

Stomatal response to nonhydraulic root-to-shoot communication of partial soil drying in relation to foliar dehydration tolerance

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Abstract

This paper culminates a series of works to: (1) compare stomatal response of several temperate, deciduous tree species to nonhydraulic root-to-shoot signals of soil drying; and (2) test whether sensitivity to nonhydraulic signaling is allied with drought avoidance/tolerance tendencies of species. Saplings were grown with roots divided between two pots. Three treatments were compared: half of the root system watered and half droughted (WD); half of the root system watered and half-severed (WS); and both halves watered (WW). Drying about half of the root system caused marked nonhydraulically induced declines in stomatal conductance (g_s) in *Nyssa sylvatica* and *Acer saccharum* but only slight declines in *Quercus alba*, *Q. rubra*, *Q. prinus* and *Q. acutissima*. Declines in g_s were significantly correlated with declining soil matric potential (Ψ_m) in three species. Soil Ψ_m when g_s of WD plants was 80% of WS controls varied from a high of -0.03 MPa in *A. saccharum* to a low of -0.18 MPa in *Q. alba*. Neither lethal leaf water potential nor osmotic adjustment was significantly correlated across all species with any measure of stomatal sensitivity to the nonhydraulic root-to-shoot signal. However, species showing considerable osmotic adjustment also tended to show little inhibition of g_s . Additionally, species showing little or no foliar osmotic adjustment also showed high stomatal sensitivity to nonhydraulic drought signaling, as indicated by relatively large changes in g_s per unit change in soil Ψ_m . Stomatal sensitivity to nonhydraulic drought signaling appears mechanistically linked to a limited extent with characteristics that define relative species drought tolerance. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Drought resistance; Nonhydraulic signaling; Partial root drying; Stomatal conductance; Temperate deciduous trees; Water relations

Abbreviations: g_s , stomatal conductance; WD, half-dried treatment; WS, half-severed treatment; WW, fully watered treatment; Ψ_m , matric potential; Ψ_π^{100} , osmotic potential at full turgor; Ψ , water potential.

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1. Introduction

The recent discovery of nonhydraulic, root-sourced stress signals is changing understanding of how plants ‘sense’ and respond to drying soil. Formerly, it was widely held that as soil moisture

becomes limiting and water uptake is restricted, shoot water deficits develop; lowered shoot water status then limits shoot growth and stomatal opening (Kramer, 1983). It is apparent that this sequence of events does occur in nature (Kramer, 1988; Boyer, 1989), but there are now many reports of instances where stomatal conductance (g_s) has been diminished in drying soils even when shoots are adequately hydrated (e.g. Davies et al., 1994). Stomatal closure resulting from soil water depletion appears to be at least partially mediated by flow of chemical information from root-to-shoot. Nonhydraulic inhibition of stomatal opening can be substantial, with declines in g_s of up to 50% or more (relative to well-watered controls) reported for both woody (e.g. Khalil and Grace, 1993; Croker et al., 1998) and herbaceous species (e.g. Zhang and Davies, 1989).

Although the mechanism of action and specific effects are still being elucidated, much evidence indicates that nonhydraulic root-to-shoot signaling is an important component of plant response to drought conditions (Davies et al., 1994). The ability to respond dynamically to changes in available soil water usually improves a plant's long-term water use efficiency, impacting growth and/or survival (Ludlow et al., 1989; Mansfield and McAinsh, 1995). Drought resistance mechanisms such as dynamic stomatal control with soil drying are often related to the geographic distribution of tree species, mainly affecting juvenile establishment and survival (Martin et al., 1987).

Nonhydraulic, root-sourced inhibition of stomatal opening likely varies among species, like other drought response characteristics (e.g. osmotic adjustment, dehydration tolerance). Plants classified as drought avoiders, which have dehydration-sensitive tissues (i.e. leaves die at relatively high leaf Ψ), may rely on nonhydraulic root-to-shoot signaling to avoid transpirational water loss and potential leaf death during drought. Conversely, plants classified as drought tolerators, having tissues better adapted to withstand dehydration (i.e. leaves die at lower leaf Ψ), might be less sensitive to nonhydraulic drought signaling because they can withstand more tissue dehydration. These functional groupings, with respect to nonhydraulic drought signaling, have not been evaluated.

Most research of nonhydraulic signaling of soil drying has focused primarily on the physiological aspects of the process (Davies et al., 1994). Our goal was to explore the ecophysiological significance of nonhydraulic signaling in the context of drought resistance. This paper culminates a series of studies aimed at comparing stomatal response of several temperate, deciduous tree species to nonhydraulic root-to-shoot signals of soil drying under standardized conditions, and then testing whether species sensitivity to nonhydraulic signaling is allied with their tendencies to avoid or tolerate drought. Stomatal responses of *Nyssa sylvatica*, *Acer saccharum*, *Quercus prinus*, *Q. rubra*, *Q. alba* and *Q. acutissima* to nonhydraulic, root-sourced signals of soil drying are reported here. Also discussed are correlations of inhibition of g_s with soil Ψ_m , lethal leaf Ψ and osmotic adjustment, physiological variables that characterize relative drought avoidance or tolerance of species (Ludlow, 1989). Stomatal sensitivity of six other tree species was previously reported (Croker et al., 1998), as was foliar dehydration tolerance of these twelve species (Augé et al., 1998).

