that allowed rapid (and thus frequent) measurements of petiole hydraulic conductivity. Although analysis of the raw data obtained by this technique is more complicated than that of data obtained by measurements made on excised segments, it has several advantages. In particular, the ability to make measurements

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rapidly and with zero storage time for excised material makes this approach useful for examining real-time physiological responses.

The mechanism by which embolism repair takes place is largely unknown, although a broad hypothesis for how this might occur has been proposed (Holbrook & Zwieniecki 1999). It is clear, however, that information on how physical or physiological factors influence the capacity for repair will increase our understanding of the underlying processes. The experimental manipulations used in this study include phloem girdling and the application of two substances (mercuric chloride and a surfactant) to the transpiration stream. There is some evidence that disruption of downstream phloem transport may decrease the extent of embolism repair (Salleo *et al*. 1996; but see Zwieniecki & Holbrook 1998), although a mechanism for this is unknown. A number of authors have suggested that xylem parenchyma cells may be involved in the repair process in angiosperms (Salleo *et al*. 1996; Canny 1997; Holbrook & Zwieniecki 1999), although stem parenchyma is apparently not involved in embolism repair in *Pinus sylvestris* (Borghetti *et al*. 1991). Because the movement of water into an air-embolized vessel is counter to the prevailing water potential gradient within the xylem, it is reasonable to hypothesize that a process involving the active movement of solutes across cell membranes is involved. We chose to alter the metabolic activity of living cells within the xylem by applying mercuric chloride both because it is a potent cellular poison and because it has a strong inhibitory effect on water channels (Schaffner 1998). Our final treatment modified the surface tension within the xylem in an attempt to alter the relation between cavitation and xylem tension.

MATERIALS AND METHODS

Study site and plant material

Field measurements were conducted at the Harvard Forest, Petersham, Massachusetts, USA, during August 1998. Three species were used: red maple (*Acer rubrum* L.) – five young trees, each approximately 4 m tall; tulip tree (*Liriodendron tulipifera* L.) – one 30-year-old tree, approximately 15 m tall; and northern fox grape (*Vitis labrusca* L.) – one multi-stemmed vine tangle possibly consisting of more than one genetic individual. Both red maple and tulip tree are diffuse-porous. Fox grape has a xylem anatomy typical of woody vines, consisting of large, solitary vessels. The plants were located on the east side of a large clearing and were exposed to direct sunlight for at least half of the day. None of the individuals included in the study showed any evidence of damage due to biotic or abiotic factors.

Petiole hydraulic conductance

Petiole hydraulic conductance (g MPa^{-1} s^{-1}) was calculated as the slope of delivery pressure versus flow rate into the petiole. The basic approach consisted of measuring the steady-state flow rate of water through a petiole while still attached to the tree (leaf blade removed). Measurements were made at two delivery pressures, allowing the conductance to be determined without explicit knowledge of the total driving force. Measurements were made under steady-state conditions, justifying the use of a linear relation between flow rate and driving gradient:

$$Q_1 = (P_{\text{delivery1}} - P_{\text{plant}})G \quad (1)$$

$$Q_2 = (P_{\text{delivery2}} - P_{\text{plant}})G \quad (2)$$

where Q_1 and Q_2 are the flow rates into the petiole for two different delivery pressures, $P_{\text{delivery1}}$ and $P_{\text{delivery2}}$, G is the conductance, and P_{plant} is the hydrostatic pressure within the xylem. If we assume that P_{plant} remains constant during the measurement cycle (i.e. it behaves as a constant pressure reservoir), then the conductance (G) can be calculated as:

$$G = (Q_2 - Q_1)/(P_{\text{delivery2}} - P_{\text{delivery1}}) \quad (3)$$

We feel that the assumption that P_{plant} remains constant during the time needed to complete one cycle (i.e. high- and low-pressure reading) is justified because the volume of water pushed into the plant is small compared to the water capacity of the branch. P_{plant} is further buffered against perturbation by the ongoing movement of water through the branch as a result of transpiration from the remaining leaves.

