

Correlations of stomatal conductance with hydraulic and chemical factors in several deciduous tree species in a natural habitat

ROBERT M. AUGÉ*, CRAIG D. GREEN, ANN J. W. STODOLA,
ARNOLD M. SAXTON, JOSHUA B. OLINICK
AND RICHARD M. EVANS

*Institute of Agriculture, Tennessee Agricultural Experiment Station,
University of Tennessee, P.O. Box 1071, Knoxville, TN 37901–1071 USA*

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SUMMARY

Recent research in whole-plant stomatal physiology, conducted largely with potted plants in controlled environments, suggests that stomatal conductance (g_s) might be more closely linked to plant chemical variables than to hydraulic variables. To test this in a field situation, seasonal g_s was examined in relation to a number of plant and environmental variables in 11 temperate, deciduous forest tree species. Stomatal conductance was generally better correlated with environmental variables (air temperature, vapor pressure deficit, PPFD) than with plant variables, and slightly better correlated with plant hydraulic variables (shoot water and osmotic potentials) than with plant chemical variables (xylem sap ABA concentration, xylem sap pH). We examined a model, developed previously for maize, which describes regulation of g_s by xylem sap ABA concentration with leaf water status acting to modify stomatal sensitivity to the ABA signal. This model explained slightly more variation in seasonal g_s in the forest trees than did single plant variables but not more variation than most single environmental variables. Response surface models, especially those incorporating environmental variables, were more consistently successful at explaining g_s across species.

Key words: abscisic acid, maples, oaks, pH of xylem sap, stomatal conductance, temperate trees, water potential.

INTRODUCTION

Stomata regulate plant carbon gain, water loss and other physiological determinants of forest productivity. Our ability to assess impacts of environmental changes on forest ecosystems relies heavily, therefore, on understanding stomatal function and control. The traditional view is that stomatal conductance (g_s) is controlled chiefly via leaf water status. It now appears that stomata, at least of plants in drying soil, might also be regulated by root-to-shoot movement of chemical factors (Zhang *et al.*, 1987; Davies *et al.*, 1994; Tardieu *et al.*, 1996; Wilkinson & Davies, 1997). Moreover, chemical root signals, such as abscisic acid concentrations ([ABA]) or pH changes in xylem fluid, have been postulated

to exert a predominant influence, with leaf water status acting to modify stomatal sensitivity to the chemical signal (Tardieu & Davies, 1993; Tardieu *et al.*, 1993). Strong 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 & Pallardy, 1998a,b).

The theory of whole-plant stomatal regulation by root-sourced, nonhydraulic factors has been advanced on the basis of a few field studies (Loveys, 1984; Wartinger *et al.*, 1990; Tardieu *et al.*, 1991) but mostly by work with potted plants in controlled conditions. There are indications that g_s and xylem sap [ABA] might be correlated in trees in field conditions (Loewenstein & Pallardy, 1998b) but additional work is needed to corroborate or refute the idea that g_s of woody species in the field is typically better correlated with chemical rather than with hydraulic plant variables. Our objectives were to: characterize seasonal g_s , shoot water relations, xylem sap [ABA] and xylem sap pH of several

*Address for correspondence (tel +1 423 974 7324; fax +1 423 974 1947; e-mail auge@utk.edu).

Abbreviations: [ABA], abscisic acid concentration; g_s , stomatal conductance; VPD, vapor pressure deficit; Ψ_π , osmotic potential; Ψ_p , turgor potential; Ψ , water potential.

deciduous tree species; determine whether g_s of tree species is correlated best with plant chemical factors ([ABA] or pH of xylem fluid) or with plant hydraulic factors (shoot water potential (Ψ) components); determine whether g_s of tree species is correlated best with plant or with environmental factors (PPFD, vapor pressure deficit (VPD), air temperature, air velocity); test a published model that incorporates both leaf Ψ and xylem sap [ABA] in describing g_s ; and search for other, empirical models that account for variation in g_s .

MATERIALS AND METHODS

Experimental site

Field data were collected between May and September, 1997, on saplings and trees of 11 species growing outdoors at the University of Tennessee Forestry Experiment Station and Arboretum (36° 1' N and 84° 13' W; c. 890 ha) in Oak Ridge, TN, USA. Longterm mean annual precipitation (30 yr normals, 1967–96) at the arboretum is 1385 mm rainfall, and mean temperature is 14.0°C. Trees on two sites with different soil characteristics were examined. The Chestnut Ridge site has strong acidic, moderately permeable forest soils classified as Fullerton cherty silt loams and in the taxonomic class of Clayey, kaolinitic, thermic Typic Paleudults. Soils on the Pine Ridge site are primarily Armuchee shaly silty clay loam in the taxonomic class of Clayey, mixed, thermic Ochreptic Hapludults and are typified as being strongly acid, eroded, infertile and low in available water capacity.

On the Chestnut Ridge site, we measured *Acer rubrum* L. (red maple), *Acer saccharum* Marsh. (sugar maple), *Cornus florida* L. (flowering dogwood), *Liriodendron tulipifera* L. (tulip poplar), *Nyssa sylvatica* Marsh. (black gum), *Oxydendrum arboreum* (L.) D. C. (sourwood), *Quercus alba* L. (white oak), *Quercus prinus* L. (chestnut oak), *Quercus rubra* L. (red oak). On the Pine Ridge site, *Liquidambar styraciflua* L. (sweet gum) and *Quercus muehlenbergii* Willd. (chinquapin oak) were measured. The same trees were tagged and measured 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 irradiance wherever possible. Tree size varied with species, with trunk diameters 1.25 m above the soil surface ranging from 1 to 17 cm. All the tree species had been growing in the ground at their current location for several years, from planted seedlings (*Q. rubra*) or from naturally distributed seed (all other species). We selected trees that were as large as possible but whose foliage was accessible to investigators standing on the ground or

on the vehicle used to transport equipment and personnel.

Plant measurements

Six to eight replicates of each species were sampled weekly throughout most of the growing season. 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 c. 8–12 leaves. Preliminary, diurnal g_s measurements of trees of each species were recorded at the start of the season to determine the time of day during which g_s was reasonably constant, and during which g_s was subsequently measured throughout the study (c. 09.30–15.30 hours US Eastern Standard Time). The following data were collected for each shoot. 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 width (at its widest point) of each sampled leaf 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 osmotic potential (Ψ_π). The entire shoot was then severed and its Ψ immediately estimated with a pressure chamber (Soilmisture Equipment 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 contamination of xylem sap by ABA exuding from phloem tissues. A piece of surgical tubing was then placed over the stem and into a microfuge tube; the pressure was raised 0.2–0.3 MPa above the balance pressure until 100–200 μ l of xylem sap had been collected from each shoot. Previously, this overpressure has not artificially altered [ABA] in xylem sap (e.g. Duan *et al.*, 1996; Ebel *et al.*, 1997). The initial 50 μ l 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 N₂. At the end of each day, syringes containing leaf samples and microfuge tubes containing xylem sap samples were transported back to the laboratory in liquid N₂ and placed in a –80°C freezer, for later analysis of pH (MI-410, Microelectrodes Inc., Londonderry, NH, USA) and [ABA].

Turgor potential (Ψ_p) was computed as $\Psi - \Psi_\pi$. Transpiration was calculated for each leaf as $(\rho_{vs} - \rho_{va}) / (r_1 - r_a)$ (Campbell, 1977): ρ_{vs} , the vapor density of the evaporative leaf surface (estimated from shoot Ψ and leaf temperature); ρ_{va} , the ambient vapor density of the air surrounding the shoot; r_1 , the leaf

resistance to water vapor (inverse of g_s , measured as already described); r_a , the boundary layer resistance to water vapor diffusion. Boundary layer resistance was calculated as $\delta^{bl}/D_{wv}:\delta^{bl}$, boundary layer thickness; D_{wv} , the diffusion coefficient of water vapor (Nobel, 1991). $\delta^{bl} = 4.0[(l/v)^{0.5}]$; l , leaf length in the downwind direction (we used leaf dimension at its widest point); v , the ambient wind speed within the shoot. $D_{wv} = [2.42 + (0.015)(\text{leaf temperature in } ^\circ\text{C} - 20)](10-5 \text{ m}^2 \text{ s}^{-1})$.

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 by dilution and spike recovery tests for each species for nonspecific interference (Walker-Simmons & 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. Fluxes of ABA in stems were calculated as $[\text{ABA}] \times E$.

Predawn shoot Ψ was measured with a pressure chamber.

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 (LI-COR, Lincoln, NE, USA) on each leaf at the time of g_s measurement. Vapor pressure deficit (vapor pressure difference between leaf and air) was calculated from leaf and air relative humidities and temperatures (Campbell, 1977).

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_1 to c_8 rep-

resenting various model parameters (e.g. intercepts and slopes), included simple linear regression

$$g_s = c_1 + c_2X + \varepsilon$$

and response surface

$$g_s = c_1 + c_2X1 + c_3X2 + c_4X1^2 + c_5X2^2 + c_6X1 * X2 + \varepsilon$$

Non-linear models included the double exponential explored by Tardieu & Davies (1993) and Tardieu *et al.* (1993)

$$g_s = c_2 \exp(c_3X1 \exp(c_4X2)) + \varepsilon$$

a sum of exponentials

$$g_s = c_1 \exp(c_2X1) + c_3 \exp(c_4X2) + \varepsilon$$

exponential response surface

$$g_s = c_7 + c_8 \exp(c_1 + c_2X1 + c_3X2 + c_4X1^2 + c_5X2^2 + c_6X1 * X2) + \varepsilon$$

and the hyperbolic

$$g_s = (c_1 + c_2X1)/(c_1c_2X1) + 1/(c_3X2) + \varepsilon$$

In all these models (except simple linear regression), many combinations of the independent variables were tested. In addition, 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 to the above models to see whether controlling these factors would improve the fit of the models of interest. Little difference was found between the fit of adjusted and unadjusted g_s , so the simpler models without adjustment were focused on.