2. Materials and methods

2.1. Plant material

Experimental plants were produced as described before (Croker et al., 1998). Two- to three-year old saplings of *N. sylvatica* Marsh. (black gum) and *A. saccharum* Marsh. (sugar maple) were planted when 0.5–1.5 m in height with the root system divided between two 11 l plastic pots (30 individuals of each species) in the fall of 1996. Standard nursery potting medium was used: 4 pine bark/1 sand, with 4 l dolomitic lime, 4 l 17N–6P–10K osmocote plus minor elements and 2 l epsom salt ($MgSO_4$) added to each m^3 . Split-root plants were allowed to overwinter and the experiment was conducted in a $24.4 \times 7.3 \times 4.1$ m ($l \times w \times h$) rain shelter (two layers of 4 ml clear polyethylene stretched over bowed aluminum supports). The shelter was covered by a 50% transmission shade cloth, to promote stomatal opening.

Also examined were four oak species: *Q. prinus* L. (chestnut oak), *Q. rubra* L. (red oak), *Q. alba* L. (white oak) and *Q. acutissima* Carruthers (saw-tooth oak). Oaks produce tap roots, thus growing oak trees with healthy, roughly equal root systems split between two pots was more difficult than for *N. sylvatica*, *A. saccharum* and the six species examined before (Crocker et al., 1998). This was accomplished by splitting the emerging radical with a razor blade 1 month after germinating acorns of each species (April 1996 for *Q. prinus*, *Q. rubra*, *Q. acutissima*; October 1996 for *Q. alba*). The two halves of the radical were divided between two square pots taped together with the acorn perched above them and soil mounded up around the acorn to prevent desiccation. After roots had grown into each of the pots (~16 weeks), trees were transplanted into larger pots and the soil was gently washed away from the acorn and top of the root mass, to remove a conductive soil bridge between pots.

Seeds of *N. sylvatica* and *Q. acutissima* were obtained from Rennerwood Nursery, Rennerwood, TX. Seeds of *A. saccharum* were obtained from Jansch Enterprises, Inc., Wartburg TN. Acorns of *Q. rubra* and *Q. alba* were obtained from Lovelace Seeds, Inc., Elsbury, MO. *Q. prinus* acorns were obtained from the East Tennessee Nursery, Tennessee Division of Forestry, Delano, TN.

2.2. Treatments and environmental conditions

Each species had three split-root treatments. Water was withheld from one of the tree's two pots for a sustained drying period for one group of trees (WD). A two-control system was used as follows. In one control group, one half of the root system was left intact and watered while the other half was severed (on 3 July 1997) prior to the drought episode (WS). In the second control group, both halves of the root system were left intact and were watered every day or two as needed throughout the experiment (WW).

The drying episode was initiated on 8 July 1997 (hereafter referred to as day 0) by withholding water from one pot of all WD individuals; the drying period continued until October 1997. The

second pot of WD plants, the remaining pot of WS plants, and both pots of WW plants were watered as needed throughout the experiment, about every other day. Air temperature and PPF were measured at 1 s intervals and averages recorded hourly throughout the experiment with a thermocouple and a quantum light sensor (LI-189, LiCor, Lincoln, NE, USA), respectively, connected to a datalogger (21x, Campbell Sci., Logan, UT, USA).

2.3. Water status measurements

Bulk soil matric potential (Ψ_m) was measured and recorded every 4 h throughout the drying period for the drying pots of WD plants, using heat dissipation sensors (SoilTronics, Burlington, WA, now available as 229-L, Campbell Sci.) connected through multiplexers (AM32, Campbell Sci.) to a datalogger (21x, Campbell Sci.) as described earlier (Augé et al., 1994). Sensors were calibrated, then dipped in a kaolinite slurry to improve sensor/soil contact and buried in pots about 8 cm from the perimeter of the pot and about 12 cm deep.

Stomatal conductance was measured with a diffusion porometer (AP3, Delta-T Devices, Cambridge, UK). Preliminary, pre-drying, diurnal g_s measurements of all plants were used to determine the time of day during which g_s was reasonably constant, and during which g_s was subsequently measured throughout the drying episode (about 1000–1230 hours EST). Measurements of g_s , which began day -15, were conducted about every other day for 10 weeks and every few days thereafter. The porometer sensor head was placed parallel to the midvein and in the center of the newest, fully expanded, unshaded leaves of each individual. Each day, 3–4 preliminary measurements of g_s were made on each replicate to determine which branches were exhibiting the highest g_s . Four measurements were then recorded for each replicate, two leaves each from the two branches with the highest g_s . To control for possible diurnal effects, g_s was sampled in a specific order each day: one WW, one WD, one WS and one WD plant were measured in that order for a species, then one WW, one WD, one WS and one

WD plant for another species, etc. This pattern was repeated until all replicates of all species were measured. Each day the species order and treatment sampling order were maintained, but the first species sampled was shifted (i.e. day one: *A. saccharum*, *N. sylvatica*, *Q. acutissima*, ...; day two: *N. sylvatica*, *Q. acutissima*, *Q. alba*, ...; etc.). Replicates were blocked in this way so that within each block, one set of WW, WD and WS replicates for each species was always measured within about 10 minutes, minimizing potential confounding effects due to environmental changes throughout the day. The drying episode lasted 48–89 days, depending upon varying soil drying rates among species. After g_s measurements ceased, roots were excavated and dry weights (80 °C for 1 week) ascertained.

2.4. Hypotheses, experimental design and statistical analysis

The experiments were designed to test three hypotheses: (1) within each species, g_s will be significantly reduced in response to nonhydraulic signals of soil drying; (2) species will vary in sensitivity to nonhydraulic signals of partial soil drying; and (3) across species, stomatal sensitivity to nonhydraulic signals of partial soil drying will be correlated with foliar dehydration tolerance.

A six (species) \times three (treatments) factorial design was used. For each species, 16 of the healthiest plants with the most evenly divided roots were identified, then eight were randomly selected for the WD treatment, four for the WS treatment and four for the WW treatment. Plants were arranged in three blocks around the datalogger with treatments and species randomized in each block.