Measurements were made using a 'two-point flow meter' constructed for this study. The instrument consists of two captive air tanks filled with de-gassed water and pressurized typically to approximately 0·15 and 0·3 MPa. Water flows from the pressure tanks through a 0·1 μm filter (Micron Separation Inc., Westborough, MA, USA) into a calibrated PEEK (polyetheretherketone) capillary tube and is delivered to the distal end of the petiole via a stiff-walled, large (2 mm) diameter tube (Fig. 1). The pressure drop across the capillary tube is measured by two pressure transducers (Omega PX236-100 Series, Omega Engineering Inc., Stamford, CT, USA). The relationship between pressure drop and flow rate (and thus the conductance of the PEEK tube) was determined empirically by directing the outflow onto an analytic balance (± 0·01 mg). The minimum resolution of the two-point flow meter was determined by the accuracy of the differential pressure measurement (± 3·5 kPa) and the conductance of the calibrated tube (0·00104 g MPa^{-1} s^{-1}) to be approximately $\pm 3{\cdot}5 \times 10^{-6}$ g s^{-1}.

Measurements began with removal of the leaf blade under water using a fresh razor blade. The petiole was immediately attached to the two-point flow meter as described below using a compression fitting designed to have a minimal effect on petiole conductance (Fig. 1). Prior to excision of the leaf blade, the distal end of the petiole was wrapped in a 0·3–0·5 mm layer of parafilm to secure and protect the petiole. Connectors were tightened by hand (finger-tight) and the final connection to the petiole contributed less than 5% of the total measured resistance. The flow rate into the petiole was determined using first the high-pressure tank, followed by the low-pressure tank. This

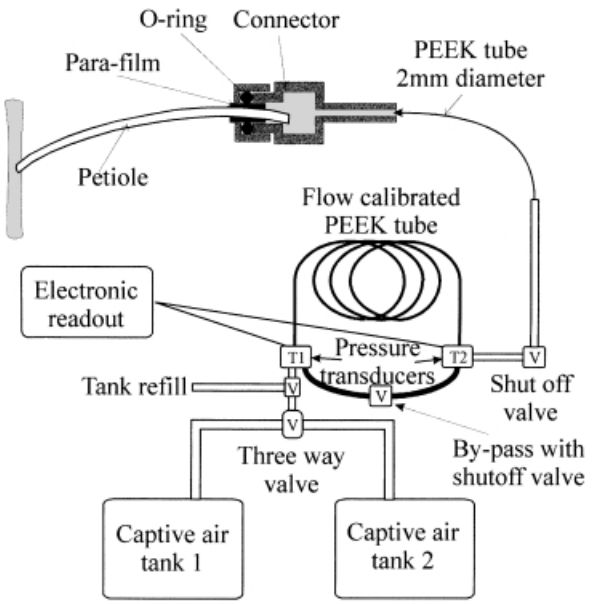

Figure 1. Schematic representation of the two-point flow meter. Water flows from one of two pressurized air tanks, through a tube of known conductance, and into the distal end of the petiole. The pressure drop across the calibrated PEEK tubing is used to determine the flow rate, while the downstream pressure transducer gives the delivery pressure into the petiole. Consecutive measurements of flow rate at two different pressures are used to calculate petiole conductance.

was repeated at approximately 40 s intervals until the calculated conductance of the petiole varied by less then 10% for three consecutive readings. Delivery pressures were typically approximately 0·12 MPa and approximately 0·07 MPa, respectively, for the high- and low-pressure inputs. Conductance was calculated as the slope of a linear relation between two consecutive measurements of flow rate and delivery pressure and the average of the final two conductance measurements recorded.

