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HYDRAULIC RESISTANCE: A DETERMINANT OF SHORT TERM STOMATAL CONDUCTANCE SIGNALING IN DISPARATE XYLEM ANATOMY OF RED MAPLE (*Acer rubrum* L.) AND SHUMARD OAK (*Quercus shumardii* Buckl.)

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HYDRAULIC RESISTANCE: A DETERMINANT OF SHORT TERM
STOMATAL CONDUCTANCE SIGNALING IN DISPARATE
XYLEM ANATOMY OF RED MAPLE (*Acer rubrum* L.) AND
SHUMARD OAK (*Quercus shumardii* Buckl.)

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Plant and Environmental Science

by
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Accepted by:
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Dr. Christina Wells
Dr. Douglas Bielenberg

ABSTRACT

Recent work has shown that stomatal conductance (g_s) and net photosynthesis (A_{net}) are responsive to the hydraulic conductance of the soil to leaf pathway (X_p). However, no study has compared xylem anatomical differences under controlled conditions to directly evaluate the whole plant hydraulic and chemical response to manipulations of X_p . Two tree species with differing xylem structures were used to study the effect of systematic manipulations in X_p that elevated xylem hydraulic resistance. Simultaneous measures of g_s , A_{net} , bulk leaf abscisic acid concentration (ABA_L), leaf water potential (Ψ_L), and whole plant transpiration (E_w) were taken under controlled environment conditions. *Quercus shumardii* Buckl. (shumard oak), a ring porous species, and *Acer rubrum* L. 'Summer Red' (red maple), a diffuse porous species, were studied to investigate the short term hydraulic and chemical messenger response to water stress. Both species decreased A_{net} , g_s , Ψ_L , and E_w in response to an immediate substrate moisture alteration. However, ABA_L was not significantly different before or after drought stress exposure. Relative to initial well-watered values, red maple A_{net} , g_s , and E_w declined more than shumard oak. However, g_s and A_{net} versus whole plant leaf specific hydraulic resistance was greater in shumard oak. In addition, the larger hydraulic resistance in red maple was attributed to higher shoot system resistance. Stomatal conductance, A_{net} , and Ψ_L of both species had a strong correlation with substrate volumetric water content (V_w), where the Ψ_L of shumard oak declined to a lower level than red maple, and A_{net} of red maple decreased more than that of shumard oak. The results indicate hydraulic

resistance differences that may be attributed to the disparate xylem anatomy between the two species. This study also provides evidence to support the short term hydraulic signal negative feedback link hypothesis between g_s and the cavitation threshold, as opposed to chemical signaling via rapid accumulation from root or leaf synthesized ABA.

ACKNOWLEDGMENTS

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INTRODUCTION

Drought stress elicits stomatal closure, which limits CO₂ diffusion into the leaf. Consequently, photosynthetic rates decline and growth is inhibited. One possible drought induced signal for stomatal closure is the chemical hormone abscisic acid (ABA) (e.g., Zhang et al. 1987; Davies and Zhang 1991). Abscisic acid is thought to be synthesized in the roots and translocated via the xylem stream to the leaves, where it causes the closure of stomates (Zhang et al. 1987; Davies and Zhang 1991; Khalil and Grace 1993; Johnson et al. 2001). Another potential source of ABA comes from biosynthesis in the leaves (Cutler and Krochko, 1999). ABA that is synthesized in the leaves may be loaded into the phloem and transported to the roots where it is either deposited in root tissues or loaded into the xylem and recirculated to the leaves (Hartung et al., 2002). ABA that travels through the xylem to the leaves does not necessarily accumulate in the leaf tissue. The ABA may be rapidly catabolized after acting on the guard cells, redistributed among leaf tissue, or re-exported (Hartung et al., 2002; Jia and Zhang, 1999). However, ABA synthesis in the leaves only increases as leaf turgor approaches zero, which suggests that ABA import via the xylem is necessary to regulate g_s under mild drought stress conditions (Hartung et al., 2002).

In addition to chemical signaling, control of stomatal conductance (g_s) may occur through hydraulic signals, such as leaf water potential (Ψ_l) and xylem physical properties (e.g., Hubbard et al. 2001, Nardini et al. 2003). Cavitation, which results

from xylem embolisms, is an example of a physical constraint that may act as a rapid hydraulic g_s signal (Tyree and Ewers 1991; Cochard et al. 1996). Alternatively, stomatal control may involve an integration of both hydraulic and chemical messages (Comstock 2002). Together or apart, hormonal and/or hydraulic signals may directly relate to the sensing of drought and variation in drought tolerance among woody species.