Daily treatment means were statistically compared within each species using linear contrasts and the General Linear Models Procedure for ANOVA (SAS, Cary, NC). Regression analyses were used to describe relationships between relative g_s and declining soil Ψ_m for each species. Correlation analyses were used to test relationships between variables defining stomatal sensitivity to partial soil drying and variables defining foliar dehydration tolerance. Pooled standard errors of the means were calculated by taking

square roots of the error mean squares and dividing them by the square root of the number of observations in a mean.

3. Results

3.1. Stomatal conductance versus time

Stomatal conductance of each WD tree is expressed relative to the average of its WS controls for that day (hereafter referred to as 'relative g_s ') in Fig. 1. Drying reduced relative g_s of WD trees of *A. saccharum* soon after water was withheld from the one pot, to 53% of WS trees by day 16. Relative g_s of *A. saccharum* then recovered to control (WS) or near control values for about the next 3 weeks, declining again near the end of its 7-week drying episode. Relative g_s of *N. sylvatica* also declined fairly rapidly, dropping to 55% of WS trees within 2 weeks of withholding water from one pot. Relative g_s remained inhibited for about 2 weeks and then, as in *A. saccharum*, recovered to control values, where it remained for the rest of the drying period. A substantial, temporary decline in g_s of WS trees immediately following severing of half the root system was probably the reason for the upward spike in g_s of WD relative to WS trees observed in *N. sylvatica* on day 0. As a group, the oaks displayed little stomatal inhibition to partial soil drying. Mean relative g_s dropped to about 80% in each *Quercus* species sometime during their 9–11 week drying periods but was significantly different from WS controls only in *Q. acutissima* and only on one day. Therefore, hypothesis (1) is accepted for only two of the six species. Hypothesis (2) is accepted: species did vary in sensitivity to nonhydraulic signals of partial soil drying, as evidenced both by number of days that g_s was reduced and the degree to which g_s was reduced.

Fig. 2 depicts how trees with half of their root systems severed and the other half watered fared in relation to trees whose root systems remained intact and fully watered; it is a characterization of how g_s responded to the root severing treatment. In the oaks, g_s of WS controls were mostly similar to those of WW controls, particularly during the

first 5 weeks of the drying period. Except for 4 days where g_s of WS plants was unusually high, this was also the case for *A. saccharum*. Severing half of the root system tended to reduce g_s in *N. sylvatica*.

Absolute g_s fluctuated in each species throughout the experiment (Fig. 3). Fluctuations were fairly consistent among treatments and across species from day to day. Irradiance and air temperature during the experiment are depicted in Fig. 4.

3.2. Stomatal conductance of WD trees versus soil matric potential

Drying rates in the one pot of WD trees differed among species and among individuals within each species (Fig. 5). Fig. 6 shows relative g_s of WD trees, grouped by progressively drier soil Ψ_m . In *A. saccharum* and *N. sylvatica*, the extent of inhibition in g_s was linked to the extent of decline in soil moisture within the one drying pot of WD trees. To a lesser extent this was also true of *Q.*

acutissima and *Q. alba*, although drying soil caused much less inhibition of g_s in the oaks. In *A. saccharum* and *N. sylvatica*, g_s began to decrease at relatively high soil moisture levels (before soil Ψ_m declined). In *A. saccharum*, for example, g_s declined to 92% of WS trees with some drying but when average soil Ψ_m was still -0.01 MPa (Fig. 6). With more drying (ordering the relative g_s values of the previous 'All -0.01 ' group by date and averaging half of the values, those of plants that had been drying the longest but whose soil Ψ_m had not yet declined below -0.01 MPa), g_s dropped to 87% of controls. Further drying of soil in the one pot, to -0.02 to -0.10 MPa, resulted in further decreases in g_s , to 73% of WS controls. Drying of soil below -0.10 MPa did not depress g_s further in *A. saccharum*. In *N. sylvatica*, g_s did decline further when soil Ψ_m dried into the range of -0.10 to -1.0 MPa. Stomatal conductance did not recover at the driest soil Ψ_m in *N. sylvatica* or *A. saccharum*, as it did in half of the tree species studied previously

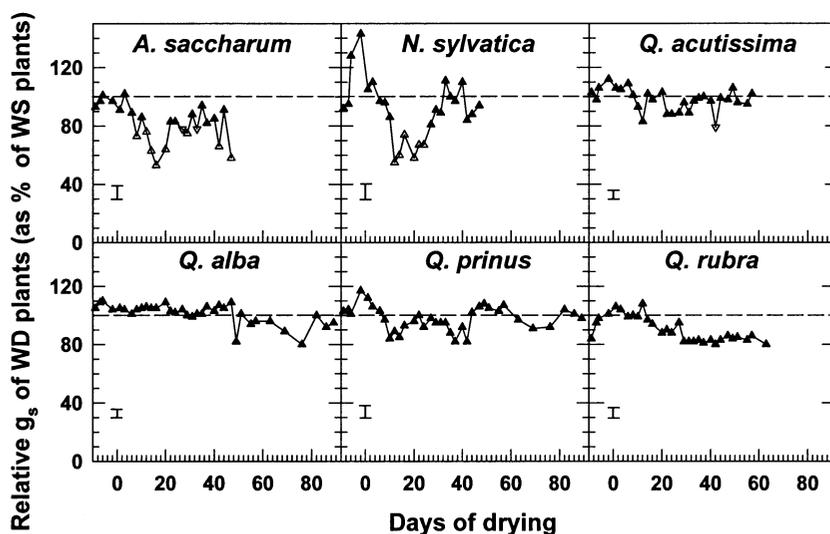


Fig. 1. Stomatal conductance (g_s) during the drying period of WD trees (about half the root system unwatered), relative to WS control trees (about half the root system severed). Daily relative g_s of each of the eight WD trees of each species was calculated as absolute g_s of an individual WD tree divided by the average absolute g_s of the four WS trees of that species for that day, expressed as percent: WD relative $g_s = (\text{WD absolute } g_s) / (\text{WS average absolute } g_s) \times 100$. Each individual tree's absolute or relative g_s value represents the average of four leaves. Roots in one pot were severed in WS trees on 3 July 1997 (day -5), and water was withheld from the one pot of WD trees beginning 8 July 1997 (day 0). Symbols show when average g_s of WD trees ($n = 8$) were significantly different from WS controls ($n = 4$; ▲ NS, △ $P \leq 0.05$, ▽ $P \leq 0.1$) as determined by ANOVA. Vertical bars in each panel represent pooled standard errors of the means.