We chose to determine petiole conductance *in situ* to minimize the time between excision and measurement. This approach eliminated problems associated with transport and storage of material to be measured. In addition, by leaving the petioles attached to the tree, we were able to use higher delivery pressures (and thus shorter equilibration times) without the danger of air emboli being flushed out of the petiole. On average, the time necessary to determine petiole conductance was approximately 3 min. During that interval, the total amount of water flowing into the plant was in the range of 0·0005–0·01 g for red maple and 0·001–0·1 g for tulip tree and fox grape, with the actual volume depending on petiole conductance. Petiole diameter and length were determined for each petiole. In all, we measured the conductance of 190 red maple petioles, 204 tulip tree petioles and 76 fox grape petioles. In addition, measurements of leaf water potential were made on the same plant material using a pressure chamber (PMS Instrument Corp., Corvallis, OR, USA). Five to seven leaves were measured at approximately 1·5 h intervals throughout the day.

Evaluation of the hydraulic measurement technique

The two-point flow meter has many elements in common with the high-pressure flow meter (Tyree *et al*. 1995), but differs in that only steady-state measurements are recorded. This avoids problems associated with transient measurements resulting from capacitance and extensibility of plant tissues. However, a true steady-state condition may never be reached because the small amount of water flowing into the plant could alter the downstream water potential. Because our goal was to make rapid measurements of petiole hydraulic conductivity, we determined the minimum time needed to obtain a stable conductance measurement. The time course of pressure changes after switching between supply tanks showed that a relatively constant delivery pressure (and thus flow rate) was achieved rapidly (Fig. 2a). In general, consecutive measurements showed a slight increase in delivery pressure; however, the calculated conductivity was fairly stable after the initial one or two measurements and remained constant for at least five or six cycles (Fig. 2b).

By pushing water into the distal end of an attached petiole we actually measure the impedance of a network of hydraulic elements (petiole, branch, stem). However, we expected that the measurement would be dominated by the hydraulic properties of the petiole by virtue of the smaller hydraulic resistance and larger volume of the branch and stem. To evaluate the contribution of these various components of the plant to the total resistance, we examined how resistance changed as the length of the total segment was sequentially shortened. For this test, petioles were attached to the instrument at midday (leaf water potential approximately – 1·4 MPa). Following the initial measurement, the branch was cut from the tree and consecutive cuts were made on the branch towards the petiole and resistance measured. Calculated total resistances indicate that there was little (< 10%) contribution associated with the branch (Fig. 2c). Furthermore, the resistance decreased linearly as the petiole was shortened, indicating that excision of the leaf blade did not result in a significant introduction of air into the xylem, and that the compression fitting had a minimal effect on petiole resistance (Fig. 2c).

Experimental treatments

Experimental manipulations were used to investigate factors influencing diurnal patterns in petiole hydraulic conductivity. These included phloem girdling and the application of either $HgCl_2$, surfactant, or water to the transpiration stream. Petiole conductance and leaf water potential of treated and control (untreated) branches were determined throughout the day.