Woody species possess different xylem anatomies (e.g., ring versus diffuse porous) with innate anatomical properties that cause variation in hydraulic conductance and resistance. The disparate hydraulic attributes are a mechanism to tolerate drought, where drought tolerant plants are capable of preventing cavitation under greater xylem tensions (Tyree and Sperry 1989). Moreover, it is widely known that cavitation and resulting embolisms not only reduce the hydraulic conductivity but further increase the xylem pathway resistance. An increase in hydraulic resistance leads to elevated xylem tension and pressure gradients that can predispose the plant to a cascade of cavitation events. In fact, hydraulic resistance of the xylem pathway can increase by a factor of two or more (Tyree and Sperry 1989; Tyree and Ewers 1991). To avoid cavitation, hydraulic signaling may induce stomatal closure to prevent embolisms and further cavitation (Tyree and Sperry 1988). Stomatal closure, whether hydraulically or chemically initiated, is a necessary mechanism to control water loss during large environmental fluctuations. However, in the event of sudden (within minutes) moisture deficits (e.g., rapid onset of elevated atmospheric vapor pressure deficit), it is unlikely that the transport of rootborne chemical signals could arrive in time to circumvent cavitation. The delinquent signaling, brought about by factors such as distance to the site of action and rate of transport, could cause

threshold cavitation (Ψ_c) and catastrophic xylem stream failure if plants did not have a rapid means to control g_s in response to severe soil dryness and/or sudden evaporative demand, especially when the vegetation's water supply is at an extreme distance e.g., tall trees (Cochard et al. 1996; Kikuta et al. 1997).

Regardless of the signal speed propagation, it is pit membrane pore size and not vessel diameter that determines cavitation susceptibility between different species (Tyree and Dixon 1986; Sperry and Sullivan 1992; Sperry et al. 1994). It is also known that the xylem hydraulic resistance is positively related to the flow through the plant, analogous to Ohm's Law, where two different xylem structures (ring versus diffuse porous) have different maximum sap flow velocities. Ring porous species range from 4 to 40 m h^{-1} , whereas diffuse porous species range from only 1 to 6 m h^{-1} (Zimmermann and Brown 1971). Although this variation in vulnerability to drought-induced cavitation has been well documented within and among species (Cochard and Tyree 1990; Cochard 1992; Sparks and Black, 1999; Sperry et al. 1994; Jarbeau et al. 1995), few studies have compared disparate xylem structure (Sperry and Sullivan, 1992; Sperry et al. 1994) and we are unaware of any that simultaneously account for rootborne hydraulic and chemical signaling.

Above Ψ_c values, g_s is not thought to be in jeopardy (Sperry and Pockman 1993; Sperry et al. 1993). The unaddressed question, however, is whether whole plant hydraulic resistance is an anatomical feature to signal stomatal closure via hydraulic signals. Alternatively, do variations in ABA_L within a species (Bauerle et al. 2003a; 2004; 2006a), pit membrane pore size, or flow rates among species (especially with respect to ring versus diffuse porous xylem structures) account for g_s control? Regardless of the mechanism(s) that control stomatal response, a plant must

maintain water movement from the soil to the leaf, and rapid stomatal responses to environmental change are a feature of this maintenance (Raven 2002). Information that addresses the above uncertainty has potential basic and applied implications because xylem and stomatal function are important components of water stress tolerance within and among species.

In this study, we used *Quercus shumardii* Buckl. (shumard oak) and *Acer rubrum* L. 'Summer Red' (red maple) to focus on the hydraulic and chemical response to rapid elevations in xylem resistance in two species with contrasting xylem anatomy. The stomatal signaling arguments in the literature and the difference in sap flow rates that could result in variation in hydraulic and chemical signaling strategies provided the impetus to investigate the response in two different xylem structures. The objective of this study was to investigate the hypothesis that cavitation induced xylem resistance functions as a short term hydraulic mechanism to decrease g_s in the absence of root-derived chemical signals.

MATERIALS AND METHODS

Plant material. Shumard oak and red maple saplings of similar age and size were grown in a common garden in 56.8-L Spin Out (Nursery Supplies Inc., United States) treated plastic pots containing a mixture of 20 pine bark: 1 sand (v:v). Plants were fertilized with 8.3 kg m⁻³ of Nutricote™ 20N-3.0P-8.3K type 360 (Chiso-Asahi Inc., Japan). Pots were spaced 1.22 m center-to-center on an outdoor gravel pad. Plant material was uniformly irrigated three times daily with pressure compensating drip emitters (ML Irrigation Inc., Laurens, SC) to avoid water stress. At the time of controlled experiment initiation, plants were randomly selected and assigned to a well-watered control or drought treatment for each of three repeated experiments.

Growth chamber conditions. Air temperature and relative humidity (RH) were measured with a Hobo Pro RH/Temperature Logger (Onset Computer Corporation, Bourne, Mass.). Air temperature was maintained at 25° C and RH was maintained at 50-55% for the length of the experiments. Four metal halide high intensity discharge lamps (Hydrofarm Inc., Petaluma, Calif.) equipped with 1000 W Agrosun® halide bulbs (Hydrofarm Inc., Petaluma, Calif.) provided a maximum photosynthetic photon flux (PPF) of 350 μmol m⁻² s⁻¹ for a photoperiod of 14 h each day. The lamps were set to come on at 0600 HR and shut off at 2000 HR.

Experimental treatments. Two experimental conditions were used to investigate the possible influence of hydraulic signals on stomatal response: 1) whole plants with roots growing under substrate moisture deficit, and 2) whole plants with roots growing in well-watered substrate. In both conditions, leaf abscisic acid

concentrations (root or shoot generated) were monitored. To investigate differences in rootborne chemical and/or hydraulic drought responses, plants were removed from the growth chamber under dark conditions, carefully removed from their pots with the root system intact, washed free of substrate, and directly transplanted into one of two disparate substrate moisture levels.