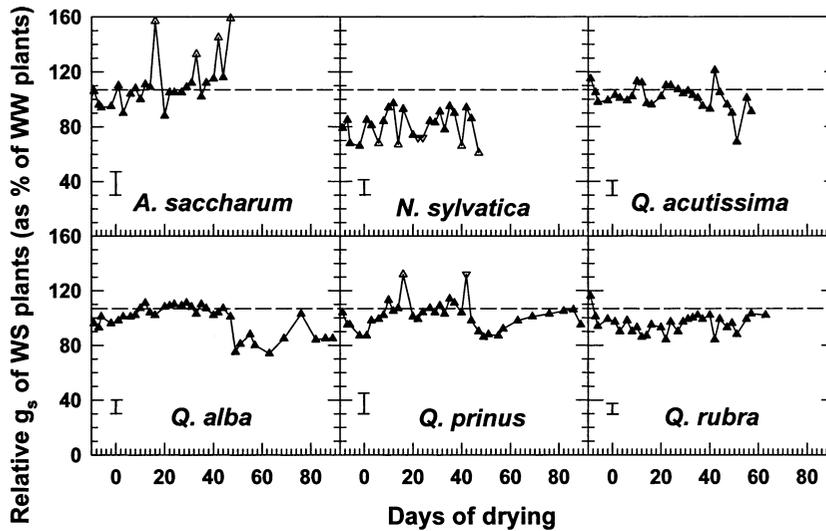


Fig. 2. Stomatal conductance (g_s) during the drying period of WS trees (about half the root system severed on 3 July 1997, day -5), relative to WW controls (both halves of the root system regularly watered). Daily relative g_s of each of the four WS trees of each species for that day, expressed as percent: WS relative $g_s = (\text{WS absolute } g_s) / (\text{WW average absolute } g_s) \times 100$. Each individual tree's absolute or relative g_s value represents the average of four leaves. Symbols show when average g_s of WS trees ($n=4$) were significantly different from WW controls ($n=4$; ▲ NS, △ $P \leq 0.05$, ▽ $P \leq 0.1$) as determined by ANOVA. Vertical bars in each panel represent pooled standard errors of the means.

(*A. rubrum*, *C. florida*, *O. arboreum*). Of the eight tree genera studied, *Quercus* consistently showed the least stomatal response to partial soil drying, whether viewed as a function of time (Fig. 1) or soil Ψ_m (Fig. 6). Each oak showed some inhibition of g_s in one or more of the dry soil ranges (Fig. 6), but inhibition was slight, never more than about 10%.

Relative g_s was positively correlated with soil Ψ_m between -0.01 and -0.10 MPa for *A. saccharum* and with soil Ψ_m between -0.01 and -0.04 MPa for *Q. acutissima* and *Q. rubra* (Fig. 7). Below -0.10 MPa relative g_s and soil Ψ_m were not correlated. Rates of decline in relative g_s as a function of soil Ψ_m (i.e. slopes of the regressions depicted in Fig. 7) varied among species, especially among oaks, with *Q. acutissima* having the largest slope. Soil Ψ_m required to cause declines in g_s of WD plants to 80% of WS controls varied from a high of -0.032 MPa in *A. saccharum* to a low of -0.182 MPa in *Q. alba* (Table 1).

3.3. Root dry weights

Average root dry weights of the watered half of WD trees were as large or larger than those of the watered side of WS trees for *A. saccharum* and *N. sylvatica* (Fig. 8). Watered root systems were similar in mass in WS and WD treatments in *Q. prinus* and *Q. rubra*. Watered root systems were smaller in WD than WS treatments in *Q. acutissima* and *Q. alba* but similar in WD and WW treatments in *Q. prinus* and *Q. rubra*. Hence, higher g_s of WS trees relative to WD trees did not appear to be linked to larger watered root systems for any species.

4. Discussion

Most experimenters using split-root systems to investigate nonhydraulic root-to-shoot signaling of soil drying have compared half-dried plants to controls that received more water (e.g. Blackman and Davies, 1985; Augé and Duan, 1991; Bano et

Table 1
Stomatal sensitivity^a of twelve deciduous tree species in relation to foliar dehydration tolerance^b

Species	Lethal ψ^c (MPa)	Leaf ^d $\Delta\psi_{\pi}^{100}$ (MPa)	Soil ψ_m at 80% g_s^e (MPa)	Slope	Maximum inhibition ^e of g_s –0.10 to 0 MPa (%)	Maximum inhibition of g_s –2 to 0 MPa (%)
<i>Nyssa sylvatica</i>	–2.04a ^f	–0.15b	–	–	97	55
<i>Liriodendron tulipifera</i>	–2.38a	–0.23bc*	–0.033 ^e	13.1	44	44
<i>Quercus rubra</i>	–3.34b	–0.604e*	–0.047	5.3	83	80
<i>Oxydendrum arborescens</i>	–3.98bc	0.23a	–0.013	10.2	62	62
<i>Halesia carolina</i>	–4.11bcd	–0.674e*	–0.027	6.7	45	45
<i>Acer rubrum</i>	–4.43cd	–0.10b	–0.044	10.6	45	45
<i>Quercus alba</i>	–4.60cd	–0.684e*	–0.182	1.4	82	82
<i>Cornus florida</i>	–4.88de	–0.52cd*	–0.032	7.0	57	48
<i>Chionanthus virginicus</i>	–5.63ef	–1.73f*	–0.026	7.9	64	64
<i>Quercus prinus</i>	–5.73f	–0.89e*	–0.068	2.2	82	82
<i>Acer saccharum</i>	–5.76f	–0.71de*	–0.032	5.6	53	53
<i>Quercus acutissima</i>	–6.14f	–1.44f*	–0.039	9.1	83	79