Ten branches per species were girdled proximal to the

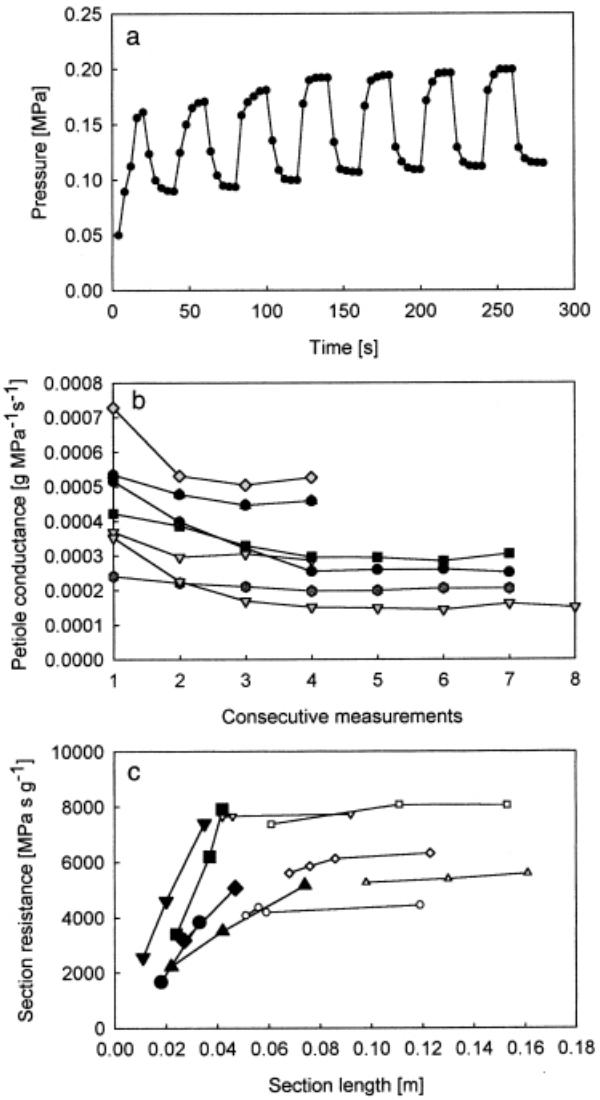

Figure 2. Evaluation of the *in situ* measurement technique. (a) Changes in delivery pressure (as recorded by pressure transducer T2; Fig. 1) with time following connection to a red maple petiole. Cycling between high and low pressures indicates manual switching between the two pressure tanks. (b) Changes in petiole conductance (calculated from sequential delivery pressure and flow measurements) with increasing numbers of measurement cycles. The time interval between changing delivery pressures was approximately 20 s. Traces for seven petioles are shown. (c) Sequential measurements of total resistance to water inflow through an attached petiole as a function of the length of the supporting branch. Following attachment of the two-point flow meter, the supporting branch was cut from the tree. Measurements of resistance to water inflow were made following sequential reduction of the length first of the branch (small open symbols) and then the petiole itself (large closed symbols). Traces from four such experiments are shown.

previous year's extension growth. Bark and phloem were removed under water from an approximately 1 cm long piece of stem, and the wound immediately covered with silicon-based grease to prevent air penetration into the stem. Girdling was done at midday when the leaf water potential was at its minimum.

Mercury chloride ($HgCl_2$, Sigma M-6529, Sigma Chemical Co., St Louis, Missouri, USA) was added to the transpiration stream of an intact plant following methods outlined by Zwieniecki & Holbrook (1998). Briefly, a plastic tube containing either a 5 mM $HgCl_2$ solution or pure water was attached to a side branch that had been cut off under water. The concentration of $HgCl_2$ applied in this experiment is higher than concentrations used in studies of water channel activity (50 μM (Henzler & Steudle 1995) to 1 mM (Kammerloher *et al*. 1994)) because we anticipated further dilution in the xylem sap. The solutions were applied during the late morning, prior to the peak in water stress, to allow them to be carried throughout the xylem of the target branch by the transpiration stream. Approximately 1–2 cm^3 of solution was taken up by each branch. Six branches per species were treated with the $HgCl_2$ and six with water.

A low surface tension solution was delivered to the transpiration stream of five branches per species according to the methods outlined above. We used a high concentration (1:1000) of surfactant (Triton X-100; Sigma Chemical Co.) because we again anticipated further dilution in the xylem sap. The surfactant solution was applied beginning on the previous evening and continuing throughout the following day to ensure that surfactant was present during the period when leaf water potentials were decreasing. Approximately 5 cm^3 of liquid was absorbed by each branch.

Data analysis

Petiole specific conductivity (g m^{-1} s^{-1} MPa^{-1}) was determined as petiole hydraulic conductivity per cross-sectional area of the petiole. We grouped individual conductivity measurements according to collection time (prior to the maximum stress and after maximum stress) and groups of leaf water potential (> – 0·5 MPa; – 0·5 to – 1·0 MPa; – 1·0 to – 1·5 MPa; < – 1·5 MPa). Tulip tree and fox grape had only three water potential groups, due to the fact that values more negative than – 1·5 MPa were not observed. Following verification that the assumptions of analysis of variance had been satisfied, these data were analysed using a two-way analysis of variance with collection time and leaf water potential as independent factors.

