Correlations of stomatal conductance with hydraulic, chemical and environmental variables in five urban tree species

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Abstract

Recent findings suggest that stomatal conductance (g_s) may be as closely linked to plant chemical variables as to hydraulic variables. To test this in an urban field setting, we examined seasonal g_s in relation to a number of plant and environmental variables in five temperate, deciduous tree species. Stomatal conductance was generally more closely correlated with abscisic acid concentrations in xylem sap than with shoot water potential, shoot osmotic potential, pH of xylem sap or environmental variables. Seasonal g_s was mostly poorly correlated with shoot water potential and osmotic potential. Among environmental variables, PPFD accounted for most variability in g_s. We tested a model, developed previously in maize, that describes regulation of g_s by abscisic acid concentration of xylem sap with leaf water status acting to modify stomatal sensitivity to the abscisic acid signal. This model explained somewhat more variation in g_s than abscisic acid concentrations alone. Response surface models, especially those incorporating environmental variables, were most successful at explaining g_s. Our findings with urban trees are consistent with the theory of regulation of g_s by root-sourced abscisic acid. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: *Acer rubrum*; *Cornus florida*; *Liriodendron tulipifera*; *Quercus acutissima*; *Quercus palustris*; Abscisic acid; Water potential; Osmotic potential; Xylem sap pH

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1. Introduction

Stomata regulate plant carbon gain, water loss and other physiological determinants of tree health and survival. Our ability to assess impacts of environmental changes on ornamental trees relies, therefore, on understanding stomatal function and control. Recent findings suggest that stomatal conductance (g_s) may be as closely linked to plant chemical variables as to hydraulic variables (Zhang et al., 1987; Davies et al., 1994; Tardieu et al., 1996; Wilkinson and Davies, 1997). Close correlations between g_s and xylem sap [ABA] have been reported under many conditions for several species, including trees (e.g. Liang et al., 1996; Loewenstein and Pallardy, 1998a,b).

The theory of whole-plant stomatal regulation by root-sourced, nonhydraulic factors has been advanced mostly by experiments with potted plants under controlled conditions. In this work we compared the strength of the correlations of g_s with both hydraulic and nonhydraulic variables in "real-life": under naturally fluctuating environmental conditions, over the course of a season, in unirrigated plants that had been growing in the ground for several years. We sought to answer the question, is the large and expected variability in g_s in the field more closely correlated with chemical or hydraulic variables? Our specific objectives were to

1. obtain concurrent measurements of g_s, shoot water relations, environmental conditions around shoots, xylem sap [ABA] and xylem sap pH of important ornamental tree species, over the course of a season;
2. determine if g_s of tree species under fluctuating field conditions is better correlated with plant chemical factors ([ABA] or pH of xylem fluid) or with plant hydraulic factors (shoot water potential (Ψ) or osmoic potential (Ψ_Π));
3. determine if g_s of tree species is better correlated with plant or with environmental factors (photosynthetic photon flux density (PPFD), vapor pressure deficit (VPD), air temperature, air velocity);
4. test a published model that incorporates both leaf Ψ and xylem sap [ABA] in describing g_s of the species addressed in this study;
5. test other, empirical models that account for variation in g_s.

2. Materials and methods

2.1. Experimental site

Field data were collected between May and September, 1997, on trees of five species growing outdoors on the University of Tennessee Agricultural Campus

(35°5′N and 83°6′W) in Knoxville, TN, USA. Normal mean annual precipitation (1961–1990) on campus was 1197 mm rainfall, and normal mean annual temperature was 14.2°C. Trees had been growing at their current location for several years in soil that had been disturbed from construction ∼60 years ago.

We examined *Acer saccharum* Marsh. (sugar maple), *Cornus florida* L. (flowering dogwood), *Liriodendron tulipifera* L. (tulip-tree, tulip-poplar), *Quercus acutissima* Carruthers (sawtooth oak), and *Quercus palustris* Muench. (pin oak), tagging and measuring the same trees throughout the season. Replicates of each species were located close to one another, to facilitate sampling and minimize microhabitat-induced variability in measured variables. For each species, we selected locations offering relatively high light wherever possible. We selected trees that were as large as available but whose foliage was accessible to investigators standing on the ground or on the jeep used to transport equipment and personnel. Trunk diameters 1.25 m above the soil surface ranged from 29 to 49 cm for *A. saccharum*, 8 to 23 cm for *C. florida*, 9 to 22 cm for *L. tulipifera*, 55 to 67 cm for *Q. acutissima* and 26 to 63 cm for *Q. palustris*.