A preliminary experiment established the substrate moisture levels, where the drought treatment substrate volumetric water content (V_w) of 14.7% = -1.5 MPa and the well-watered control V_w of 40% = -0.1 MPa. In order to relate substrate moisture to plant water status, we paired a sub-sample of full sun solar noon leaf water potential (Ψ_L) measures (Plant Moisture Status Console, Soil Moisture Equipment Corp., Santa Barbara, Calif.) to bulk moisture content of the substrate (Theta Probe type ML2, Delta-T Devices, Cambridge, England).

For water potential readings, leaves were removed with a sharp razor blade and times between excision and initial pressurization rarely exceeded 20 s. To account for resistances in the pathway from the bulk soil to the point of leaf harvest, we followed the protocol of Bauerle et al. (1999) and Fulton et al. (2001) for equilibration of Ψ_L under both predawn and solar noon field conditions.

For each sample date and tree, two sets of leaves were selected for water potential measurements. Two individuals were covered with aluminum foil before photoperiod termination (“covered”) and two were not (“uncovered”). Before dawn, leaves of both uncovered and covered sets were removed and their water potential was measured. At solar noon, the second sample set was measured in a similar fashion. A comparison of water potential in two nearby leaves, one covered and the other uncovered, is a possible way to examine the role of xylem hydraulic capacity on

friction potential (after Richter 1973; Bauerle et al. 1999). The measurement of water potential in the covered leaf provides an estimate of the local water potential of the xylem stream to which the second, exposed leaf is attached. The relationship between Ψ_L under full light solar noon conditions and substrate volumetric water content was linear (Bauerle et al. 2006b). Thus, the method provided us with a non destructive rapid means to assess plant water status and repeatedly and promptly predict plant water stress without sacrificing excessive plant tissue (Fulton et al. 2001, Shackel et al. 2001, Bauerle et al. 2006b).

A Ψ_L value of -1.5 MPa was assumed sufficient to cause xylem cavitations in both species due to the fact that a value of -1 to -1.4 MPa caused cavitation in *Laurus nobilis* L. (Salleo et al. 2000) and -1.02 to -1.5 MPa caused cavitation in three populations of *Populus trichocarpa* (Sparks and Black 1999). To minimize potential artifacts of elevated hydraulic resistance caused by transplantation, transplanting took place under dark conditions at 2100 HR when transpiration was not detectable via the sap gauges (approximately 1 h after the photoperiod had ended).

Substrate moisture measurements. After transplant, substrate moisture measurements followed methods used in Bauerle et al. (2003b). Briefly, V_w of each container was measured in four locations with a Theta Probe at 11 and 23 cm below the substrate surface. The readings were taken in pre-drilled locations on opposite sides of the pot between 1000 and 1100 HR. Drilled holes were large enough to allow the probe adequate movement and contact with the substrate in the container. The four readings per container were then averaged and converted to Ψ . Additional Ψ_L measurements were periodically made with a pressure chamber under well-watered and water stress conditions and used as linear cross reference verification

against substrate volumetric water content (the linear relationship was persisted throughout the experiment).

Gas exchange measurements. Gas exchange was measured randomly from on two of the youngest fully expanded mature leaves. Measurements were taken daily for the length of the experiment. Net photosynthesis and g_s were measured with a portable steady state gas exchange system (CIRAS-I, PP Systems, Haverhill, Mass.) fitted with a temperature and light controlled cuvette (model PLC5 (B); PP Systems, Haverhill, Mass.). Leaf temperature was maintained at 25° C and PPF at saturation ($1200 \mu\text{mol m}^{-2} \text{s}^{-1}$). Measurements were taken only after the plants had received between five and six hours of continuous day light each day. To confirm stomatal responsiveness to ABA in both species, a subsample of leaves were cut under distilled water and allowed to rehydrate for 24 hr. Leaf petioles were placed in test tubes with either pure distilled water (control) or pH-buffered ABA solutions ($5 \mu\text{mol/L}$ ABA). Stomatal conductance was monitored with a LI-1600 porometer (LiCor Inc., Lincoln, Nebr.). Leaf g_s was continually monitored for 1 hr after ABA treatment.

Sap flow measurements. Commercially available sap flow gauges (Dynamax, Inc., Houston, Texas) were used for sap flow measurements. The gauges have been described in detail elsewhere (Steinberg et al. 1990). We followed the protocol of Bauerle et al. (2002). Sap flow gauges were installed on the main stem of shumard oak and red maple. Stem diameter at the point of gauge attachment ranged from 8.6 to 18.3 mm. For the first and second replication of the experiment, four gauges were on well-watered control plants and ten were on plants subjected to a drought

treatment. For the third experiment replication, four gauges were on well-watered control plants and four were on plants subjected to drought.

Sap flow steady-state. To accomplish consistent steady state flow among individual trees and species, sap flows of each plant were brought to within 15 g h^{-1} of each other under growth chamber conditions. Lower canopy leaves were removed until sap flow was uniform across replicates and species. Regardless of inherent xylem anatomy flow rate differences, we established a uniform water loss rate among species to reduce the potential differences in the rate of root sourced ABA transport to leaf tissue. After arrival at steady-state, plants were acclimated and monitored via sap flow and gas exchange for no less than 48 h before experiment initiation. In no case were leaf level gas exchange or whole plant transpiration influenced by transplanting (see additional precautionary measures below).