R -square values were calculated for all models by model sums of squares divided by corrected total sums of squares. This penalizes the non-linear models without an explicit intercept ('correction factor'), but was carried out to make all models comparable and to make the results show how well models fit beyond just an overall mean.

RESULTS

Seasonal behavior

Soils gradually dried throughout the season on both the Chestnut Ridge and Pine Ridge sites, as estimated by predawn shoot Ψ (Fig. 1). At the end of June, predawn shoot Ψ was near -0.1 MPa or above for all species except *A. saccharum*. Subsequent declines were subtle in *Liq. styraciflua*, *N. sylvatica*, *O. arboreum*, *Q. muehlenbergii* and *Q. prinus* (to $c.$ -0.2 MPa), moderate in *Q. rubra* and *Q. alba* (to $c.$ -0.3 MPa in the latter part of the season), and most

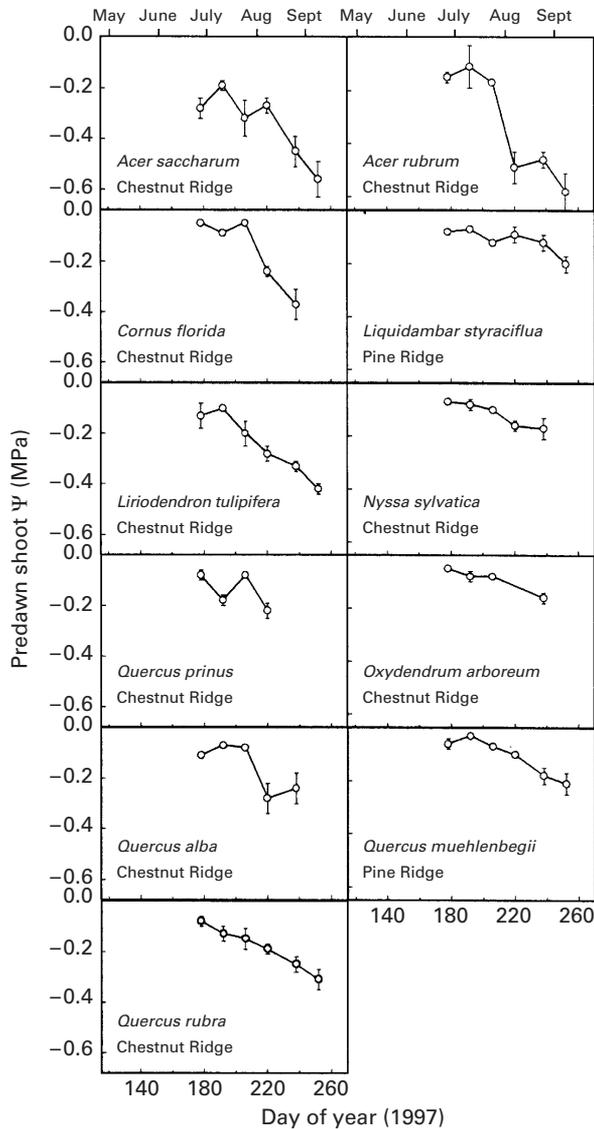


Fig. 1. Seasonal predawn shoot water potentials (Ψ), of trees whose stomatal conductance and other measurements are portrayed in Figs 2–14. Symbols represent daily means ± 1 SE ($n = 4$ trees). Chestnut Ridge and Pine Ridge refer to two University of Tennessee Forestry Experiment Station sites (see the Materials and Methods section for site descriptions).

pronounced in *A. rubrum*, *A. saccharum*, *C. florida* and *Lir. tulipifera* (declining to -0.4 to -0.6 MPa). Environmental conditions around the shoots during sampling of water status are depicted in Fig. 2.

Stomatal conductances remained mostly < 200 $\text{mmol m}^{-2} \text{s}^{-1}$ throughout the season in the two maples, *C. florida* and *O. arboreum* (Fig. 3). In *Q. muehlenbergii* and *Q. rubra*, g_s ranged between 100 and 300 $\text{mmol m}^{-2} \text{s}^{-1}$. In the remaining species, g_s oscillated between *c.* 100 and 400 $\text{mmol m}^{-2} \text{s}^{-1}$. Across species, g_s was generally lower at the start and end of the season (May and Aug/Sept) than during June and July. Typically, changes in transpiration (Fig. 4) and g_s were not closely related. Xylem sap [ABA] were lowest in *A. rubrum*, *A. saccharum*, *C. florida*, *Liq. styraciflua*, *O. arboreum* and *Q. muehlen-*

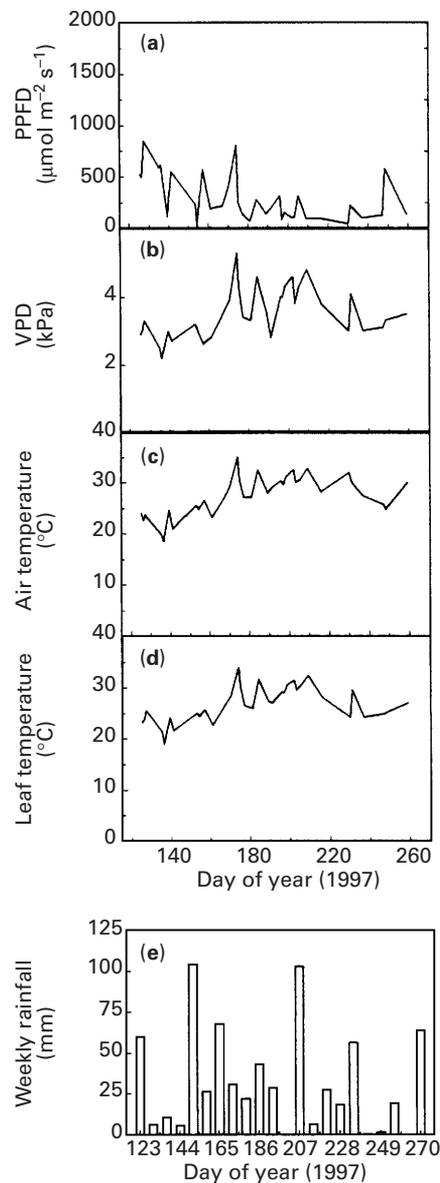


Fig. 2. (a–d) Micro-environmental conditions around shoots at time of sampling for water status and ABA measurements, throughout the season. Lines describe weekly means of all replicates of all species. PPFD, vapor pressure deficit (VPD) and leaf temperature were each measured on four leaves per shoot, and air temperature was measured once within each shoot, for each replicate of each species each day. PPFD and VPD for individual species are depicted in Figs 11 and 12. (e) Rainfall, measured at the end of each week, during the season.

bergii, remaining < 600 nM during the season (Fig. 5). Except for the first sampling date in May, [ABA] also remained < 600 nM in *Q. alba*. [ABA] was somewhat higher (*c.* 1000 nM) in *N. sylvatica*, *Q. prinus* and *Q. rubra*, and > 2000 nM during the first half of the season in *Lir. tulipifera*. These xylem sap [ABA] are within the ranges observed previously in these and other, well watered or moderately droughted, tree species (Wartinger *et al.*, 1990; Jackson *et al.*, 1995; Loewenstein & Pallardy, 1998a,b): for example 50 to *c.* 500 nM for *Quercus*

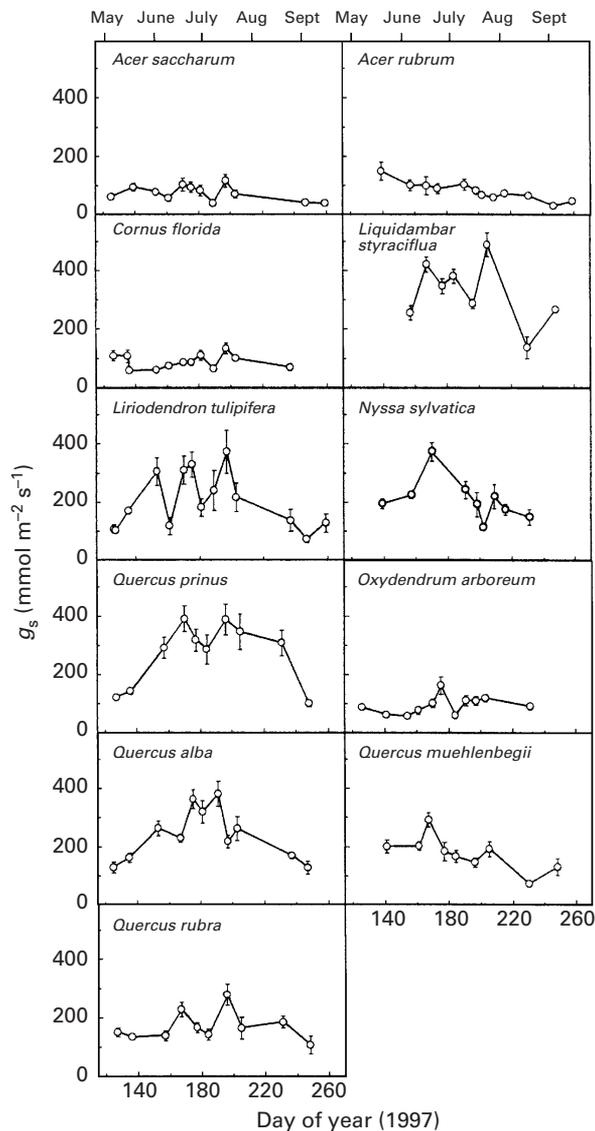


Fig. 3. Seasonal stomatal conductance (g_s) in 11 tree species. Symbols represent weekly means ± 1 SE ($n = 6-8$ trees, four leaves per shoot, one shoot per tree).

species (Triboulot *et al.*, 1996; Fort *et al.*, 1997; Loewenstein & Pallardy, 1998a,b) and 65–185 nM in sugar maple (Loewenstein & Pallardy, 1998a,b). In most species, xylem sap [ABA] was higher in May than later in the season. Fluxes of ABA remained $< 10 \text{ pmol m}^{-2} \text{ s}^{-1}$ in *A. rubrum*, *A. saccharum*, *C. florida* and *O. arboreum* (Fig. 6), but were somewhat higher in *N. sylvatica*, *Q. muehlenbergii* and *Q. rubra* ($\leq 30 \text{ pmol m}^{-2} \text{ s}^{-1}$). ABA fluxes were also in this range for *Liq. styraciflua*, *Q. alba*, and *Q. prinus*, except for higher values in May or the beginning of June. Fluxes of ABA were consistently highest in *Lir. tulipifera* ($100-120 \text{ pmol m}^{-2} \text{ s}^{-1}$) for the first half of the season.