2.2. Plant measurements

Six to eight individual trees of each species were sampled weekly throughout most of the growing season (total samples = 447). Two to three investigators worked together in making plant and environmental measurements for each replicate of each species. All measurements for each replicate were made on one terminal shoot, consisting of about 8–12 leaves. Measurements were made between 10:00 am and 3:00 pm each day to minimize diurnal influences. All data were collected for each shoot before moving on to the next tree.

Stomatal conductance and leaf temperature were measured with diffusion porometers (AP4, Delta-T Devices, Cambridge, UK) on four leaves from each shoot, avoiding the two or three most recently expanded, still maturing leaves, as well as any leaves showing insect or other damage. The dimension of each leaf at its widest point was recorded. Immediately following g_s measurements, two of the leaves were excised, sealed in separate 3 ml syringes and placed in liquid nitrogen for later measurement of bulk leaf Ψ_Π. The entire shoot was then severed and its Ψ immediately estimated with a pressure chamber (Soilmoisture Equip. Corp., Santa Barbara, CA, USA). Immediately following measurement of shoot Ψ, the phloem was stripped from the cut end of the stem protruding from the pressure chamber to avoid pollution of xylem sap by ABA exuding from phloem tissues. One end of a piece of surgical tubing was placed over the stem and the other end into a microfuge tube, then the pressure was raised 0.2–0.3 MPa above the balance pressure until 50–200 μl of xylem sap was collected from each shoot. This overpressure has not previously artificially

altered [ABA] in xylem sap (e.g. Duan et al., 1996; Ebel et al., 1997). The initial 50 µl of sap extracted from each shoot was discarded to avoid contamination from cells at the cut surface. Sap samples were sealed in the tubes and immediately frozen in liquid N_2. At the end of each day, syringes with leaf samples and microfuge tubes with xylem sap samples were transported back to the lab in liquid N_2 and placed in a $-80°C$ freezer, for subsequent analysis of pH (MI-410, Microelectrodes Inc., Londonderry, NH, USA) and ABA concentration.

Transpiration was calculated for each leaf as $(\rho_{vs} - \rho_{va})/(r_l - r_a)$ (Campbell, 1977), where ρ_{vs} was the vapor density of the evaporative leaf surface (estimated from shoot Ψ), ρ_{va} the ambient vapor density of the air surrounding the shoot, r_l the leaf resistance to water vapor (inverse of g_s, measured as described above) and r_a the boundary layer resistance to water vapor diffusion. Boundary layer resistance was calculated as δ^{bl}/D_{wv}, where δ^{bl} was the boundary layer thickness and D_{wv} is the diffusion coefficient of water vapor (Nobel, 1991). The δ^{bl} was computed as $4.0(l/v)^{0.5}$, where l was the leaf length at its widest point and v was the ambient wind speed within the shoot. $D_{wv} = (2.42 + (0.015)(\text{leaf tempera-ture in }°C - 20))(10^{-5}\ m^2\ s^{-1})$.

Syringes were removed from the freezer and allowed to thaw until no longer cold to the touch (10–15 min) before measuring Ψ_{Π} with a vapor pressure osmometer (Model 5500 XR, Wescor Inc., Logan, UT, USA). The osmometer was calibrated before each use with graded NaCl solutions. Xylem sap [ABA] was measured by an indirect ELISA (Walker-Simmons, 1987) using monoclonal antibody to ABA (Agdia, Elkhart, IN, USA) and ($\pm$) ABA as a standard, as described previously (Duan et al., 1996). Validation of the ELISA assay for use with unpurified xylem exudate was confirmed for each species by dilution/spike recovery tests for nonspecific interference (Walker-Simmons and Abrams, 1991). Comparison of plots of undiluted, standard ABA with plots for standard ABA diluted by xylem sap (four incremental dilutions) indicated only negligible nonspecific interference. As a further check, three serial dilutions were assayed for every xylem sap sample in the experiment, to ensure that each dilution gave similar final values to undiluted sap. Duplicate ABA standards were assayed for each plate.