ABA sampling Leaf disks were collected six times during each experiment immediately following gas exchange measurements. Using a 1 cm inside diameter cork borer, leaf disks were collected from five of the first fully expanded undamaged mature leaves measured from the terminal tip of the main leader. Each day, one 1-cm-diameter disk was removed from each of the five leaves. The protocols were simple modifications of Alves and Setter (2000) as modified by Bauerle et al. (2003a).

ABA assay. ABA was assayed by enzyme linked immunosorbent assay (ELISA) as described by Alves and Setter (2000) and modified by Bauerle et al. (2003a). Each well of a 96-well microtiter plate (Corning/Costar High Binding #3366) was coated with $5 \mu\text{L}$ of ABA-bovine serum albumin conjugate. After incubation, for 24 h at 5°C , the plate was decanted and washed four times with TBST (Tris Buffered Saline with 0.02% v/v Tween-20) with 5-minute incubations

per wash. One hundred μL of TBSA (Tris buffered Saline + Bovine Serum Albumin) and 10 μL of eluted sample were added to each well. Then 100 μL of anti-ABA monoclonal antibody (clone 15-I-C₅, Mertens et al. [1983], currently available from Agdia Inc., Elkhart, IN) was added to each well. The plate was incubated for 24 h at 5° C. After incubation, the plate was again decanted and washed with TBST a total of four times. One hundred and eighty μL of diluted secondary antibody (anti-mouse-alkaline phosphatase conjugate, Sigma product A-3562, in TBST with 0.1% [w/v] BSA) was added to each well. The plate was incubated at 5° C for 24 h.

Once the final incubation was complete, the plate was decanted and again washed four times. Colorimetric reagent, containing para-nitrophenylphosphate, PNPP (Sigma # N3129) in diethanol-amine buffer was added and the plate was left to develop for 30 min at room temperature. After 30 min, the plate was read with a plate reader at a wavelength of 405 nm (model BS10000, Packard BioScience, Meriden, CT). (+) ABA content was determined by calculations based on (+) ABA calibration standards. A spreadsheet macro written in Excel (Microsoft, Seattle, WA) provided a logit-transformed plot of the standard curve, calculated regressions, and predicted pmol ABA per well. Samples were replicated three times in the assay and averaged.

Basic resistance equations. An estimate of the hydraulic resistance of the plant (R_p), root system (R_r), and shoot (R_s) were derived according to the simple Ohm's Law hydraulic analogue, originally proposed by van den Honert (1948). The whole plant transpiration data were used to calculate the hydraulic resistance based on the evaporative flux method:

$$R_p = (\Psi_{pd} - \Psi_m)/E_m \quad (1)$$

$$R_r = (\Psi_{pd} - \Psi_{nf})/E_m \quad (2)$$

$$R_s = (\Psi_{nf} - \Psi_m)/E_m \quad (3)$$

where Ψ_{pd} is pre-dawn plant Ψ , Ψ_m is midday Ψ , Ψ_{nf} is Ψ in the absence of local friction, and E_m is the midday transpiration rate. Due to the fact that van den Honert's unbranched catena model does not account for non-steady state flow conditions i.e. an inelastic hydraulic system, we incorporated resistances in the branched pathway (e.g. Bauerle et al. 1999). Richter (1973) proposed an equation to incorporate the resistances in the branched pathway

$$\Psi_p = \Psi_s + \rho gh + \sum J_i \bullet r_i \quad (4)$$

$$I = s$$

where total water potential at a given point p in the plant is the sum of the three potentials: soil water (Ψ_s), gravitational ($\rho =$ density due to gravity, $g =$ acceleration due to height, $h =$ height gradient) and frictional ($\sum J_i \bullet r_i$). The frictional potential is equal to the sum of the products of partial fluxes, J_i , and partial resistances, r_i , along the branched pathway from the bulk soil, s, to the point p in the plant.

Experiment replication, design, and analysis. The experiment was replicated three times. The first and second experimental replication consisted of seven red maple and seven shumard oak replicates. Five replicates of red maple and shumard oak were subjected to drought and two were well-watered controls. The third experiment was identical to the first two except that it consisted of four red maples and four shumard oaks. In the third experimental replication, two of each species were well-

watered controls and the remaining plants were subjected to drought. Prior to all three replications, plants were placed into the growth chamber and allowed to acclimate to growth chamber conditions.

Plants for each experiment were arranged in a completely randomized unbalanced design. Differences between the measurements of each experiment were tested with the GLM (SAS Institute Inc., Cary, NC) procedure. No differences between experiments were found, so data from the experiments were pooled for further analysis. Treatment least squares means and differences in least squares means among species were determined by least squares analysis using the MIXED procedure. The GLM procedure was used for regression analysis. Differences in means and slopes were declared significant at $p \leq 0.05$.

RESULTS

Leaf water potential and substrate moisture level. There were no significant differences in Ψ_L between species before imposing drought (Table 1). Both species showed significantly lower ($P \leq 0.05$) Ψ_L after transplant into dry substrate. Moreover, Ψ_L for shumard oak was significantly lower than that of red maple after five continuous days of substrate drought conditions. Although V_w was significantly lower ($P \leq 0.05$) in the drought treatment as compared to initial well-watered conditions, there were no significant differences in V_w between species either within the well watered control or drought treatment either before or after transplant.

Leaf ABA concentration. Leaf ABA levels were not significantly different within or between red maple and shumard oak either before or during drought. Furthermore, no significant differences in ABA_L concentrations were found between well-watered controls and water stressed red maple and shumard oak (mean $ABA_L = 11.54 \pm 7.41$ pmol cm²).