Xylem sap pH varied during the season; ranging from 6.0 to 7.0 in *A. rubrum*, *A. saccharum*, *Liq. styraciflua*, *N. sylvatica*, *Q. muehlenbergii* and *Q. rubra*; from 6.0 to 6.5 in *Q. prinus*; from 5.5 to 6.5 in *C. florida*, *Lir. tulipifera* and *Q. alba*; and from 5.0 to

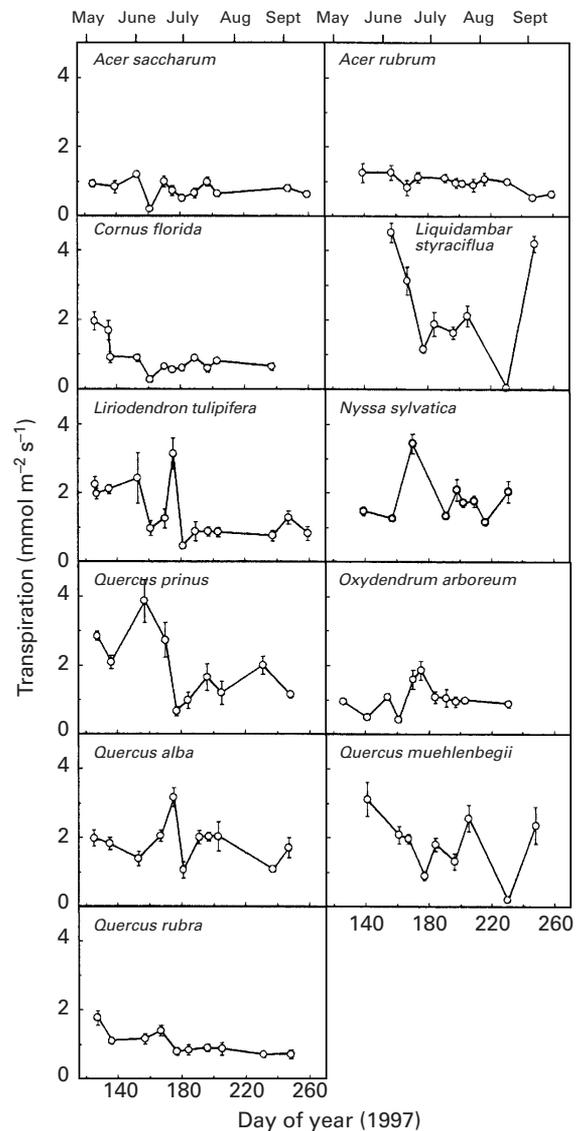


Fig. 4. Seasonal transpiration rates at the time of g_s measurements in 11 tree species. Symbols represent weekly means ± 1 SE ($n = 6-8$ trees, four leaves per shoot, one shoot per tree).

5.5 in *O. arboreum* (Fig. 7). In many species, xylem sap was slightly more alkaline at the end than at the start of the season.

Mean shoot Ψ decreased *c.* 1 MPa during the season in *A. rubrum*, *A. saccharum*, *Q. alba*, *Q. muehlenbergii* and *Q. prinus*, from *c.* -1.0 MPa early in the season to -2.0 MPa or just below by the end of August (Fig. 8). Declines in shoot Ψ of *c.* 0.5 MPa were observed in *Liq. styraciflua* and *N. sylvatica* during the course of the season, and declines of *c.* 0.8 MPa were observed in *O. arboreum* from June until the end of the season. These changes are mostly consistent with declines in predawn shoot Ψ during that time. Shoot Ψ_p was relatively low in May for *Q. alba*, *Q. muehlenbergii* and *Q. prinus*, increasing in the latter two species during June and then falling again (in all three species) by the end of the season (Fig. 9). For *A. rubrum* and *A. saccharum*, Ψ_p values appeared unreliable and were therefore not reported.

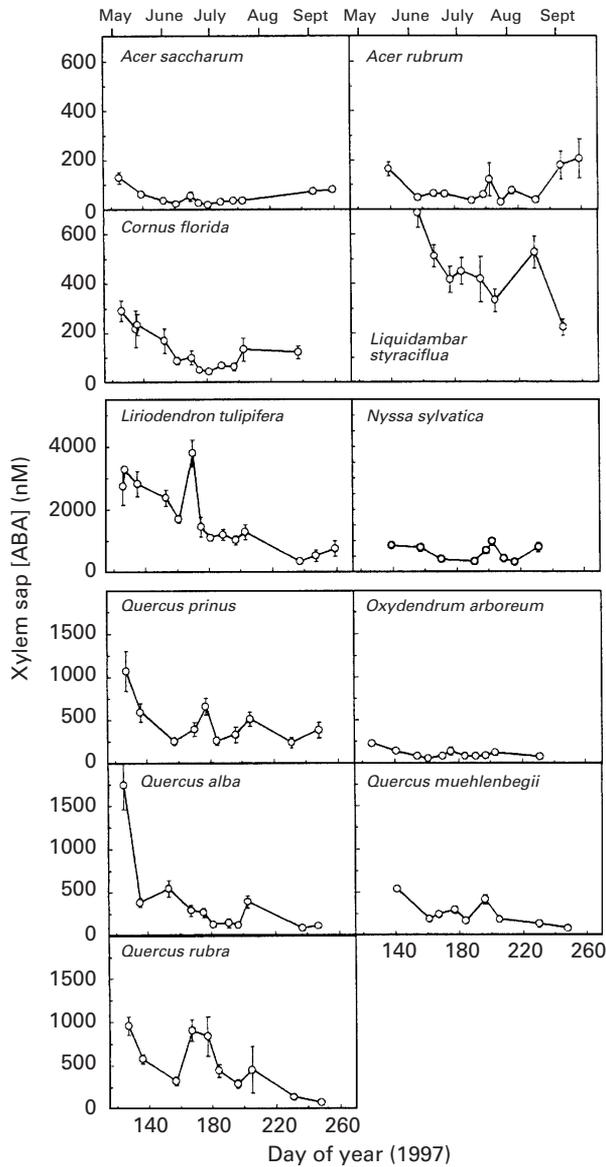


Fig. 5. Seasonal ABA concentrations in xylem sap at time of g_s measurements in 11 tree species. Symbols represent weekly means ± 1 SE ($n = 6-8$ trees).

Shoot Ψ and Ψ_p remained mostly constant during much of the season in *C. florida*, *Lir. tulipifera* and *Q. rubra*, and did not reflect the declines in predawn shoot Ψ observed in these species. Fluctuations in shoot Ψ_π were relatively small in all species during the season (Fig. 10). Shoot Ψ_π remained relatively constant throughout the season in *C. florida*, *Liq. styraciflua*, *Lir. tulipifera* and *N. sylvatica*. Other species did show seasonal declines in shoot Ψ_π , of c. 0.3–0.5 MPa.

Mean PPFD and VPD during porometry are shown in Figs 11 and 12.

Correlations of g_s with hydraulic and chemical variables

Table 1 is a multiple variable model including all the variables listed. It summarizes how much of the

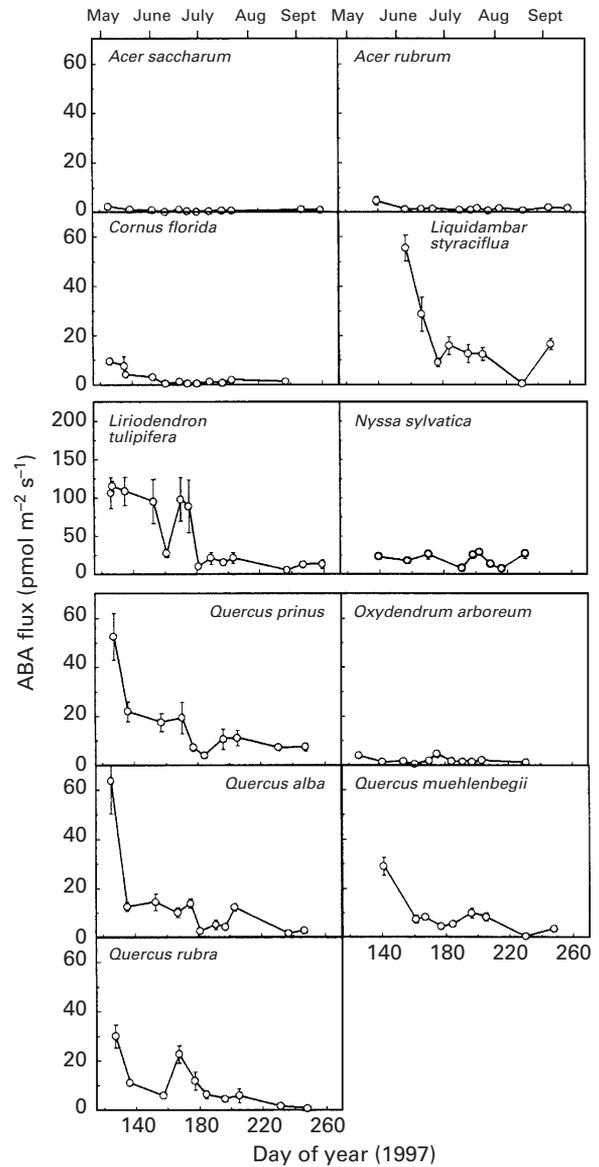


Fig. 6. Seasonal ABA fluxes in xylem sap at time of g_s measurements in 11 tree species. Symbols represent weekly means ± 1 SE ($n = 6-8$ trees).