2.3. Environmental measurements

Air temperature (platinum resistor; model RH-20C, Omega Engineering Inc., Stanford, CT, USA), relative humidity (dielectric sensor; model RH-20C, Omega Engineering Inc.) and wind speed (hot wire anemometer; model 100-VT, Davis Instruments, Hayward, CA, USA) were measured within each shoot, during each set of g_s measurements. PPFD was measured with a quantum sensor (LiCor, Lincoln, NE, USA) on each leaf at the time of g_s measurement. VPD was

calculated from relative humidity, and leaf and air temperatures (Campbell, 1977).

2.4. Statistical analysis

Various models were fitted to each species subset of the g_s data, using single and multiple independent variables (X). Linear models, with c_i representing various intercept and slope model parameters, included simple linear regression

$$g_s = c_1 + c_2 X + \varepsilon, \tag{1}$$

and response surface

$$g_s = c_1 + c_2 X1 + c_3 X2 + c_4 X1^2 + c_5 X2^2 + c_6 X1 \times X2 + \varepsilon \tag{2}$$

where ε is the residual error inherent in every statistical model. Nonlinear models included the double exponential explored by Tardieu and Davies (1993) and Tardieu et al. (1993):

$$g_s = c_1 + c_2 \exp(c_3 X1 \exp(c_4 X2)) + \varepsilon, \tag{3}$$

a sum of exponentials

$$g_s = c_1 \exp(c_2 X1) + c_3 \exp(c_4 X2) + \varepsilon, \tag{4}$$

exponential response surface

$$g_s = c_7 + c_8 \exp(c_1 + c_2 X1 + c_3 X2 + c_4 X1^2 + c_5 X2^2 + c_6 X1 \times X2) + \varepsilon, \tag{5}$$

and the hyperbolic

$$g_s = \frac{(c_1 + c_2 X1)}{(c_1 c_2 X1)} + \frac{1}{(c_3 X2)} + \varepsilon. \tag{6}$$

In all of the above models (except simple linear regression), many combinations of the independent variables were tested. Additionally, preliminary multiple regression analyses were run on the g_s data, using various environmental measures as explanatory variables. Residuals from these analyses were then fitted by the above models. The purpose was to see if controlling for these factors would improve the fit of the models. It was found that there was little difference between the fit of adjusted and unadjusted g_s, so the simpler models without adjustment were used.

The R^2 values were calculated for all models by model sums of squares divided by corrected total sums of squares. This penalizes the nonlinear models without an explicit intercept ("correction factor"), but was done to make all models comparable and to make the results show how well models fit beyond just an overall mean.

3. Results

3.1. Seasonal responses

During the experimental period there was sufficient variability in rainfall and other environmental conditions to provide a range in values of g_s and other plant and environmental variables.

Throughout the season, g_s ranged between 100 and 200 mmol m^{-2} s^{-1} in *A. saccharum* and *C. florida*, and between about 100 and 500 mmol m^{-2} s^{-1} in the other three species. Transpiration remained mostly in the range of 1–3 mmol m^{-2} s^{-1} for *A. saccharum*, *C. florida* and *L. tulipifera*, and 2–4 mmol m^{-2} s^{-1} for the two *Quercus* spp.

Xylem sap [ABA] remained below 250 nM for most of the season in *A. saccharum*, *C. florida* and *Q. acutissima*. [ABA] were highest in *L. tulipifera*, often above 2000 nM. [ABA] in *Q. palustris* ranged between 250 and 1000 nM. Xylem sap [ABA] of these species were consistent with the ranges observed in these and other well-watered or moderately droughted tree genera (Wartinger et al., 1990; Jackson et al., 1995; Loewenstein and Pallardy, 1998a,b), e.g. 50 to ∼500 nM for *Quercus* species (Triboulot et al., 1996; Fort et al., 1997; Loewenstein and Pallardy, 1998a,b) and 65–185 nM in sugar maple (Loewenstein and Pallardy, 1998a,b).

Xylem sap pH varied during the season between 5.5 and 6.5 in *C. florida*, *Q. palustris* and *Q. acutissima*; between 5.8 and 6.5 in *L. tulipifera*; and between 6.5 and 7.0 in *A. saccharum*.