Sap flow. After adjustment to steady-state, E_w rates were not statistically different among species prior to drought (Fig. 1A). Upon transfer into dry substrate, mean daily E_w decreased significantly ($P \leq 0.05$) from that of well watered controls. Although diffuse porous species had significantly higher E_w rates than ring porous controls on days four, five, and six, no differences between E_w rates of water stressed plants were observed during the experiment.

Table 1. Leaf water potential (Ψ_L) (MPa) and volumetric substrate water content (V_w) ($\text{m}^3 \bullet \text{m}^{-3}$) of stressed red maple and shumard oak. Results are given as the mean \pm SE of 12 red maple and shumard oak replicates. Both Ψ_L and V_w were measured prior to and five days after imposing drought.

Species	Ψ_L		V_w	
	Before Stress	After Stress	Before Stress	After Stress
Red Maple	-0.48 ± 0.21^a	-3.01 ± 0.21^b	0.50 ± 0.025^a	0.085 ± 0.025^b
Shumard Oak	-0.54 ± 0.21^a	-3.87 ± 0.21^c	0.51 ± 0.025^a	0.088 ± 0.025^b

Least squares means were used to determine significant differences between treatments. Significant differences between species at $P \leq 0.05$ are designated by different letters.

Gas exchange. Both species showed decreased g_s and A_{net} in response to drought (Fig. 1B, 1C). Leaf level gas exchange corroborated E_w , where diffuse porous controls had significantly higher g_s ($P \leq 0.05$) compared to ring porous oak (Fig. 1C). Water-stressed red maple replicates showed significantly higher g_s and A_{net} than that of shumard oak on days one and two ($P \leq 0.05$), after which no further differences in g_s and A_{net} were observed between water-stressed red maple and shumard oak. All water-stressed groups showed significantly lower g_s and A_{net} ($P \leq 0.05$) than well-watered controls after only one day of drought exposure.

Whole plant transpiration was correlated with g_s for all species with an $r^2 = 0.76$ and 0.85 for shumard oak and red maple respectively (Fig. 2A). The slopes between species showed significant differences ($P \leq 0.05$), with red maple having the greater slope. Net photosynthesis also showed a strong relationship with g_s for both species (Fig. 2B). The slopes for red maple and shumard oak were significantly different ($P \leq 0.05$). Of the two species, A_{net} of shumard oak declined faster than red maple with respect to g_s .

Substrate water content. The correlation between A_{net} and V_w was significant and resulted in differences ($P \leq 0.05$) between slopes of red maple and shumard oak (Fig. 3A). The correlation between g_s and V_w showed a decrease in g_s as V_w decreased (Fig. 3B). The slope for the relationship of g_s and V_w for shumard oak was significantly less ($P \leq 0.05$) than red maple. Both species showed a strong correlation between Ψ_L and V_w (Fig. 3C). The slope for shumard oak was significantly greater ($P \leq 0.05$) than the slope for red maple.

Hydraulic resistance. The calculated hydraulic resistance of the R_p , R_r , and R_s is illustrated in Figure 4. The non-linear difference between maple and oak is explicitly fit with a power curve to positive value data sets. Below transpiration rates of approximately 20 g h^{-1} , hydraulic resistance in the shoot and whole plant were visually different between oak and maple (Fig. 4A and B). In contrast, hydraulic resistance of the roots were similar among species and xylem anatomies within the calculated range of resistances (Fig. 4C).

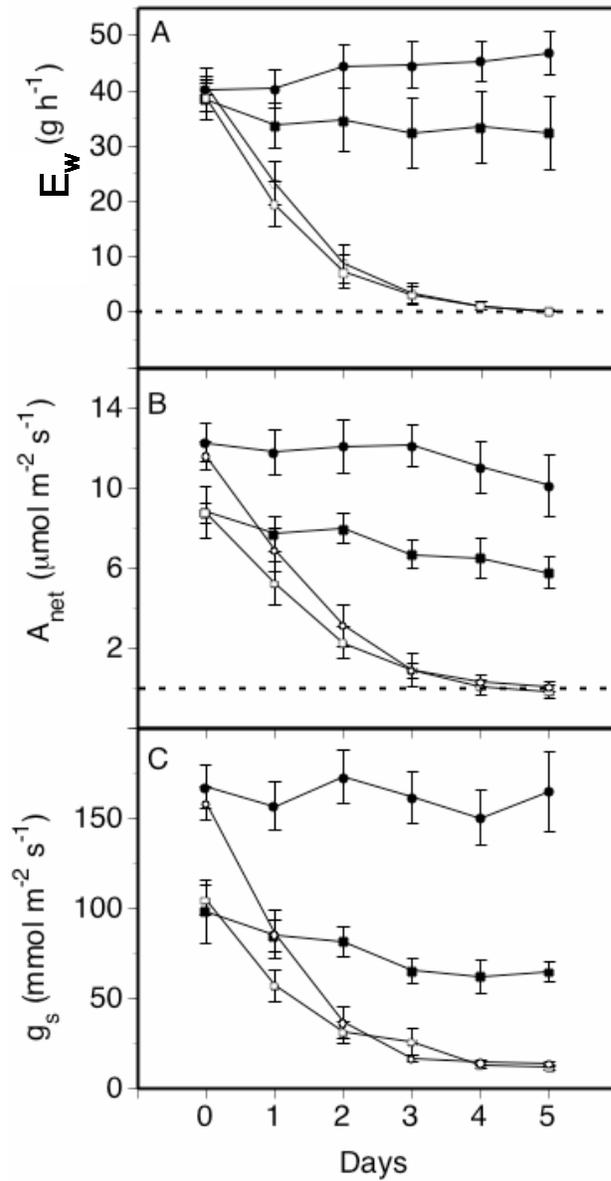


Fig. 1. (A) Transpiration (E_w), (B) net photosynthesis (A_{net}), and (C) stomatal conductance (g_s) during the time course for red maple (●) and shumard oak (■) well watered controls and the red maple (○) and shumard oak (□) drought treatment.