^a Stomatal sensitivity to nonhydraulic root signals of soil drying was characterized as soil ψ_m required to inhibit g_s of WD trees by 20% (i.e. bring g_s to 80% of WS controls), and by extent of change in relative g_s of WD trees per unit change in soil ψ_m (slopes of the regressions depicted in Fig. 7). Relative g_s (g_s of WD trees relative to WS trees) is defined in Fig. 1. Stomatal sensitivity to nonhydraulic root signals of soil drying was also characterized as maximum inhibition of g_s , which refers to the lowest daily average relative g_s observed during the drying episode when soil ψ_m was in the range of –0.10 to 0 MPa or –2.0 to 0 MPa. Stomatal data for *A. rubrum*, *C. florida*, *C. virginicus*, *C. florida*, *C. virginicus*, and *H. carolina* from Croker et al. (1998).

^b Foliar dehydration tolerance was characterized previously as lethal leaf water potential (ψ) and active osmotic adjustment ($\Delta\psi_{\pi}^{100}$) occurring during a lethal drying episode (from Augé et al., 1998).

^c ‘Lethal’ refers to measurements made on the last surviving leaves of plants subjected to a continuous drying episode.

^d Within the $\Delta\psi_{\pi}^{100}$ column, numbers highlighted by an asterisk signify that final ψ_{π}^{100} were significantly different ($P \leq 0.05$) than initial ψ_{π}^{100} for that species (i.e. osmotic adjustment occurred).

^e Values of soil ψ_m at 80% g_s were calculated from regressions of relative g_s against soil ψ_m .

^f Within columns, numbers followed by the same letter are statistically similar (Duncan’s Multiple Range Test, $P > 0.05$). $N = 8$ to 20 for lethal leaf ψ and $\Delta\psi_{\pi}^{100}$ (except $n = 5$ for *Q. rubra*).

^g g_s values represent daily means, $n = 8$.

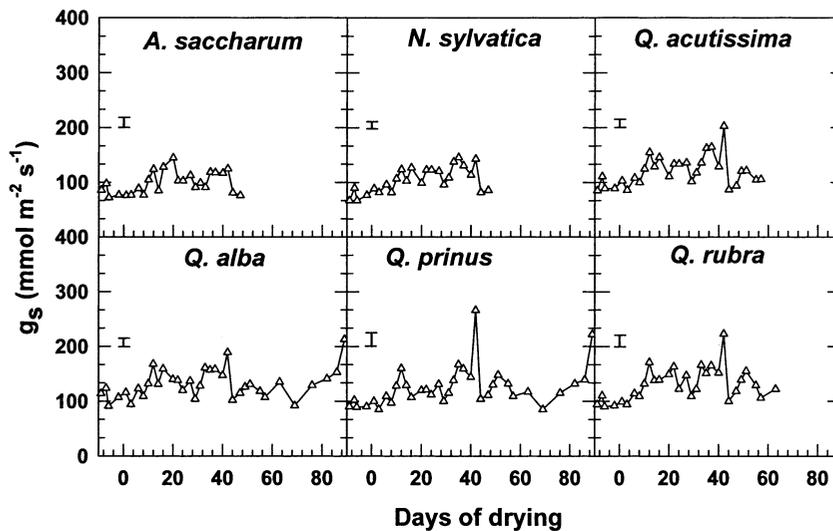


Fig. 3. Stomatal conductance (g_s) of fully watered (WW) trees (both halves of the root system watered regularly) during the experiment. Symbols represent daily tree averages ($n = 4$), four leaves per tree. Vertical bars in each panel represent pooled standard errors of the means.

al., 1993). This approach usually includes occasional measurements of leaf water status, which, if statistically similar in the two groups, is offered in support of the conclusion that differences in g_s between half-dried and fully watered plants must result from some hormonal or other biochemical means. However, relying on measurements of leaf Ψ and a control that receives about twice as much water as half-dried plants has at least three serious limitations: (1) instruments might not be sensitive enough to measure small fluctuations in leaf water status; (2) measurements of water status are sporadic and invasive, not continuous, thus overlooking possible temporary differences in leaf water status between WD and WW plants; and (3) stomatal opening in some plants is regulated during mild drought to maintain relatively stable leaf Ψ , and so leaf Ψ may not change much even when drought is severe enough that leaves have begun to dehydrate.

A more conclusive approach is to compare g_s of WD plants to a control group receiving about the same amount of water and having a similar mass of watered roots. The purpose of the WS controls in this experiment was to control for potential effects on leaf hydration caused by reducing the

water supply of the WD plants by half, relative to WW plants. This provides an unequivocal test of nonhydraulically induced stomatal inhibition (e.g. Saab and Sharp, 1989). Half-dried and WS trees

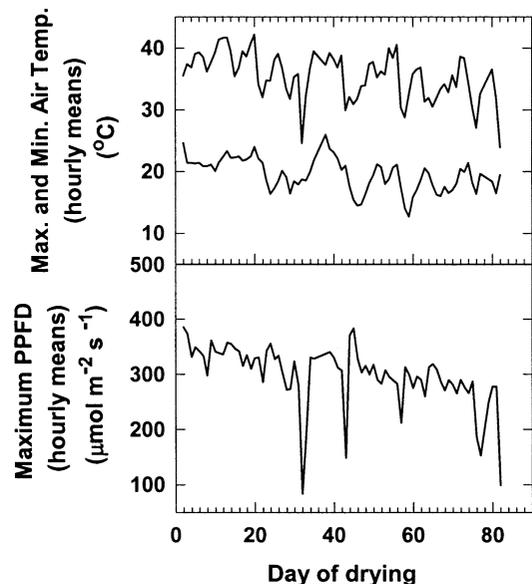


Fig. 4. Air temperatures and PPFD in the rain shelter during the experiment.