Shoot Ψ ranged between ∼−1.0 and −2.5 MPa during the season in *A. saccharum*, *Q. acutissima* and *Q. palustris*, and remained above −1.6 MPa in *C. florida* and *L. tulipifera*. Ψ_Π remained mostly between −1.5 and −2.0 MPa for *L. tulipifera* and *Q. acutissima*, near −1.5 MPa for *A. saccharum* and *C. florida*, and between −2.0 and −2.5 MPa in *Q. palustris*.

At time of g_s measurements throughout the season, VPD ranged between 2 and 5 kPa, PPFD between 50 and 1000 mmol m^{-2} s^{-1}, and air temperature between 20 and 30°C.

3.2. Correlations of g_s with hydraulic and chemical factors

Table 1 shows a multiple variable model including all the variables listed. It summarizes how much of the variability in g_s can be explained by plant and environment effects. It serves as a "control" model with which to compare the models described subsequently. Table 1 also serves as a source for some chosen variables, e.g. temperature and VPD, and it indicates which physical variables were important. Transpiration was calculated for comparison with g_s (Tables 2 and 3).

Table 1
Regression analyses of stomatal conductance using plant and environmental variables[a]

Species	Regression variables						
	Trunk diameter	Air temperature	Leaf temperature	Air velocity	VPD	PPFD	Model R^2
Acer saccharum		*			*	*	0.34
Cornus florida	*						0.12
Liriodendron tulipifera				*			0.11
Quercus acutissima		*			*	*	
Quercus palustris							0.10

[a] Models included all variables listed, and significant variables are indicated by * ($P < 0.05$). Date of sampling and leaf size was also examined but not included in regression analyses as it was not significant for any species given the other variables.

Table 2
Correlation coefficients (R) for single variable models explaining g_s

Species[a]	Shoot Ψ	Shoot Ψ_Π	[ABA]	Xylem pH	VPD	PPFD	Air temperature	Air velocity
Acer saccharum	0.00	0.06	−0.30[**]	−0.02	0.04	0.51[***]	0.05	0.07
Cornus florida	0.11	−0.11	−0.03	−0.08	0.07	−0.01	0.15	0.13
Liriodendron tulipifera	0.00	−0.04	−0.18	0.04	−0.03	−0.11	0.05	0.24[*]
Quercus acutissima	0.02	−0.06	−0.31[***]	−0.19[*]	−0.04	0.17	0.04	0.11
Quercus palustris	−0.20	−0.03	−0.40[***]	−0.09	−0.01	0.26[*]	0.05	0.10
Average, g_s[b]	0.07	0.06	0.24	0.08	0.04	0.21	0.07	0.13
Average, transpiration[c]	−[d]	0.13 (1)	0.23 (4)	0.13 (1)	–	0.42 (4)	–	–

[a] Models were fitted to each species separately, using all data collected during the season.

[b] Average correlation coefficients across species for each variable were computed from absolute values of species correlation coefficients.

[c] For comparative purposes, average correlation coefficients across species are also provided for transpiration; number in parentheses indicates how many species showed significant correlation ($P < 0.05$).

[d] Indicates regressions not computed, as the variable was used in calculating transpiration.

[*] Refers to significance of regression (R^2) at $P < 0.05$.

[**] Refers to significance of regression (R^2) at $P < 0.01$.

[***] Refers to significance of regression (R^2) at $P < 0.001$.

Table 3
Multiple correlation coefficients (R, always positive) for various two-variable models explaining g_s[a]

Species	Tardieu	[ABA]/shoot Ψ	pH/shoot Ψ	[ABA]/pH	Air temperature/PPFD	VPD/PPFD	[ABA]/Air temperature	[ABA]/VPD	[ABA]/PPFD
Acer saccharum	0.32**	0.33*	0.12	0.33	0.60***	0.51***	0.34*	0.38*	0.63***
Cornus florida	0.20	0.25	0.20	0.21	0.37*	0.39**	0.37*	0.40**	0.21
Liriodendron tulipifera	0.22	0.27	0.13	0.24	0.37*	0.34*	0.34*	0.28	0.21
Quercus acutissima	0.32**	0.35*	0.30	0.42***	0.43***	0.50***	0.42***	0.47***	0.38**
Quercus palustris	0.45***	0.55***	0.40*	0.50***	0.49***	0.42**	0.51***	0.49***	0.62***
Average, g_s[b]	0.30	0.35	0.23	0.34	0.45	0.43	0.40	0.40	0.41
Average, transpiration[c]	–[d]	–	–	0.31 (1)	–	–	–	–	0.55 (4)