The effects of the experimental treatments on petiole specific conductivity were examined using a two-way analysis of variance with treatment ($HgCl_2$, girdling, surfactant, water and control) and leaf water potential as independent variables. The assumption of homogeneity of variance was not satisfied, requiring that the data be square root-transformed. In all cases, *post hoc* comparisons were made using contrast analysis.

Staining procedure

Individual leaves from all studied species were excised under water at pre-dawn and midday and placed in small vials containing a 2% solution of safranin in water. A total

of 96 leaves were measured (16 leaves per collection time per species). Petioles were submerged approximately 1·5 cm into the solution and the leaves placed outdoors in full sun for 2 h. Each petiole was then removed from the safranin solution, washed, and preserved in 50% glycerin and 50% ethanol. Cross-sections (one per petiole) were made approximately 1 cm above the stain delivery line, and the total number of stained and unstained vascular bundles in the cross-section were counted. Vascular bundles containing at least one vessel with safranin dye were scored as stained. The average number of stained bundles was compared using a *t* test for independent samples.

RESULTS

Petiole specific conductivity varied between 0·5 and 19 g s^{-1} m^{-1} MPa^{-1} in red maple, 12–220 g s^{-1} m^{-1} MPa^{-1} in tulip tree, and 12–100 g s^{-1} m^{-1} MPa^{-1} in fox grape. High variability in conductivity was observed within each species at all times during the experiment. Leaf water potential ranged during the day from – 0·2 to – 1·8 MPa in red maple, – 0·1 to – 1·25 MPa in tulip tree, and – 0·25 to – 1·45 MPa in fox grape. Leaf water potential of all three species recovered almost to pre-dawn values by late afternoon or early evening, indicating a good soil water supply.

Two-way analysis of variance indicated a significant effect of leaf water potential on petiole conductivity in red maple (F = 8·87 with 7 and 82 degrees of freedom; $P < 0·0005$; Fig. 3a) and tulip tree ($F = 13·51$ with 5 and 112 degrees of freedom; $P < 0·00005$; Fig. 3b). There were no differences in mean petiole specific conductivity between leaf water potential groups in fox grape ($F = 0·81$ with 5 and 65 degrees of freedom; $P = 0·45$; Fig. 3c). There was no significant interaction between leaf water potential and collection time (red maple F = 2·61 with 7 and 82 degrees of freedom, P = 0·056; tulip tree F = 0·43 with 5 and 112 degrees of freedom, $P = 0·65$; fox grape $F = 2·78$ with 5 and 65 degrees of freedom, P = 0·069). Thus, there was no evidence for hysteresis in the relation between mean petiole specific conductivity and leaf water potential: increases in petiole conductivity during the afternoon when leaf water potentials were increasing paralleled losses in conductivity during the morning when leaf water potentials were decreasing.

Two-way analysis of variance indicated significant effects of leaf water potential and experimental treatment (girdling, $HgCl_2$, surfactant) on petiole specific conductivity of red maple (Table 1). Contrast analysis suggests that the untreated group was significantly different from $HgCl_2$ ($P < 0·001$), girdling ($P < 0·001$) and surfactant ($P < 0·001$) treatments, but that it was not different from the water treatment ($P > 0·05$). Recovery from loss of specific conductivity was not observed following girdling or application of $HgCl_2$ (Fig. 4a). Surfactant application led to higher conductivity at high leaf water potential. In contrast to our initial expectation, decreasing the surface tension and thus the tensile strength of water in the transpiration stream did not result in a greater loss of specific conductivity.