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.

Single variable regression analyses. Single variable regression analyses of g_s and several plant and environmental variables are summarized in Table 2, with some examples depicted in Fig. 13. Stomatal conductance was generally better correlated with environmental variables than with plant variables. Mean correlation coefficients (R values) of g_s with air temperature, VPD and PPFD across species were 0.29, 0.25 and 0.20, respectively, each higher than mean correlation coefficients of g_s with shoot Ψ , shoot Ψ_p and [ABA] and pH of xylem sap. Stomatal

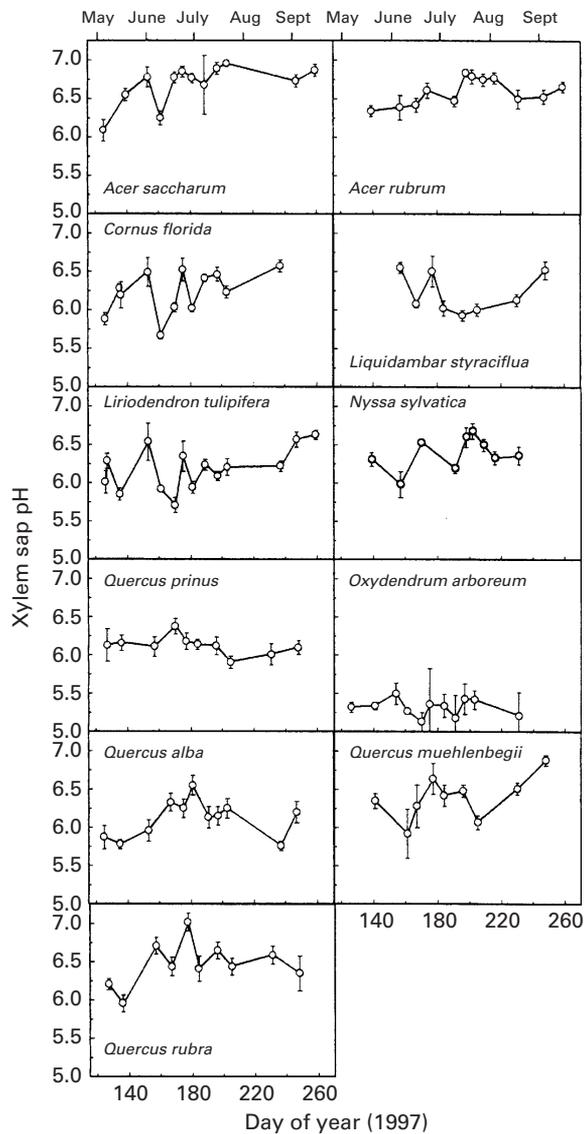


Fig. 7. Seasonal xylem sap pH at time of g_s measurements in 11 tree species. Symbols represent weekly means ± 1 SE ($n = 6-8$ trees).

conductance was also significantly correlated with environmental variables for more species than for plant variables. Stomatal conductance was significantly correlated with air temperature, VPD and PPFd for seven, six and five species, respectively, while g_s was significantly correlated for no more than four species with any of the five plant variables. Significant correlations between g_s and each environmental variable were positive in every instance. Among environmental variables, g_s was best correlated with air temperature and least well correlated with air velocity, in terms of mean R value across species and number of species showing significant correlations. Transpiration was much better correlated with PPFd than was g_s (Table 2).

Among plant variables, g_s was best correlated with shoot Ψ_π and least well correlated with pH of xylem sap, as characterized by the mean R value across species and number of species showing significant

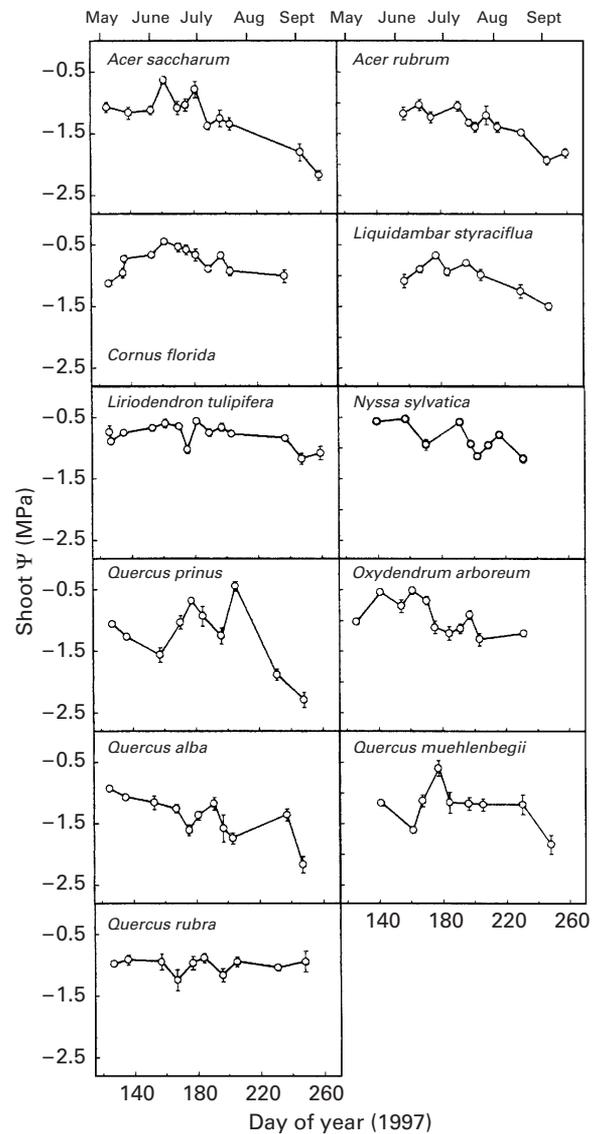


Fig. 8. Seasonal shoot water potential (Ψ) at the time of g_s measurements in 11 tree species. Symbols represent weekly means ± 1 SE ($n = 6-8$ trees).

correlations with g_s . Stomatal conductance was somewhat better correlated with each of the plant hydraulic variables (shoot Ψ_π , shoot Ψ_p and total shoot Ψ) than with either of the plant chemical variables ([ABA] or pH of xylem sap), in terms of mean R values across species. Between plant chemical variables, g_s was better correlated with xylem sap [ABA] than with xylem sap pH, both in terms of mean R value and number of species showing significant correlations. The one species in which g_s was significantly correlated with xylem pH, *O. arboreum*, had the lowest xylem pH and a relatively stable pH throughout the season. The relationship was negative: g_s declined as pH increased.

Significant single-variable correlations between each Ψ component and g_s , and between xylem sap [ABA] and g_s , were positive for some species and negative for others. Stomatal conductance was significantly correlated with xylem [ABA] for four of

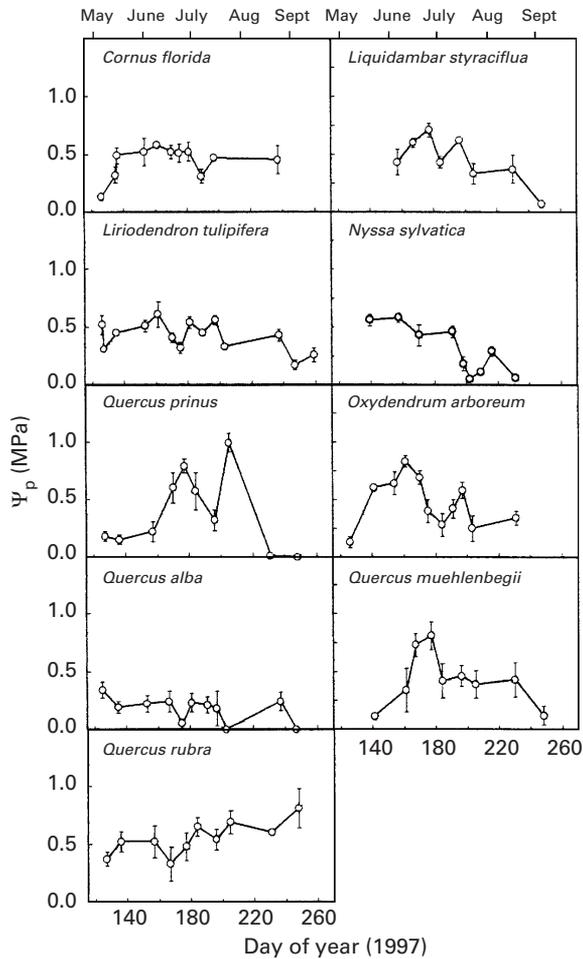


Fig. 9. Seasonal shoot turgor potential (Ψ_p) at time of g_s measurements in 11 tree species. Symbols represent weekly means ± 1 SE ($n = 6-8$ trees, and determinations on two leaf samples per tree).

the 11 species. Viewed throughout the season, increases in xylem [ABA] were correlated with decreases in g_s for *N. sylvatica*, *Q. alba* and *Q. prinus*, as indicated by negative correlation coefficients. Xylem [ABA] was positively, significantly correlated with g_s in *Lir. tulipifera*.