[a] Tardieu represents the double exponential model developed for maize (Tardieu and Davies, 1993; Tardieu et al., 1993). All other models are response surfaces. See Section 2 for model descriptions. Models were fitted to each species separately, using all data collected during the season.

[b] Average correlation coefficients across species for each variable were computed from absolute values of species correlation coefficients.

[c] For comparative purposes, average correlation coefficients across species are also provided for transpiration, for models with variables not used in computing transpiration. Number in parentheses indicates how many species showed significant correlation ($P < 0.05$).

[d] Indicates regressions not computed, as the variable was used in calculating transpiration.

* Refers to significance of regression (R^2) at $P < 0.05$.

** Refers to significance of regression (R^2) at $P < 0.01$.

*** Refers to significance of regression (R^2) at $P < 0.001$.

3.2.1. Single variable regression analyses

Single variable regression analyses of g_s and several plant and environmental variables are summarized in Table 2. As often observed in the field, much variability in g_s could not be accounted for by a single plant or environmental variable. Nonetheless, g_s correlations with some variables were significant, and R-values indicate with which variables g_s was most strongly correlated.

Stomatal conductance was better correlated with xylem sap [ABA] than with shoot Ψ or Ψ_Π. Stomatal conductance was also better correlated with xylem sap [ABA] than with xylem sap pH. Viewed across species, xylem sap [ABA] was best correlated with g_s among the four plant and four environmental variables. The g_s/[ABA] correlation was significant for three of the five species (*A. saccharum* and both oaks), and the g_s/[ABA] correlation had the highest average correlation coefficient (R) across species. The only other single variable g_s correlation that was significant for more than one species was with PPFD (*A. saccharum* and *Q. palustris*), which was more closely correlated with g_s than was shoot Ψ, shoot Ψ_Π or xylem sap pH. Average R of g_s/air velocity correlations was also higher than that of g_s with shoot Ψ, shoot Ψ_Π or xylem sap pH. Stomatal conductance was not significantly correlated with shoot Ψ, shoot Ψ_Π, VPD or air temperature in any species. The highest correlation of g_s with any plant or environmental variable for an individual species was with PPFD, in *A. saccharum* ($R = 0.51$).

3.2.2. Multiple variable regression analyses

A model developed for maize that relates g_s to xylem sap [ABA] and leaf Ψ (Tardieu et al., 1993; Tardieu and Davies, 1993, see Section 2 for equation) accounted for more variation in g_s than did any of the single variable correlations, in terms of average R across species (Tables 2 and 3). We dropped c_1 ($g_{s\ min}$) from the Tardieu model, as R's were higher without it. The Tardieu double exponential model explained a significant amount of seasonal variation in g_s in three of the five tree species. Several other variables gave as high or higher R values than [ABA] and shoot Ψ when substituted into the Tardieu equation. Modifying sensitivity of g_s to [ABA] by air temperature rather than shoot Ψ, for example, gave an R of 0.40 averaged across species, and the [ABA]/PPFD double exponential model gave an average R of 0.41, compared to R of 0.30 for the Tardieu model. Two-variable double exponential models gave the highest R values when they incorporated PPFD.

Stomatal conductance was better explained by two-variable response surface models than by the Tardieu model, indicated by higher average R values and more significant correlations among species (Table 3). Other multiple variable models were also tested (see Section 2) with the same combinations of variables as for the double exponential. All non-linear models performed no better than the double exponential, with R's generally low and only occasionally significant (data

not shown). It became apparent that the variability in the data could not be explained by a model with few parameters. Therefore, the more flexible response surface models were tried. Exponential response surfaces and linear response surfaces performed similarly, so the latter were used.