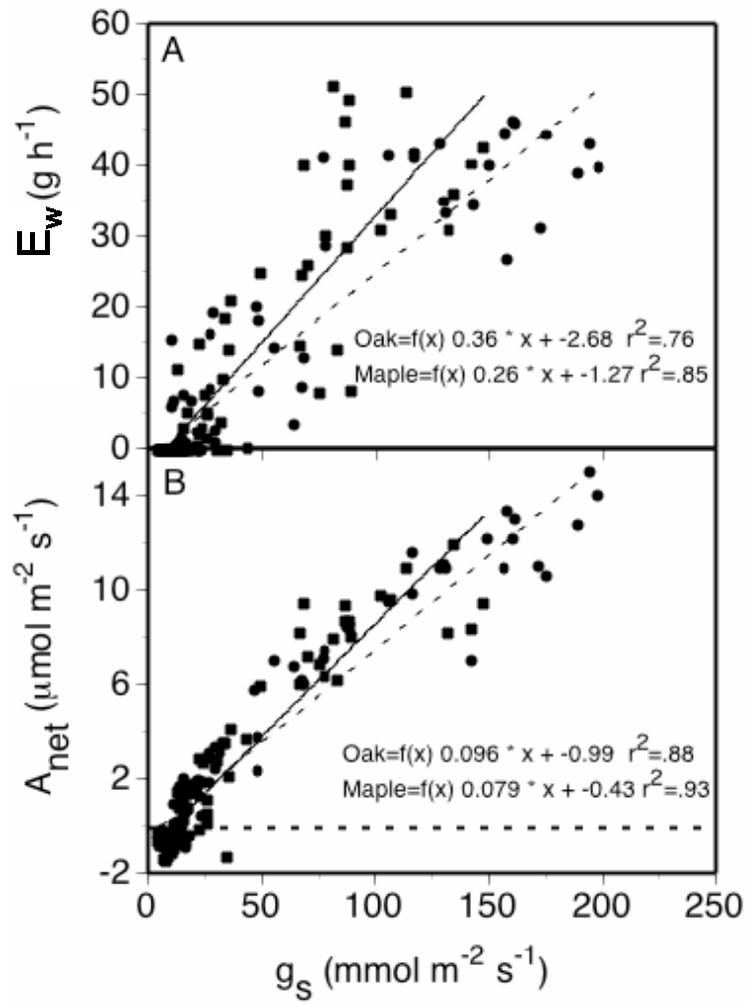


Fig. 2. The relationship of (A) Transpiration (E_w) and (B) net photosynthesis (A_{net}) with stomatal conductance (g_s) for red maple (—●) and shumard oak (—■) exposed to drought stress conditions.