Stomatal conductance was significantly correlated with shoot Ψ for four of the 11 species. Stomatal conductance declined as shoot Ψ declined in *A. rubrum* and *Liq. styraciflua*, and shoot Ψ declined as g_s increased in *O. arboreum* and *Q. rubra*. The latter relationship suggests that high g_s and transpiration were driving shoot Ψ in *O. arboreum* and *Q. rubra*, rather than vice versa, which is reasonable as shoot Ψ remained above -1.5 MPa in these species (above levels that would evoke much drought response).

The best g_s correlations for individual species were with air temperature in *Q. prinus* ($R = 0.52$) and *Q. alba* ($R = 0.49$). The best correlation of g_s with a plant variable was for shoot Ψ_π in *Liq. styraciflua* ($R = 0.46$).

Multiple variable regression analyses. A model developed for maize relating g_s to xylem sap [ABA] and

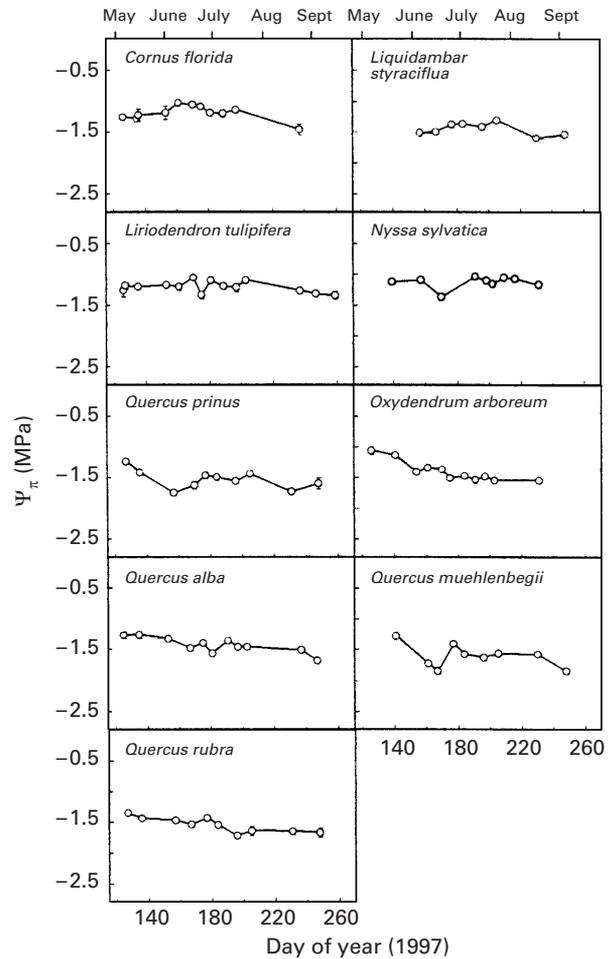


Fig. 10. Seasonal shoot osmotic potential (Ψ_π) at time of g_s measurements in 11 tree species. Symbols represent weekly means ± 1 SE ($n = 6-8$ trees, and determinations on two leaf samples per tree).

leaf Ψ (Tardieu & Davies, 1993; Tardieu *et al.*, 1993 see the Materials and Methods section for equation) did not account for as much variation in g_s (Table 3) as most of the single environmental variable linear regressions (Table 2). The Tardieu model explained a significant amount of seasonal variation in g_s in four of the 11 tree species, whereas air temperature, for example, explained a significant amount of seasonal variation in g_s in seven of the 11 tree species. The Tardieu model was fairly successful in accounting for variation in g_s for *Liq. styraciflua* and *Q. prinus* ($R = 0.45$ for each); $c1(g_{s \text{ min}})$ was removed from the Tardieu model, as values of R were higher without it.

Other variables were just as successful as [ABA] and shoot Ψ when substituted into the Tardieu double exponential equation (data not shown). Modifying sensitivity of g_s to [ABA] by shoot Ψ_π rather than shoot Ψ , for example, gave an R of 0.29 averaged across species, versus the mean R of 0.25 for the [ABA]:shoot Ψ relation. The pH:shoot Ψ double exponential model gave a mean R value of 0.22. Modifying stomatal sensitivity to either [ABA] or pH by VPD, PPFD or air temperature gave

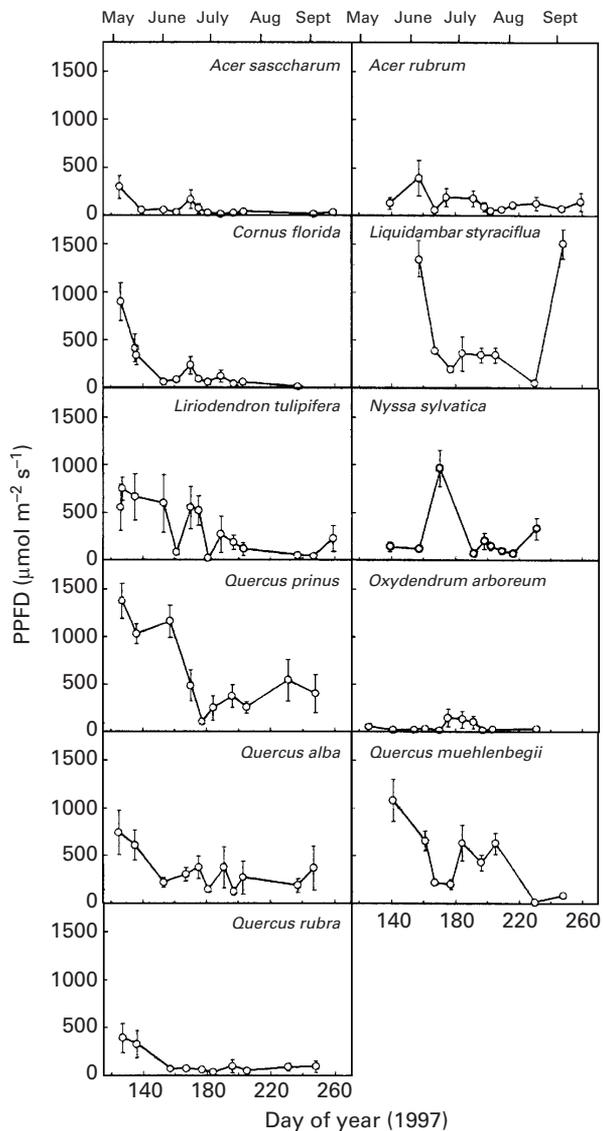


Fig. 11. Photosynthetic photon flux density (PPFD) on leaves at time of g_s measurements in 11 tree species. Symbols represent weekly means ± 1 SE ($n = 6-8$ trees, four leaves per shoot, one shoot per tree).

higher R values than modifying stomatal sensitivity to [ABA] or pH by shoot Ψ . PPFD:VPD gave the highest mean R value for the double exponential model (0.36), with nine species showing significant correlations.

Other multiple variable models were also tested with the same combinations of variables as for the double exponential. All non-linear models performed no better than the double exponential, with R values being generally low and only occasionally significant (data not shown). It became apparent that the variability in the data (e.g. Fig. 13) could not be explained by a model with only a few parameters. Therefore, the more flexible response-surface models were tried. The exponential response surface gave no better performance than the linear response surface, so the latter models were focused on.

Correlation coefficients for the [ABA]:shoot Ψ response surface (mean of 0.36 across species) and

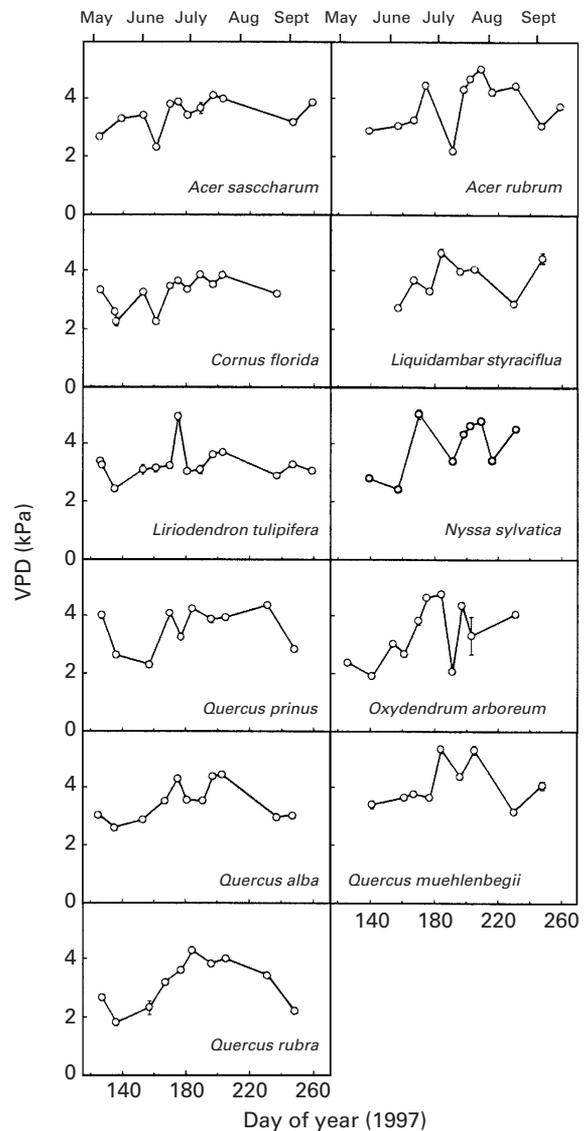


Fig. 12. Vapor pressure deficit (VPD) within shoots at time of g_s measurements in 11 tree species. Symbols represent weekly means ± 1 SE ($n = 6-8$ trees, one shoot per tree).

the pH:shoot Ψ response surface (mean of 0.34) were higher than corresponding R values for the Tardieu models based on those variables (0.25 and 0.22, respectively). Again, substituting shoot Ψ_π for shoot Ψ improved the correlation of g_s with [ABA] and one Ψ component. As in the double exponential models and the single-variable models, response surfaces based on the environmental variables accounted for the greatest variation in g_s (columns 6-9 of Table 3; R (averaged across species) = 0.44-0.46). The response surface showing a significant correlation for the most number of species (10 of the 11 species) was the shoot Ψ_π :air temperature model, which incorporated the best single plant predictor (shoot Ψ_π) and the best single environmental predictor (air temperature).