The [ABA]/shoot Ψ response surface had a slightly higher average R (0.35) than the double exponential [ABA]/shoot Ψ model of Tardieu (0.30). Incorporating PPFD into the two-variable response surfaces generally increased the strength of correlations. Response surfaces composed of environmental variables were generally better correlated with g_s than models composed of plant variables, with the air temperature/PPFD and VPD/PPFD response surfaces having the highest average correlation coefficients of all two-variable models. The air temperature/PPFD, VPD/PPFD and [ABA]/air temperature response surfaces each accounted for significant variation in g_s for all five species. Four of the five species showed significant g_s correlations for the [ABA]/VPD response surface. [ABA] response surfaces accounted for more variation in g_s than pH response surfaces. The two-variable model explaining the most variation in g_s within an individual species was the [ABA]/PPFD response surface, for *Acer rubrum* ($R = 0.63$) and *Q. palustris* ($R = 0.62$). Variation in g_s of the two oaks tended to be explained to the same degree within the same models.

4. Discussion

The existence of strictly nonhydraulic root signals that can influence g_s has been demonstrated or suggested in several herbaceous species and a few woody species, including *Acer pseudoplatanus* (Khalil and Grace, 1993), *Prunus avium × pseudocerasus* (Gowing et al., 1993), *Malus × domestica* (Gowing et al., 1990), *Prunus dulcis* (Fußeder et al., 1992), *Rosa hybrida* (Augé and Duan, 1991), some coniferous species (Jackson et al., 1995) and *C. florida*, *L. tulipifera*, *A. rubrum*, *Chionanthus virginicus*, *Halesia carolina* and *Oxydendrum arboreum* (Croker et al., 1998). The theory of control of g_s by nonhydraulic signals such as xylem [ABA] was initially advanced to explain stomatal behavior of drying plants, and it has since been used to describe g_s across essentially the entire range of soil moisture and leaf hydration occurring naturally in the field (Tardieu et al., 1993; Tardieu and Simonneau, 1998). ABA moving to guard cell complexes may originate in leaves or roots (Saliendra et al., 1995; Liang et al., 1997; Hartung et al., 1998; Thompson et al., 1997). Regardless of origin, g_s has been more closely correlated with concentrations of ABA in leaf and shoot xylem than with leaf Ψ or soil Ψ, under controlled conditions. We also found this to be true in these urban trees under naturally varying, outdoor circumstances.

As is typically observed in the field, g_s was not strongly correlated with any one variable in these urban trees. Environmental conditions and plant characters

fluctuate widely in the field, with differing time-scales and differing quantitative influences on g_s, so a confusion of effects is likely (Tardieu and Simonneau, 1998). However, among the many variables for which we obtained concurrent measurements, xylem sap [ABA] was the single variable that most strongly correlated with seasonal g_s in the experimental trees. The g_s/[ABA] correlation coefficient was negative for all five species, in accord with the hypothesis of nonhydraulic root-to-shoot communication via ABA: more ABA in xylem sap results in a reduction in stomatal aperture (Davies et al., 1994). Correlation coefficients for the g_s/[ABA] relation, ranging from 0.30 to 0.40 for sugar maple and the two oaks, were lower than those observed previously in *Juglans nigra* ($R = 0.61$), *A. saccharum* ($R = 0.50$) and *Quercus alba* ($R = 0.53$) (Loewenstein and Pallardy, 1998b) and higher than those observed before in *Q. robur* and *Q. petraea* ($R = 0.00$ to 0.14) (Triboulot et al., 1996).

The more easily measured environmental variable PPFD was the single variable having the next-best correlation with g_s. Strong relationships between g_s and PPFD (with the relation plateauing once saturating PPFD has been reached) are well known for several species (e.g. Jones, 1983). Other environmental variables and plant hydraulic variables (shoot Ψ and Ψ_Π) did not explain much variation of seasonal g_s in our study. These findings support the theory that stomata are more closely regulated by xylem [ABA] than by hydraulic variables, but the literature is inconsistent as regards large trees. Some data support the dependency of seasonal woody plant g_s on xylem [ABA], or at least better correlation of g_s with xylem [ABA] than with measures of leaf water status (Wartinger et al., 1990, *P. dulcis*; Khalil and Grace, 1993, *A. pseudoplatanus*; Correia et al., 1995, *Vitis vinifera*; Jackson et al., 1995, *Picea sitchensis*, *Pinus sylvestris*; Liang et al., 1996, *Acacia confusa*, *Litsea glutinosa*; Loewenstein and Pallardy, 1998a,b, *J. nigra*, *Salix nigra*). In other work, g_s has been much better correlated with leaf Ψ than with xylem sap [ABA] (Triboulot et al., 1996, *Q. petraea*, *Q. robur*) or significantly associated with changes in both leaf Ψ and xylem [ABA] (Loewenstein and Pallardy, 1998a,b, *A. saccharum*, *Q. alba*).