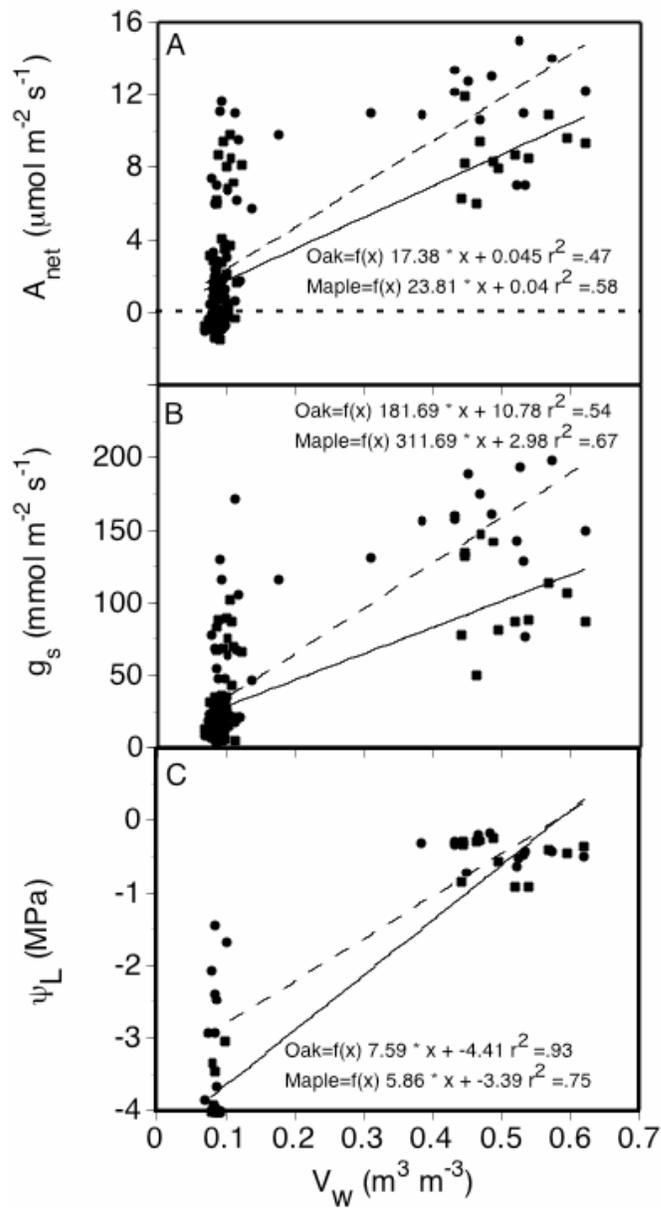


Fig. 3. The relationship of (A) net photosynthesis (A_{net}), (B) stomatal conductance (g_s), and (C) leaf water potential (Ψ_L) with volumetric substrate water content (V_w) for red maple (--- ●) and shumard oak (___ ■) exposed to drought stress conditions.

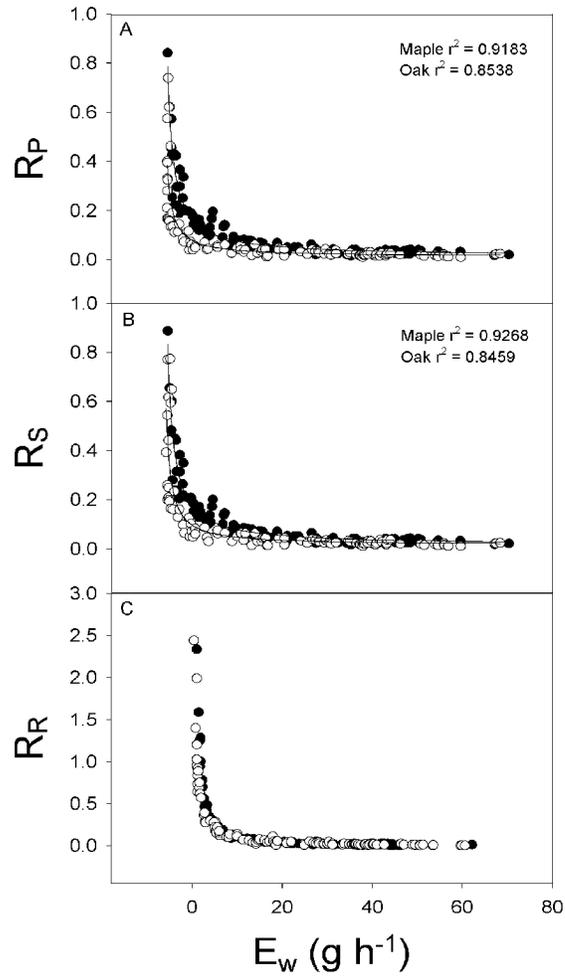


Fig. 4. The relationship of (A) plant (R_p), (B) shoot (R_s), and (C) root (R_r) hydraulic resistance to whole plant transpiration (E_w) for red maple (●) and shumard oak (○). Values are calculated estimates Based on equations 1-4 in the text.

DISCUSSION

Results indicate a sudden exposure to severe drought leads to significant changes in g_s , A_{net} , Ψ_L , R_p , R_s , R_r and sap flow. However, we observed no change in ABA_L concentration under the conditions we imposed. In addition, the drought response of ring-porous (shumard oak) and diffuse-porous (red maple) tree species differed with respect to g_s , A_{net} , R_p , R_s and Ψ_L without a change in ABA_L concentration, and these differences may be due to the variation in xylem anatomy between the species.

Under well watered conditions, plants transpire at a rate that is close to the point at which xylem cavitation and runaway embolism would occur if the plants were under drought stress conditions (Tyree and Sperry 1988). The trees used in this study were taken from a well-watered substrate and placed into a dry substrate within minutes. Even though the transplanting took place at night after the plants were dark acclimated for a minimum of one hour and transpiration was not detectable by the sap flow system, the sudden reduction in substrate moisture may have caused catastrophic cavitation and embolism when stomates opened and transpiration occurred at the next photoperiod. As we observed, the xylem flow path would have experienced drastic increases in resistance as the xylem conduit becomes less conductive. In fact, within 48 h, there was a 4-fold decrease in sap flow rates of both species, which possibly prevented any root-sourced ABA from reaching the leaves. This might explain why exposure to a rapid short term substrate moisture deficit caused significant decline in g_s , A_{net} , and Ψ_L , but did not cause ABA_L to elevate. It

has been argued that the absence of stomatal control by shoot water potential would prove dangerous to plants and that root-sourced signals such as ABA could not respond to cavitation events in distant shoots (Comstock 2002). The sudden and rapid water stress most likely resulted in cavitation and, therefore, caused a substantial drop in Ψ_L , leading to guard cell turgor loss and stomatal closure. In the absence of root-sourced ABA signals to regulate g_s , increased guard cell sensitivity to prevailing ABA concentration via pH adjustment, or localized chemical signals such as release of apoplastic ABA would possibly be a function of hydraulic signals such as lower Ψ_L and reduced hydraulic conductance. Although we did not have the capacity to measure pH changes, no evidence from the whole potted plant experiments indicated that ABA_L was elevated in response to the probable xylem cavitation.