The response surface giving the highest correlation coefficient for g_s within an individual species

Table 1. Regression analyses for stomatal conductance using plant and environmental variables

Species	Regression variables								Model R^2
	Leaf size	Trunk diameter	Time of day	Air temperature	Leaf temperature	Air velocity	VPD	PPFD	
<i>Acer rubrum</i>			a*						0.34
<i>Acer saccharum</i>							*		0.17
<i>Cornus florida</i>	*		*			*			0.37
<i>Liquidambar styraciflua</i>			*	*	*	*		*	
<i>Liriodendron tulipifera</i>			*					*	0.27
<i>Nyssa sylvatica</i>								*	0.23
<i>Oxydendrum arboreum</i>		*							0.31
<i>Quercus alba</i>	*								0.50
<i>Quercus muehlenbergii</i>					*		*		0.30
<i>Quercus prinus</i>	*		*	*					0.55
<i>Quercus rubra</i>	*								0.23

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

was air temperature: VPD for *Q. alba*, with an R value of 0.65 (Table 3). The response surface based on plant variables that gave the best correlation with g_s within an individual species was pH:shoot Ψ for *O. arboreum*, with a mean R value of 0.56. Fig. 14 depicts the Tardieu relationship for some representative species, as well as the best overall two-variable response surface models involving plant variables (xylem sap [ABA]:shoot Ψ_p), environmental variables (air temperature:PPFD) and one plant and one environmental variable (shoot Ψ_p :air temperature). Constructing response surfaces that model g_s with more than two variables generally increased correlation coefficients somewhat, but did not increase the number of species showing significant correlations (data not shown). The three-variable response surfaces having the highest R values averaged across species were the air temperature: VPD:PPFD ($R = 0.55$; three environmental variables), shoot Ψ_p :PPFD: VPD ($R = 0.54$) and shoot Ψ :PPFD:air temperature ($R = 0.54$) response surfaces. Mean correlation coefficients did not differ among four-variable response surfaces, ranging from 0.56 to 0.61.

DISCUSSION

Seasonal fluctuations in plant variables are summarized in the Results section to give a sense of tree response to the environmental conditions of 1997, which proved to be a reasonable year to test our hypotheses. There were no dramatic climatic extremes, yet there was sufficient variability in precipitation and soil moisture to provide a range in values of g_s and other plant and environmental variables.

The recent discovery of non-hydraulic, root-sourced stress signals is changing our understanding of the way in which plants 'sense', and stomata respond to, fluctuations in soil moisture. Previously,

it had been widely held that g_s was hydraulically regulated by leaf Ψ or turgor (Kramer & Boyer, 1995), at least in anisohydric plants (Tardieu *et al.*, 1996; Tardieu & Simmoneau, 1998). However, there have been several reports of instances where g_s was inhibited in drying soils even in the absence of perturbations in leaf water status (see Davies *et al.*, 1994, for review). Such studies suggest that stomatal closure resulting from soil water depletion is mediated by changes in root water status through effects on the chemical flow of information from root to shoot. The existence of 'nonhydraulic' root signals has been demonstrated or suggested in several woody species, including *Acer pseudoplatanus* (Khalil & Grace, 1993), *Prunus avium* \times *pseudocerasus* (Gowing *et al.*, 1993), *Malus* \times *domestica* (Gowing *et al.*, 1990), *Prunus dulcis* (Fußeder *et al.*, 1992), *Rosa hybrida* (Augé & Duan, 1991), some coniferous species (Jackson *et al.*, 1995) and *C. florida*, *O. arboreum*, *L. tulipifera*, *A. rubrum*, *Chionanthus virginicus* and *Halesia carolina* (Crocker *et al.*, 1998). Much evidence supports the hypothesis that the movement of ABA from roots to shoots in xylem acts as the stress signal (Davies *et al.*, 1994). Other evidence suggests that stomata might be responding to changes in apoplastic pH that occur in response to changes in soil moisture (Hartung *et al.*, 1998). Whether or not the ABA moving to guard cell complexes is predominantly of leaf or root origin (Saliendra *et al.*, 1995; Liang *et al.*, 1997; Thompson *et al.*, 1997; Hartung *et al.*, 1998), g_s has often been more closely correlated with concentrations of ABA in leaf and shoot xylem than with leaf Ψ or soil Ψ .

Our purpose was to determine if seasonal g_s of forest trees is better correlated with plant chemical factors ([ABA] or pH of xylem fluid), plant hydraulic factors (shoot Ψ components) or environmental factors (PPFD, VPD, air temperature, air velocity). Xylem sap [ABA] was not strongly associated with g_s in the trees studied here. Stomatal conductance:

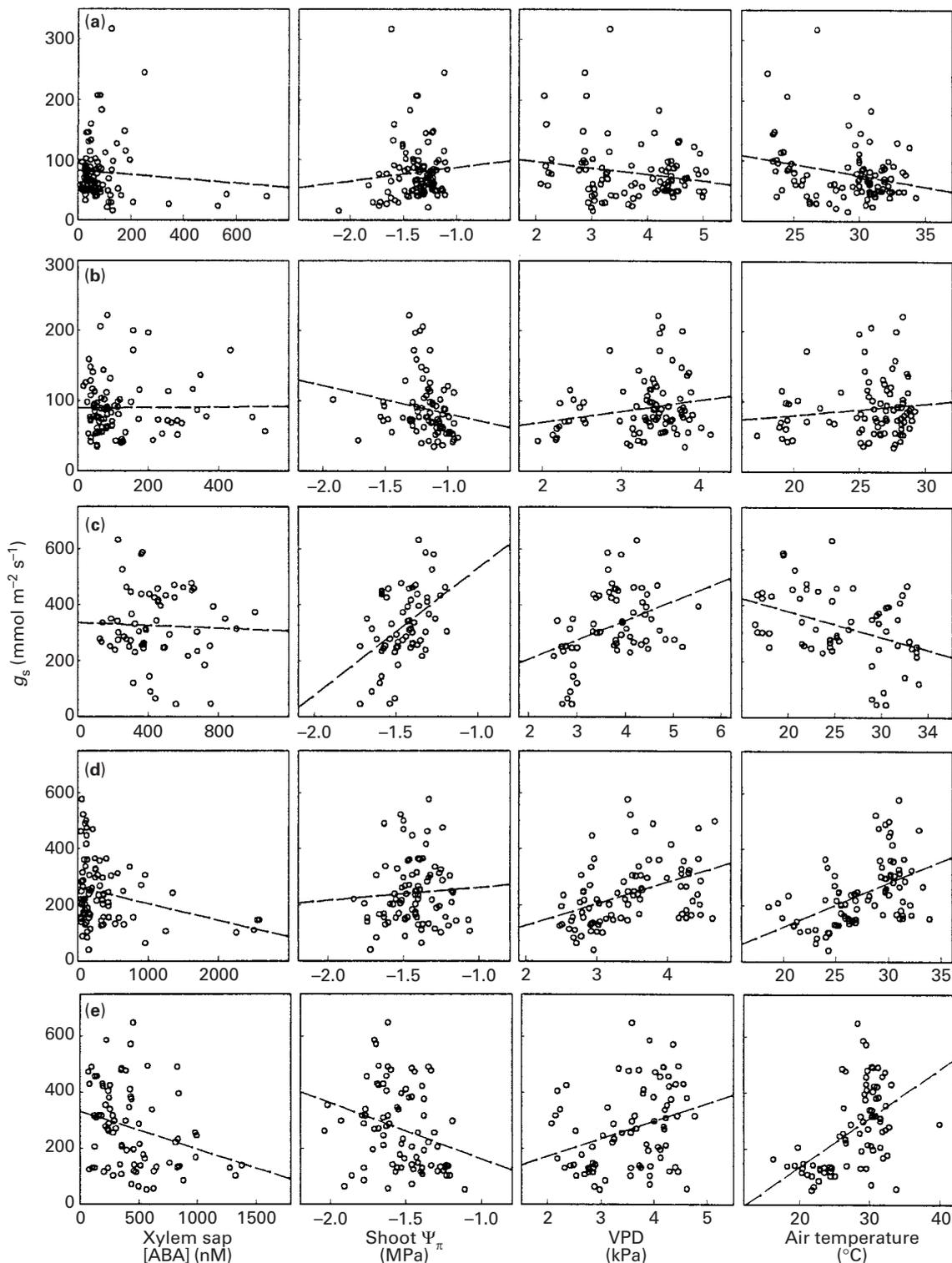


Fig. 13. Relationship between seasonal stomatal conductance (g_s) and some plant and environmental variables, for five representative tree species (*Acer rubrum* (a), *Cornus florida* (b), *Liquidambar styraciflua* (c), *Quercus alba* (d) and *Q. prinus* (e)). Ψ_π , osmotic potential; VPD, vapor pressure deficit; [ABA], ABA concentration in xylem sap. Each point represents a sample from one tree and 1 d (mean of four leaves for the tree); g_s was measured from May to September 1997. The dashed line represents the 1–1 line of perfect prediction. Correlation coefficients and significance of correlation for these and other single variable regressions are listed in Table 2.