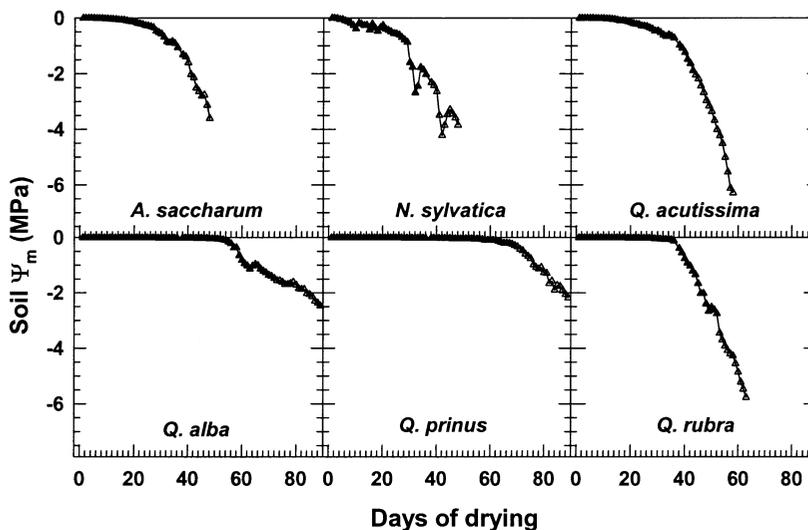


Fig. 5. Average soil Ψ_m in dried pots of WD trees during the drying episode. Symbols represent daily averages of eight WD trees and of measurements recorded every 4 h. Water was withheld from WD trees beginning day 0. Symbols (Δ) indicate the first day soil Ψ_m of dried WD pots was different from soil Ψ_m of fully watered soil ($P \leq 0.05$). On all subsequent days, soil Ψ_m of dried WD pots was significantly different from fully watered soil, for each species.

had mostly similar amounts of roots receiving water regularly, so decreases in g_s of WD trees relative to WS were not due to a direct hydraulic signal linked to a difference in water gathering capacity. Further, severing half of the root system caused g_s to decline somewhat in plants of several species, at least temporarily; hence, measures of g_s of WD as a percentage of g_s of WS plants probably underestimate WD declines somewhat, and so represent conservative estimates.

In a prior study of six temperate tree species, each showed significant and marked nonhydraulically induced inhibition of g_s , with maximum inhibition of daily average relative g_s ranging from 40 to 60% of WS controls (Croker et al., 1998). Maximum inhibition of g_s in *A. saccharum* and *N. sylvatica* in the current study was also within this range. In contrast, the oaks showed little stomatal sensitivity to the soil drying, consistent with the perception that *Quercus* is a relatively drought tolerant genus among temperate deciduous trees (e.g. Bahari et al., 1985).

Plants tend to possess mechanistically linked combinations of drought responses and morphological characteristics, and based on these groupings drought resistance of perennials has been

categorized in terms of avoidance or tolerance (Levitt, 1980; Turner and Kramer, 1980; Ludlow, 1989). Plants that have tissues with low dehydration tolerance (operationally defined as lethal leaf Ψ , Ludlow, 1989) and that depend on avoiding water deficits for survival are known as drought avoiders (Turk et al., 1980; Ludlow et al., 1983). Drought avoiders have mechanisms that maximize water uptake and/or minimize water loss (e.g. through early stomatal closure). In contrast, plants with tissues having high dehydration tolerance and only moderate avoidance mechanisms are known as drought tolerators. These plants may rely primarily on osmotic adjustment to survive drought (Ludlow et al., 1985), which aids in turgor maintenance and allows stomata to remain open to greater leaf water deficits (Ludlow, 1980; Flower and Ludlow, 1986).

Stomatal response to nonhydraulic signaling of soil drying was characterized in this study in three ways (Table 1). The lowest points in Fig. 1 depict the maximum declines in daily average relative g_s of WD trees. Soil Ψ_m required to depress relative g_s by 20% is an index of how much soil drying was necessary to initiate inhibition of g_s . Perhaps the best gauge of stomatal sensitivity to the non-