Although previous studies have shown that ABA is an important component in the regulation of stomatal closure in response to water deficit, and it is well accepted that ABA is produced in the roots and translocated to the shoots, where it accumulates in the guard cell apoplast and causes stomatal closure (Zhang et al. 1987; Davies and Zhang 1991; Khalil and Grace 1993; Johnson et al. 2001), the results of our study show that transpiration decreased as hydraulic resistance increased in the absence of any increase in ABA_L , either root or shoot generated, and support the hypothesis that the g_s response to short term water stress is primarily a hydraulically derived response.

Under drought stress conditions, it could be argued that ABA located in the mesophyll and epidermis may be released to the apoplast and serve as a source of ABA that acts on the guard cells and induces stomatal closure (Hartung et. al., 1988;

Daeter and Hartung, 1995; Popova et. al., 2000). Moreover, changes in xylem sap pH in response to water deficits can increase guard cell sensitivity to free ABA (Wilkinson and Davies, 1997; Thompson et. al. 1997). Though our bulk leaf ABA levels did not increase within the pmol range, it is still possible that the g_s response to low V_w may have resulted from the release of previously sequestered leaf ABA acting on the guard cells and/or guard cell sensitivity to ABA may have increased in conjunction with xylem sap pH changes. Alternatively, ABA that travels from the roots to the shoots in the xylem stream does not necessarily accumulate in leaf tissue and may be rapidly degraded in the leaf after it has acted on the guard cells to reduce g_s (Hartung et. al., 2002; Sauter et. al., 2001). It may therefore be that the rate of catabolism exceeded biosynthesis, which could also further explain the consistently low concentration of ABA_L in the drought stressed plants. In addition, free ABA released due to a pH shift is often below assay detectable levels. Thus, the inability for us to detect a change in ABA_L was possibly an artifact of the rapid drought onset that lowered the rate of ABA arrival to the leaf. The observation only indicates that root sourced ABA may have not been responsible for the decline in g_s , whereas release of previously derived ABA or the response of stomata with elevated ABA sensitivity cannot be ruled out. The possible alternative explanations indicate that care should be taken when trying to deduce hydraulic from chemical responses.

Variation in A_{net} between species was evident during the first 48 h of drought stress and these differences may be due to the species contrasting xylem anatomy. Although the sap flow rates for both species remained the same during the first two days of drought exposure, estimated resistance was higher for red maple. The higher resistance reflects the smaller vessel diameters of the xylem of the red maple versus

the oak. Our results are similar to those found by Tognetti et al. (1998) who reports a higher resistance for *Quercus pubescens* (a ring porous species) than that reported for the diffuse porous species *Fagus sylvatica* by Magnani and Borghetti (1995). This observation may indicate a xylem anatomical advantage of diffuse over ring porous anatomy, the result of which would allow maintenance of photosynthesis and carbon assimilation during a short-term drought stress. In addition, the observation may in fact be a further extension of Zimmermann's (1983) hypothesis that wider conduits are more vulnerable to cavitation than narrower ones. Even though the hypothesis has since been confirmed (e.g. Lo Gullo et al. 1995), the consequence of xylem anatomy variation on carbon assimilation and sequestration in the face of short term drought stress still warrants further investigation.

The observation that A_{net} and sap flow were strongly correlated with g_s for red maple and shumard oak supports the hypothesis that stomatal factors are more important in limiting photosynthesis under drought stress than are non-stomatal factors (Blum 1996). To our knowledge, this is the first report to tease apart leaf ABA from short term hydraulic signals in tree species with contrasting xylem anatomies. However, there were differences in the photosynthesis response to drought in the two species. The results demonstrate differences in the effect drought stress had on both A_{net} and sap flow rates of tree species with different xylem structures. However, the relationship of A_{net} and g_s with V_w shows that both A_{net} and g_s decline more in red maple than shumard oak. Although one possible explanation could be the increase in hydraulic resistance brought about smaller vessel diameters of the xylem of the red maple, red maple A_{net} is still maintained at a higher rate with a given level of g_s as compared to shumard oak. Furthermore, red maple, a diffuse

porous species, maintains higher A_{net} and sap flow during stomatal closure for the first few days of drought than shumard oak. Coupled with the ability of red maple to maintain higher A_{net} and g_s values at the onset of drought and with potentially higher resistance in the xylem conduit under drought stress, physiological differences are apparent between ring porous and diffuse porous water stress response.

CONCLUSIONS

In conclusion, this set of experiments demonstrates that variation exists between tree species with different xylem structures in relation to their physiological response to a severe short term drought. The physiology of these species may differ in more characteristics than xylem structure alone. However, we propose that the differences in drought stress response may in part be due to their different xylem anatomies. Results show that red maple, a diffuse porous species, had higher rates of g_s and A_{net} during the first few days of drought, which may indicate the ability to maintain carbon assimilation during periodic short term drought episodes – a frequent incidence in mesic habitats. Red maple also maintained higher sap flow rates in relation to g_s for the first few days of drought. We observed no significant differences in ABA_L levels indicating that hydraulic resistance factors may have induced stomatal closure. Alternatively, increased guard cell sensitivity to ABA and the release of free ABA due to a pH shift in the xylem sap may have caused stomatal closure. Further research is warranted to explain potential drought tolerance mechanisms that exploit xylem anatomical differences and explain the variation in stress response observed in this study.

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