[ABA] correlation coefficients were mostly higher than those observed previously in large *Quercus* trees under natural conditions (Triboulet *et al.*, 1996) and lower than those observed previously in *Juglans nigra*, *A. saccharum* and *Q. alba* (Loewenstein &

Pallardy, 1998b). Averaged across species, g_s correlation coefficients were slightly higher with shoot Ψ than with xylem [ABA], but effectively g_s appeared to be similarly linked with shoot Ψ and xylem [ABA] throughout the season. Strength of the correlation

Table 2. Correlation coefficients (R) for single variable models explaining stomatal conductance

^a Species	Shoot Ψ	Shoot Ψ_{π}	Shoot Ψ_p	[ABA]	Xylem pH	VPD	PPFD	Air temperature	Air velocity
<i>Acer rubrum</i>	^b 0.27**	0.16	—	−0.08	−0.03	−0.18	0.24*	0.28**	0.19
<i>Acer saccharum</i>	0.01	0.17	—	−0.06	0.12	0.25**	0.23*	0.17	0.01
<i>Cornus florida</i>	−0.17	−0.17	−0.06	0.01	−0.05	0.20	0.17	0.13	0.24*
<i>Liquidambar styraciflua</i>	0.37**	0.46**	0.21	−0.04	−0.15	0.39**	−0.11	0.39**	0.17
<i>Liriodendron tulipifera</i>	−0.07	−0.12	−0.00	0.31*	−0.15	0.43***	0.41***	0.33***	0.18
<i>Nyssa sylvatica</i>	0.14	−0.27*	0.27*	−0.25*	−0.02	0.05	0.28*	0.05	0.24*
<i>Oxydendrum arboreum</i>	−0.21*	−0.23*	−0.06	0.17	−0.30**	0.14	0.19	0.37***	0.06
<i>Quercus alba</i>	−0.02	0.07	−0.16	−0.27*	0.14	0.44***	0.13	0.49***	0.06
<i>Quercus muehlenbergii</i>	−0.01	−0.23	0.10	0.02	−0.12	0.01	0.28*	0.23	0.23
<i>Quercus prinus</i>	−0.27	0.31*	−0.34	−0.34**	0.09	0.32**	−0.18	0.52***	0.06
<i>Quercus rubra</i>	−0.33**	−0.17	−0.26*	−0.02	0.04	0.29**	0.01	0.27	0.11
^c Mean, g_s	0.17	0.21	0.16	0.14	0.11	0.25	0.20	0.29	0.14
^d Mean, transpiration	—	0.22 (5)	—	0.15 (3)	0.16 (4)	—	0.54 (10)	—	—

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

^b Asterisks indicate significance of regression (R^2) at $P < 0.05$ (*), 0.01 (**) or 0.001 (***).

^c Mean correlation coefficients for g_s across species for each variable were computed from absolute values of species correlation coefficients.

^d For comparative purposes, mean correlation coefficients across species are also provided for transpiration. Numbers in parentheses indicate the number of species that showed significant correlation, $P = 0.05$. — indicates regression not computed, as the variable was used in calculating transpiration (or, in the case of shoot Ψ_p , shared a variable).

Table 3. Multiple correlation coefficients (R; always positive) for various two-variable models explaining stomatal conductance

Species	^a Tardieu	[ABA]/ shoot Ψ	[ABA]/ shoot Ψ_{π}	pH/ shoot Ψ	Air temp/ PPFD	Air temp/ VPD	VPD/ PPFD	Shoot Ψ_{π} / air temp
<i>Acer rubrum</i>	0.14	^b 0.52***	0.47***	0.36*	0.53***	0.44**	0.44***	0.37*
<i>Acer saccharum</i>	0.21	0.22	0.28	0.30	0.42**	0.28	0.43***	0.35*
<i>Cornus florida</i>	0.24	0.24	0.36	0.34	0.25	0.33	0.30	0.37
<i>Liquidambar styraciflua</i>	0.45***	0.47*	0.46*	0.39	0.49**	0.62***	0.61***	0.60***
<i>Liriodendron tulipifera</i>	0.24	0.32	0.28	0.30	0.55***	0.48***	0.52***	0.38*
<i>Nyssa sylvatica</i>	0.30*	0.34	0.48**	0.22	0.35	0.26	0.34	0.43*
<i>Oxydendrum arboreum</i>	0.22	0.29	0.36	0.56***	0.42**	0.44**	0.29	0.42**
<i>Quercus alba</i>	0.32*	0.42**	0.41**	0.31	0.61***	0.65***	0.57***	0.54***
<i>Quercus muehlenbergii</i>	0.04	0.30	0.43*	0.35	0.43*	0.49*8	0.47***	0.41*
<i>Quercus prinus</i>	0.45***	0.46**	0.45**	0.23	0.62***	0.56***	0.48*	0.61***
<i>Quercus rubra</i>	0.17	0.41*	0.31	0.40*	0.36	0.39*	0.38*	0.39*
^c Mean, g_s	0.25	0.36	0.39	0.34	0.46	0.45	0.44	0.44

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

^b Asterisks indicate significance of regression (R^2) at $P < 0.05$ (*), 0.01 (**), or 0.001 (***).

^c Mean correlation coefficients across species for each variable were compared from absolute values of species correlation coefficients.

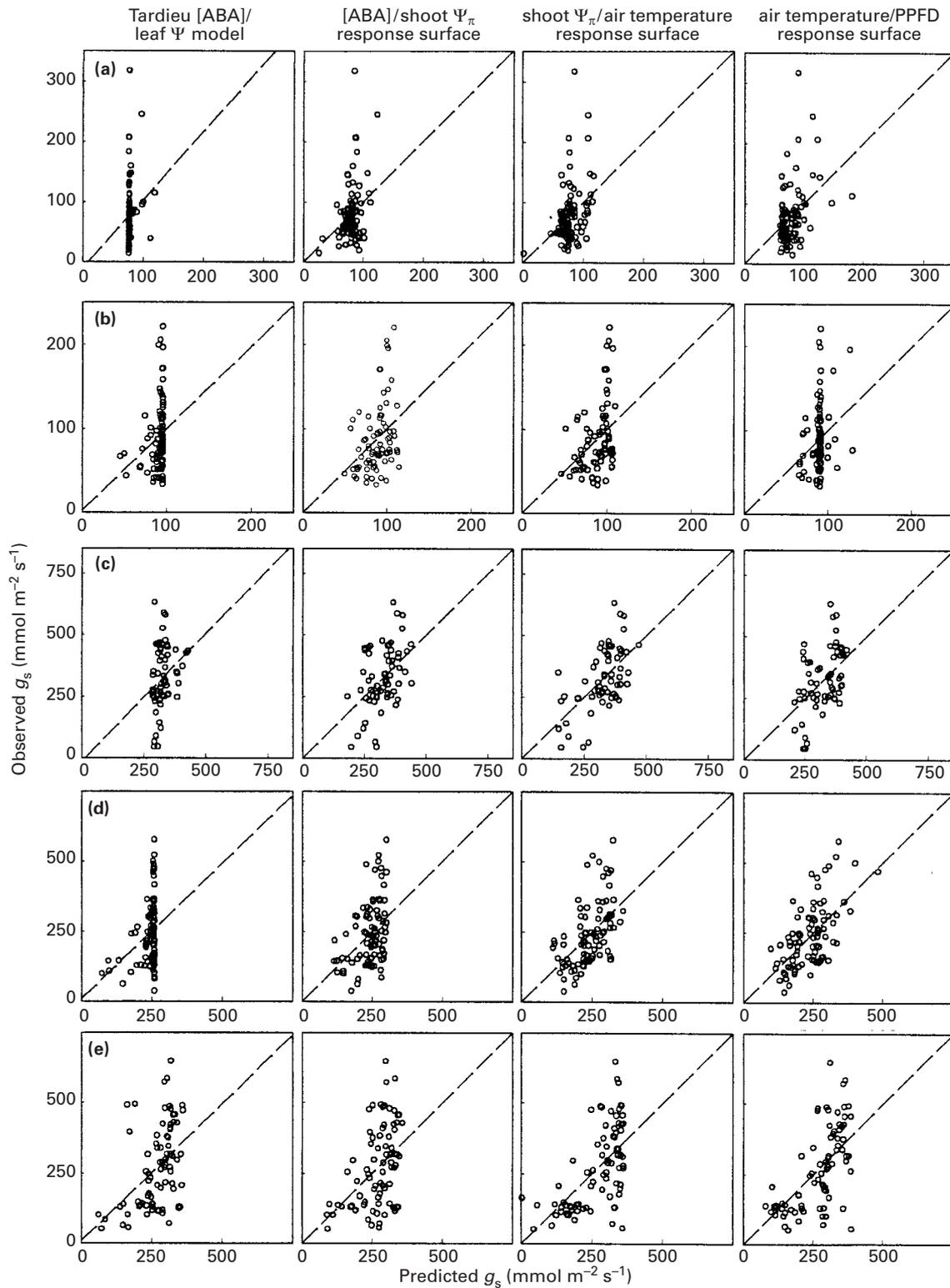


Fig. 14. Relationship between observed seasonal stomatal conductance (g_s) and predicted g_s , for five representative tree species (*Acer rubrum* (a), *Cornus florida* (b), *Liquidambar styraciflua* (c), *Quercus alba* (d) and *Q. prinus* (e)). Each point represents g_s of one tree and 1 d (mean of four leaves for the tree); g_s was measured from May to September 1997. Stomatal conductance was predicted with a double exponential model published for maize (Tardieu & Davies, 1993; Tardieu *et al.*, 1993), and with several response surfaces. The two-variable response surfaces with the best overall correlations that incorporate a plant chemical and plant hydraulic variable ([ABA]:shoot osmotic potential (Ψ_π)) and a plant and environmental variable (shoot Ψ_π :air temperature), as well as the response surface giving the best correlation among any two plant or environmental variables (air temperature:PPFD) are shown. The dashed line represents the 1–1 line of perfect prediction. Correlation coefficients and significance of correlation for these and other models are listed in Table 3.

varied considerably with tree species, but for individual species the best correlations of g_s with either Ψ or [ABA] were similar: 0.37 for g_s :shoot Ψ in *Liq. styraciflua* and 0.34 for g_s :[ABA] in *Q. prinus*. Moreover, the same number of species showed significant correlations for g_s :shoot Ψ and g_s :[ABA]. Shoot Ψ_π better explained g_s than either shoot Ψ or xylem sap [ABA], and xylem pH generally most poorly explained g_s relative to other plant and environmental variables.