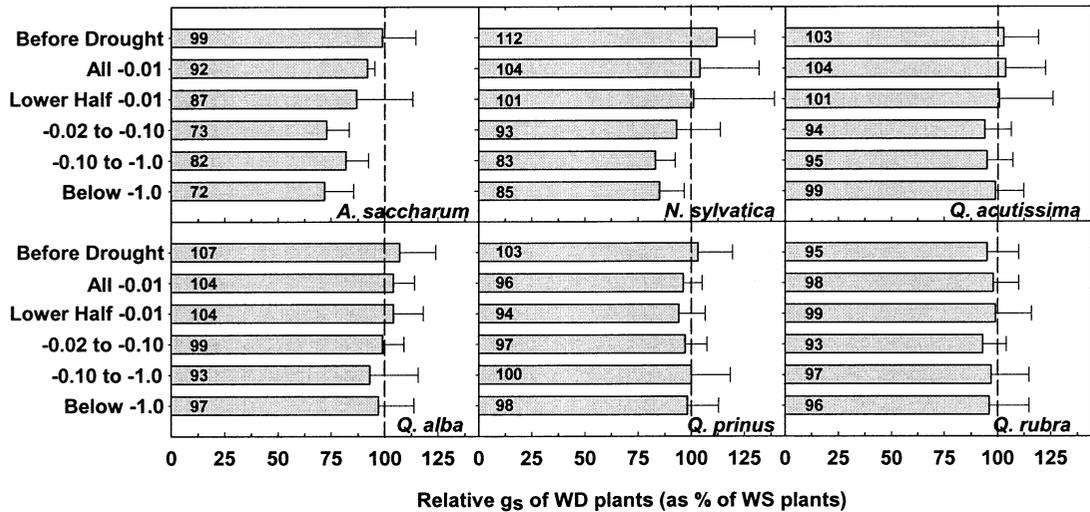


Fig. 6. Relative g_s (defined in Fig. 1 legend) of WD trees as a function of soil moisture. Each soil moisture grouping shows average relative g_s of all WD individuals within a particular soil Ψ_m range, for each species. The first group shows g_s before water was withheld from WD trees (before day 0). The next group (All -0.01) shows g_s after day 0 (after water was withheld from one pot of WD trees) but before soil lost enough water for its Ψ_m to decline (soil Ψ_m still at -0.01 MPa). The individual tree g_s of this second group were then ordered by time (days water had been withheld from the one WD pot) and g_s averaged for half of the trees of each species that had been drying the longest (i.e. the drier half of the All -0.01 MPa group), yielding values for the third group (Lower Half -0.01). Remaining groups show the average g_s of all WD individuals when soil Ψ_m was within different Ψ_m ranges: -0.02 to -0.10, -0.1 to -1.0, and below -1.0 MPa. Values within shaded bars give bar lengths. $N = 7$ to 114. Lines at right of each bar represent standard errors.

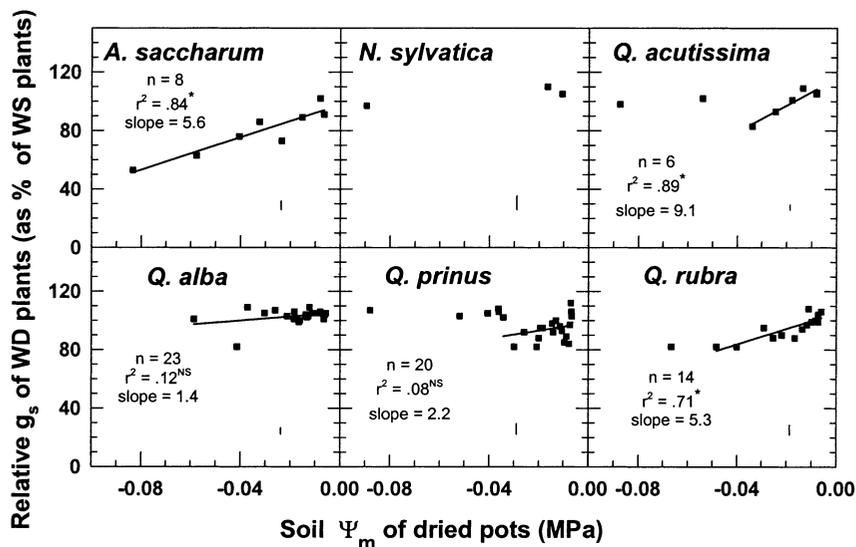


Fig. 7. Relationship between relative g_s (see Fig. 1 legend) and soil Ψ_m of the one dried pot of WD trees. Symbols represent daily averages, for both g_s and soil Ψ_m , of eight WD trees for all days after day 0 that soil Ψ_m averages fell within the range depicted. Relative g_s values (WD as % of WS trees) above 130 were not included in regressions, as these were considered to be artificially high due to temporary inhibition of g_s of WS trees following excision of half of their roots. Number of points regressed (n), correlation coefficients (r^2) and slopes are given for each species. Asterisks indicate significance of correlations ($*P \leq 0.05$).