Little support was found for the suggestion that g_s may be regulated by xylem sap pH (Thompson et al., 1997; Wilkinson and Davies, 1997) or that stomatal sensitivity to [ABA] may be modified by xylem sap pH (Jia and Zhang, 1997; Zhang et al., 1997; Hartung et al., 1998; Wilkinson et al., 1998). Stomatal conductance was significantly correlated with xylem sap pH in only one of the five species, and the average correlation coefficient of g_s with pH was quite low. Stomatal conductance has previously been more strongly correlated with [ABA] than with pH of xylem sap, in *J. nigra*, *S. nigra*, *A. saccharum* and *Q. alba* (Loewenstein and Pallardy, 1998a,b). R-values for the g_s/xylem sap pH correlation were, however, negative in four of five species, suggesting at least a tendency for more alkaline pH to result in inhibition of g_s, as predicted. Changes in xylem sap pH have been better correlated with g_s in herbaceous species than

in trees, and it is possible that xylem pH may play a more important role in smaller plants.

Some investigators have concluded that g_s is probably affected by both chemical and hydraulic factors (e.g. Tardieu and Davies, 1993; Saliendra et al., 1995; Thomas and Eamus, 1999). A model of stomatal behavior has been developed, using field-grown corn, which incorporates both hormonal and hydraulic factors, linking g_s to xylem [ABA] with a sensitivity dependent upon leaf Ψ (Tardieu et al., 1993; Tardieu and Davies, 1993). This model accounted for slightly more variation in g_s than did xylem [ABA] alone in our trees, viewed across species. Correlation coefficients were not as high as those observed for maize (Tardieu et al., 1993), and for several reasons we would expect more variation in the model under our conditions than under the conditions for which the model was developed. In the maize field, Tardieu and colleagues obtained measurements on genetically similar plants of the same age, over a short time period (3 days), under relatively high illumination. In the urban landscape, we examined genetically more variable plants of differing ages, seasonally from May to September, often under non-saturating illumination and non-limiting soil moisture. Further, Tardieu et al. (1993) were able to correct non-saturating g_s values to g_s at light saturation, according to the well known relationship between g_s and PPFD corroborated for maize (Tardieu and Davies, 1993). Even though g_s was better correlated with PPFD than with other environmental and plant hydraulic variables, we did not observe a robust correlation between g_s and PPFD in most species, even within one tree with measurements made very closely together in time.

The double exponential model for g_s developed by Tardieu combined equations describing the generation and effects of chemical signals and classical equations of water flux (Tardieu and Davies, 1993). We tested numerous models incorporating many environmental and plant variables, both chemical and hydraulic, for the best empirical correlation with g_s and found that response surfaces provided the closest fits. The xylem [ABA]/shoot Ψ response surface accounted for slightly more variation in g_s than the Tardieu model. However, response surfaces that modified stomatal sensitivity to xylem [ABA] by environmental variables accounted for more variation. Of all models incorporating a plant variable, the [ABA]/air temperature response surface accounted for the most variation in seasonal tree g_s, resulting in significant g_s correlations for all five species. Using strictly environmental variables in the response surfaces tended to explain as much or more variation in g_s than using plant variables. The air temperature/PPFD response surface had the highest average R in this study.

We recognize the limitations of empirical statistical modeling and suggest that the most important contribution of our work lies in identifying how well the g_s of these trees was correlated with single variables. Viewed across these five tree taxa and across the many environmental and phenological changes of a growing

season, xylem sap [ABA] was the variable best correlated with seasonal g_s. Therefore, our findings with urban trees are consistent with the theory of regulation of g_s by root-sourced abscisic acid.

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