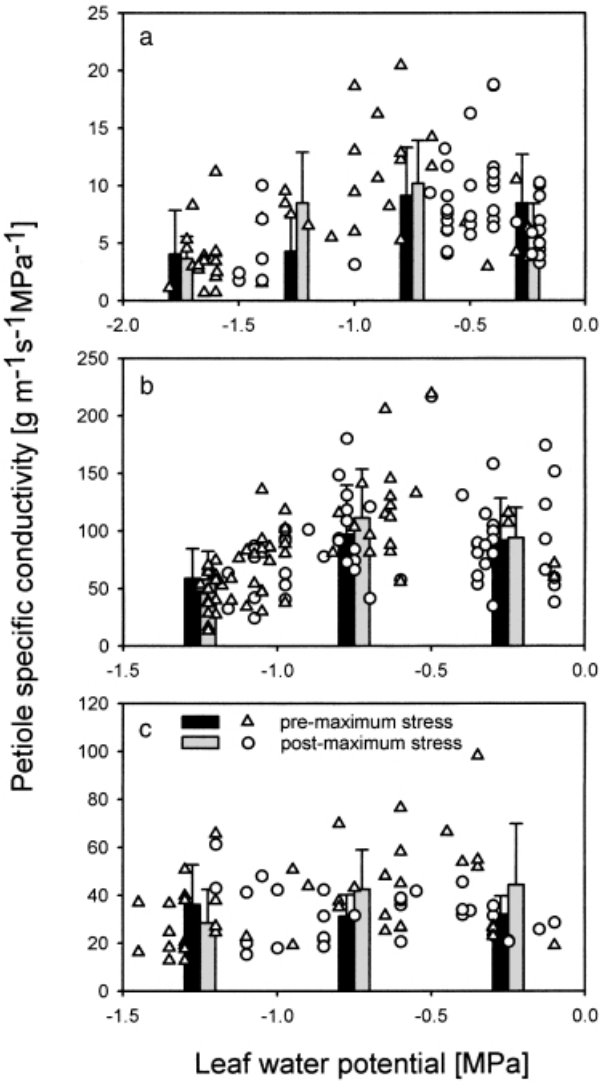

Figure 3. Relation between leaf water potential and petiole specific conductivity in red maple (a), tulip tree (b) and fox grape (c). Open symbols represent individual measurements (circles, measurements made before maximum stress; triangles, measurements collected during and after maximum stress). Solid bars represent average values for leaf water potential classes ($\Psi > -0·5$ MPa, $-1·0 < \Psi < -0·5$ MPa, $-1·5 < \Psi < -1·0$ MPa, $-2·0 < \Psi < -1·5$ MPa) used in two-way analysis of variance (details in text). Error bars indicate one standard deviation.

A significant effect of the experimental treatments (but not leaf water potential) was found in tulip tree (Table 1). Contrast analysis suggests that the untreated group differed significantly from the $HgCl_2$ ($P < 0·001$) and girdling ($P < 0·001$) treatments, but that it was not significantly different from the water and surfactant treatments ($P > 0·05$). Girdling and $HgCl_2$ eliminated the afternoon recovery of specific conductivity despite increases in leaf water potential (Fig. 4b).

Measurements of specific conductivity loss between morning and midday were supported by the results of the staining experiment. The number of stained vascular

Table 1. Results of two-way analysis of variance of petiole specific conductivity.

Source of variation	d.f.	Sums of squares	Mean squares	*F* statistic	*P* value
Red maple					
Experimental treatment	4	23·43	5·857	12·89	< 0·0001
Leaf water potential	3	13·94	4·647	10·22	< 0·0001
Interaction term	12	4·44	0·370	0·82	0·63
Within groups	171	77·34	0·454		
Total	190	119·15			
Tulip tree					
Experimental treatment	4	150·84	37·71	11·60	< 0·0001
Leaf water potential	2	14·74	7·37	2·26	0·106
Interaction term	8	66·24	8·28	2·54	0·012
Within groups	190	617·5	3·25		
Total	204	849·32			

Experimental treatments and leaf water potential classes are described in the text. A square root transformation was used to satisfy the condition of homogeneity of variance

bundles was significantly higher in petioles collected during early morning than in petioles collected during midday (red maple d.f. = 30, *t* value = 2·09, 1-sided *P* = 0·022; tulip tree d.f. = 29, *t* value = 2·48, 1-sided *P* = 0·009; fox grape d.f. = 30, *t* value = 2·49, 1-sided *P* = 0·009; Fig. 5). Variability in the percentage of stained vascular bundles was extremely high during both collection times. This high variability agrees with what was observed in the direct specific conductivity measurements.