The more easily measured environmental variables were generally better indicators of g_s than either the plant hydraulic or plant chemical variables. For example, g_s was significantly correlated with the air temperature surrounding leaves for each of the four species having significant g_s :shoot Ψ correlations, as well as for an additional three species. More species showed significant g_s correlations with PPF and VPD than with either [ABA] or shoot Ψ . Stomatal conductance was significantly correlated with air temperature for more species than with any other single plant or environmental variable. Moreover, the nature of the g_s correlation was more predictable for environmental than for plant variables. Unlike [ABA] or shoot Ψ components, higher PPF, VPD, air temperature and air velocity were always correlated with higher g_s , when correlations were significant. It was found that g_s was somewhat better related to changes in VPD than PPF. Transpiration, however, was relatively well correlated with PPF. Other investigators have observed that VPD explained more variation in transpiration than did PPF (e.g. Oren *et al.*, 1999).

Many investigators favoring the ideas of either chemical or hydraulic control of g_s concede that g_s is probably affected by both factors. In refining their hypotheses of regulation of g_s by xylem [ABA], Tardieu & Davies (1993) noted that an integration of hydraulic and chemical signals controlling g_s is more likely than control based on chemical signals or water relations alone. In describing the method by which leaf hydration controls g_s , Saliendra *et al.* (1995) noted that both hydroactive and hydropassive processes are likely to be important and would involve both hormonal and hydraulic mechanisms. Correia & Pereira (1995) and Thomas & Eamus (1999) also noted the likely interaction of both hormonal and hydraulic influences in modulating g_s .

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 & Davies, 1993; Tardieu *et al.*, 1993). This model accounted for more variation in g_s than either xylem [ABA] or shoot Ψ alone, in many of the forest trees studied here. Correlation coefficients were not nearly as high as those observed for maize (Tardieu *et al.*, 1993), and we would expect more variation in the model under conditions studied here than under

the conditions for which it was developed, for several reasons. In the maize field, Tardieu obtained measurements on genetically similar plants of the same age, over a short time period (3 d), under relatively high illumination. In the forest, we examined plants with much greater genetic variability, of widely different ages, seasonally from May to September, often under non-saturating illumination. 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 PPF corroborated for maize (Tardieu & Davies, 1993). We attempted to develop such plots for our tree species, both in the forest and under higher illumination using urban trees, but a strong correlation between g_s and PPF was not observed, even within one tree with measurements made over a short time period.

The mechanism by which stomata perceive an ABA signal has yet to be clearly defined, and others have addressed the question of whether the stomatal response is directly linked to xylem [ABA] or to a measure of the amount of ABA entering the leaf. The xylem sap concentration of ABA was evaluated here because most investigators have found that g_s is better correlated with [ABA] than with flux of ABA to leaves, in both herbaceous plants (e.g. Tardieu *et al.*, 1992, 1993; Tardieu & Davies, 1993; Jia *et al.*, 1996) and trees (Gowing *et al.*, 1993; Liang *et al.*, 1996; Loewenstein & Pallardy, 1998a). Fluxes of ABA were measured here and their seasonal trends were compared with [ABA]. *R* and *P* values are not summarized in Tables 2–3 for models incorporating ABA flux, because auto-correlation effects artificially inflate those values: g_s was used to compute ABA flux (g_s in computing transpiration, and transpiration in computing ABA flux).

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 & 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 best fits. The xylem [ABA]:shoot Ψ response surface was significantly correlated with g_s in more species than was the Tardieu model, and the mean correlation coefficient across species was higher. However, a response surface that modified stomatal sensitivity to xylem [ABA] by shoot Ψ_π rather than by shoot Ψ accounted for even greater variation. Of all models incorporating a plant variable, the shoot Ψ_π :air temperature response surface accounted for the most variation in tree g_s .

The shoot Ψ_π :air temperature response surface also resulted in significant g_s correlations in 10 of the 11 species, more than for any other model. As in the double exponential models, using environmental

variables in the response surfaces explained as much or more variation in g_s than using plant variables. Two-variable response surfaces incorporating any two of these three environmental variables – air temperature, PPFD, VPD – were equally robust in describing seasonal g_s and better described g_s than any two-variable combination of [ABA], pH, shoot Ψ , shoot Ψ_π or shoot Ψ_p .

Others have suggested that g_s might be regulated by xylem sap pH (Thompson *et al.*, 1997; Wilkinson & Davies, 1997) or that stomatal sensitivity to [ABA] might be modified by xylem sap pH (Jia & Zhang, 1997; Zhang *et al.*, 1997; Hartung *et al.*, 1998; Wilkinson *et al.*, 1998): pH gradients in the leaf control ABA distribution in the leaf and ABA concentration at guard cells, and thereby influence stomatal aperture. In the trees in this study, seasonal g_s was generally not associated with xylem sap pH. Stomatal conductance was significantly correlated with xylem sap pH in only one of the 11 species, and the mean correlation coefficient of g_s with pH was lower than for the other plant variables, including xylem [ABA]. Stronger correlations of g_s with [ABA] than with pH of xylem sap have been observed before, in *J. nigra*, *Salix nigra*, *A. saccharum* and *Q. alba* (Loewenstein & Pallardy, 1998a,b). We tested the hypothesis that xylem sap pH might modify stomatal sensitivity to [ABA] or to shoot Ψ and found that [ABA]:pH models, shoot Ψ :pH and Ψ component:pH models did not explain seasonal variations in g_s as well as [ABA]:shoot Ψ and [ABA]:shoot Ψ_π models.

Air humidity surrounding leaves can modify g_s (Lange *et al.*, 1971; Schulze *et al.*, 1987), and in some species g_s can be well correlated with the difference in absolute humidities between leaf and air (e.g. Assman & Grantz, 1990; Aphalo & Jarvis, 1991). In other species, g_s is relatively insensitive to VPD (Schulze *et al.*, 1987), perhaps especially in the field (Tardieu & Simonneau, 1998). Stomatal conductance of over half of the tree species here showed significant seasonal correlations with VPD, and VPD was one of the best descriptors of seasonal g_s , better than any of the plant variables. Stomatal conductance of large *Q. alba* trees has previously been shown to be better correlated with VPD than with xylem [ABA] (Loewenstein & Pallardy, 1988b), as in our study, and we found that among the 11 species, *Q. alba* had the highest g_s :VPD correlation coefficient. When g_s is related to VPD (not always the case in woody species, e.g. Turner *et al.*, 1984), the relationship is typically a negative one, g_s decreasing as VPD increases (e.g. Turner *et al.*, 1984; Ball *et al.*, 1987; Thomas & Eamus, 1999). However, in our study, in every case of significant correlation between seasonal g_s and VPD (*A. saccharum*, *Liq. styraciflua*, *Lir. tulipifera*, *Q. alba*, *Q. prinus* and *Q. rubra*), g_s increased as VPD increased. Vapor pressure deficit is greatly affected by temperature, and temperature

also affects g_s . Sometimes the g_s :air temperature relation is negative, sometimes it is positive, depending on species and temperature range (Wilson, 1948; Meyer & Anderson, 1952; Wuenscher & Kozłowski, 1971). Stomatal conductance has previously been positively correlated with temperature in sugar maple in the air temperature range of 26–30°C (Pereira & Kozłowski, 1977), and we also observed positive correlations between g_s and air temperature in each of the seven instances for which individual species correlations were significant. It is likely, therefore, that the positive g_s :VPD relationships were driven by the positive g_s :air temperature relationships. Air temperature was the single strongest descriptor of g_s among all plant and environmental variables examined.

Tardieu *et al.* (1993) also tested a model involving [ABA] and VPD and determined that in their maize system leaf Ψ better described stomatal sensitivity to ABA than did VPD. We found that, overall, VPD better described stomatal sensitivity to [ABA] than did shoot Ψ . Nine tree species showed significant correlations of g_s with the [ABA]:VPD response surface, versus five species for the [ABA]:shoot Ψ response surface. Mean correlation coefficients across all 11 species were 0.42 and 0.36 for the [ABA]:VPD and [ABA]:shoot Ψ response surfaces, respectively. Correlation coefficients were similar for the double exponential (Tardieu) [ABA]:shoot Ψ and [ABA]:VPD models, each with four species having significant correlations.

The limitations of statistical empirical modeling are recognized. The response surface models were used to identify influential variables among species in this study but not as a means of predicting g_s in the future. The response surface models used here have many parameters, which allow them to fit almost any data. However, this flexibility has the price of making the results specific to the current data. Thus, while certain variables might be important in explaining g_s , the relationships among the variables, and thus the prediction model, could very well change with field setting. As an illustration, a subset of four plants from each species was fitted with a response surface in shoot Ψ_π and air temperature, and this equation was used to predict the other plants in that species. The mean R value was 0.54 in the fitted data, but only 0.22 for predicting other plants. Many of the predicted R values were negative, indicating data with a reversed relationship compared with the four-plant subset. When this was repeated with [ABA] and shoot Ψ , the mean R value went from 0.48 to 0.10, suggesting an even less predictable relationship.

Further, the types of models chosen are highly dependent on the types of data used. The biologically-based double exponential model of Tardieu might actually be correct, but only for conditions in which the environment and plant characteristics

have been standardized. In a more variable forest situation, variables such as xylem sap [ABA] might still be operating to influence g_s , but, as we observed, other variables might be more influential. Finding the single best model might be an elusive goal.

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