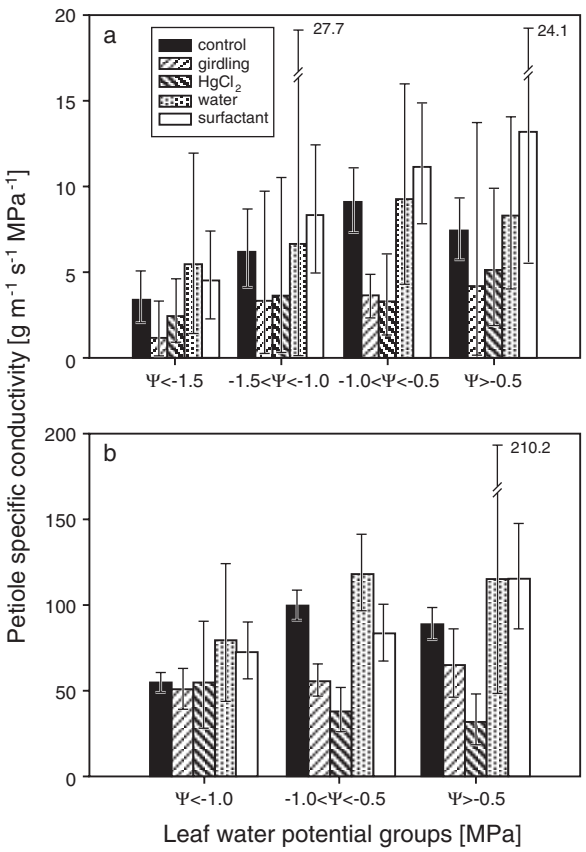

Figure 4. Mean specific conductivity of red maple (a) and tulip tree (b) petioles from branches subject to experimental treatments in relation to leaf water potential. Data were square root-transformed to satisfy the assumptions of analysis of variance, thus the back-transformed means are presented here. Vertical bars represent back-transformed 95% confidence limits.

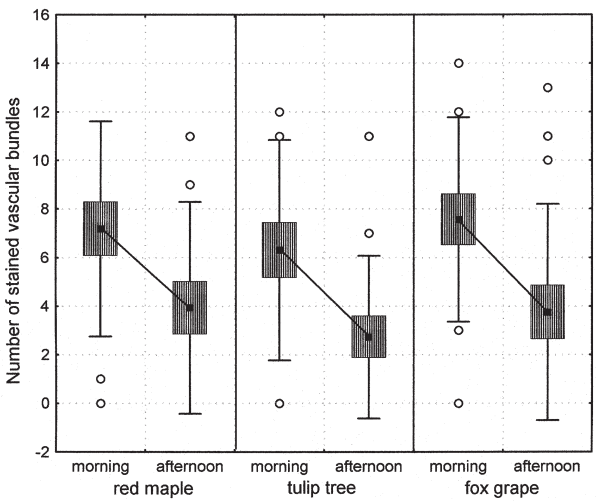

Figure 5. Mean number of stained petiole vascular bundles during the morning and afternoon. Boxes represent standard errors, 'whiskers' indicate one standard deviation, and circles denote outliers. The sample size was 16 leaves per species per collection time.

DISCUSSION

The results of this study provide evidence for short-term changes in petiole specific conductivity in red maple and tulip tree that are related to the current leaf water potential. Our findings are consistent with recent reports of short-term changes in hydraulic conductivity of several woody species (Salleo *et al*. 1996; Zwieniecki & Holbrook 1998; Tyree *et al*. 1999), as well as with diurnal changes in the number of water-filled conduits (Canny 1997; McCully *et al*.

1998; Melcher 1999). Because our *in situ* technique measures only the current conductance, we cannot be sure that the decreases in petiole specific conductivity resulted from cavitation. However, the staining experiment indicated that, on average, fewer vascular bundles were active in water transport at midday than at the start of the day, consistent with the formation of embolism.

Our study suggests a lack of hysteresis in the relationship between leaf water potential and petiole specific conductivity. Conductivity declined with decreasing leaf water potential and followed the same path (although with much variability) in reverse while leaf water potential was increasing from its midday minimum. If decreases in petiole conductivity result from the formation of air embolisms, then the subsequent recovery in conductivity may indicate refilling of xylem vessels under tension. The co-occurrence of embolism removal and xylem tension (Salleo *et al*. 1996; McCully *et al*. 1998; Zwieniecki & Holbrook 1998) may be difficult to understand given the physical requirement of positive pressure to force the gas phase back into solution (Tyree & Yang 1992; Lewis *et al*. 1994; Tyree 1997). Holbrook & Zwieniecki (1999) outline a mechanism for embolism removal in which the hydraulic compartmentalization required for local pressurization is permitted by a non-zero contact angle of water on the interior vessel surface and the formation of a convex meniscus within bordered pits.

Regardless of the actual mechanism by which embolism removal takes place, the requirement for positive pressures makes this an energy-consuming process. Living cells are the most likely transducers for this and thus are hypothesized to play an essential role (Holbrook & Zwieniecki 1999). Although longitudinal transport of water takes place within non-living components of the wood, the xylem is both surrounded by the vascular cambium and interlaced with a well-connected network of xylem parenchyma cells (Zimmermann & Tomlinson 1966; Core *et al*. 1976; Zimmermann 1983). The abundance of living cells within a tissue specialized for long-distance transport suggests that they may play a role in water transport. This is supported by the observation that the experimental treatments whose effects should be mediated solely by living cells (girdling and application of $HgCl_2$) resulted in a marked decrease in the restoration of petiole specific conductivity during the afternoon. By contrast, application of water or a surfactant, two compounds that should not interfere with the metabolic processes of living cells, did not impede the recovery of hydraulic conductivity. Nevertheless, exactly how girdling and $HgCl_2$ influenced the living cells cannot be determined from the measurements presented here and further studies are needed to explore the involvement of metabolic processes in short-term changes in hydraulic conductivity.

Basipetal girdling may halt the export of sugars and other compounds from mature, photosynthesizing leaves; however, it also represents a substantial injury to the stem. Wounding leads to the production of both electrical and hydraulic signals that, in turn, may prompt metabolic responses in apically located tissues (Wildon *et al*. 1992). Our observation that girdling resulted in a reduced ability to restore petiole specific conductivity could be a wound response. Salleo *et al*. (1996) attribute the effect of girdling on embolism repair to an altered delivery rate of a chemical signal transported in the phloem. While we cannot rule out such a mechanism, a more generalized wounding signal remains an equally likely possibility.

Short-term exposure to Hg^{2+} ions is known to reversibly block water channels (Chrispeels & Maurel 1994; Kammerloher *et al*. 1994; Steudle & Henzler 1995; Maurel 1997); prolonged exposure leads to more serious damage, including cell death. In our experiment, branches were allowed to take up $HgCl_2$ for several hours and thus we were unable to determine the extent to which the reduction in conductance restoration is due to a specific effect on water channels or a general inhibition of metabolic processes. We used a high concentration of $HgCl_2$, thus we expect that the effect on hydraulic conductivity restoration was due to cell death rather than deactivation of water channels. However, because immuno-detection assays indicate that water channels are abundant in the plasma membranes of xylem parenchyma cells (Barrieu *et al*. 1998), their contribution to the observed phenomena should be further examined.

This study presents evidence for diurnal changes in petiole conductivity that are correlated with leaf water potential, as well as experimental manipulations indicating that living cells are involved in the recovery in xylem conductance. The high variability in both petiole specific conductivity and the number of stained vascular bundles suggests that the underlying process depends on many factors. We believe that a dynamic balance between loss and repair of xylem hydraulic conductivity exists in plants and that we need to include such a possibility in discussions of long-distance water transport properties of plants. Incorporation of a dynamic perspective might lead to a change in our perception of the xylem as primarily a dead tissue, prone to permanent dysfunction, into an actively maintained and physiologically robust structure.

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