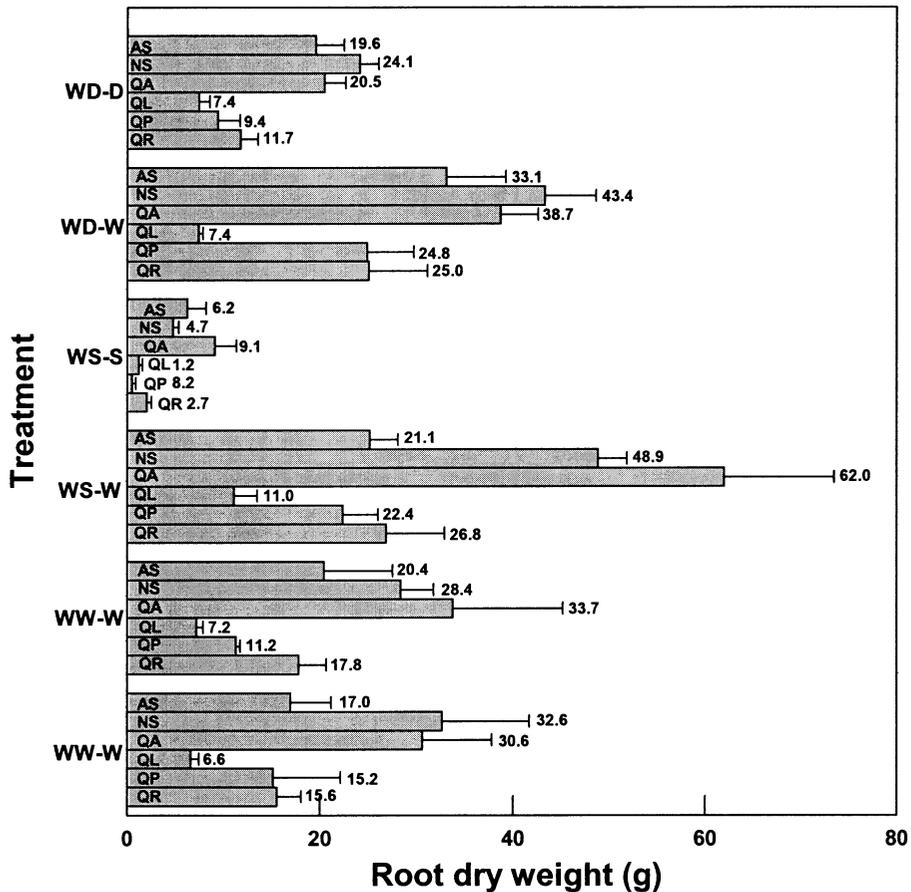


Fig. 8. Average root dry weight for dried and watered pots of half-dried trees (WD, $n = 8$), severed and watered pots of half-severed trees (WS, $n = 4$), and both watered pots of well-watered (WW, $n = 4$) trees. Lines at right of each bar represent standard errors, and values to the right of standard errors give numerical values for bar lengths. AS, *Acer saccharum*; NS, *Nyssa sylvatica*; QA, *Quercus acutissima*; QL, *Quercus alba*; QP, *Quercus prinus*; QR, *Quercus rubrum*.

hydraulic drought signaling process is the initial slope of the relative g_s /soil Ψ_m regression, which indicates how quickly g_s declined as soil Ψ_m changed in the one drying pot. The goal was to determine if these measures of stomatal inhibition by nonhydraulic root signals of soil drying were allied with the characters that define physiological drought resistance, e.g. if ability to ‘sense’ declining soil moisture, transmit this information to leaves and cause stomata to close would be greater in drought avoiders, which have evolved mechanisms to resist drought by minimizing water loss.

The 12 tree species (six in this study, six in Croker et al., 1998) represented a fairly wide range

of intrinsic drought tolerance/avoidance, with lethal leaf Ψ ranging from -2 to -6 MPa and osmotic adjustment from essentially 0 to -1.7 MPa (Table 1; Augé et al., 1998). Neither lethal leaf Ψ nor osmotic adjustment was significantly correlated across all species with any measure of stomatal sensitivity to the nonhydraulic root-to-shoot signal of soil drying (Table 2). Therefore, we reject hypothesis (3): stomatal sensitivity to nonhydraulic signals of partial soil drying was not significantly correlated with foliar dehydration tolerance across species. However, in other ways stomatal sensitivity to nonhydraulic drought signaling did appear to be inversely

Table 2

Correlation analysis of parameters listed in Table 1, characterizing the likelihood that species stomatal sensitivity to nonhydraulic signals of partial soil drying was correlated with species foliar dehydration tolerance

Correlation	Correlation coefficient (<i>R</i>)	Probability of significance (<i>P</i>)
Lethal leaf Ψ vs. soil Ψ_m at 80% g_s	0.06	0.87
Lethal leaf Ψ vs. slope (% $g_s/0.01$ MPa)	0.41	0.21
Lethal leaf Ψ vs. slope maximum inhibition of g_s from -2 to 0 MPa	-0.36	0.24
Leaf $\Delta\Psi_{\pi}^{100}$ vs. soil Ψ_m at 80% g_s	0.07	0.84
Leaf $\Delta\Psi_{\pi}^{100}$ vs. slope (% $g_s/0.01$ MPa)	0.31	0.36
Leaf $\Delta\Psi_{\pi}^{100}$ vs. maximum inhibition of g_s from -2 to 0 MPa	-0.44	0.16

linked to dehydration tolerance. Consistent with the hypothesis, those species that showed considerable osmotic adjustment also tended to be those species that showed the least inhibition of g_s as portrayed in Fig. 1. The four species whose g_s was least inhibited by nonhydraulic signaling (the oaks) each had Ψ_{π}^{100} of over 0.6 MPa. Also consistent with the hypothesis, species that did not osmotically adjust much during the lethal drought episode also possessed stomates that were relatively sensitive to the drought signals. *L. tulipifera*, *A. rubrum* and *O. arboreum*, mesic hardwoods often found in moist, bottomland habitats, showed little to no osmotic adjustment; these three species also had the highest relative g_s /soil Ψ_m slopes (i.e. high drought sensitivity).

Within genera, there was some indication that stomatal sensitivity to nonhydraulic root-to-shoot signaling was loosely allied with foliar dehydration tolerance. *A. saccharum* was more dehydration tolerant than *A. rubrum*, as indicated by significantly lower lethal leaf Ψ and markedly greater osmotic adjustment (Table 1). Correspondingly, stomates were more sensitive to partial soil drying in *A. rubrum* than in *A. saccharum*, as indicated by the higher change in g_s per unit change in soil Ψ_m ; steepness of the g_s /soil Ψ_m slope in *A. rubrum* was about twice that in *A. saccharum*. The same trend tended to occur in the native oaks. Discounting the exotic species *Q. acutissima*, foliage of *Q. rubrum* was least tolerant of dehydration and also had the greatest slope of the g_s /soil Ψ_m relation. Maximum inhibition of g_s within particular ranges of soil Ψ_m (Table 2) was

also similar within genera. The maples had similar values, 45 and 53% of controls, much lower than the four oaks which each had maximum inhibition of about 80% of controls.

These data indicate that stomates of temperate tree saplings are sensitive to small changes in water content of forest soils. In all species except the oaks, substantial inhibition of g_s occurred when only part of a root system was dried. Further, in all species except the oaks, inhibition of g_s had begun to occur before soil in the drying pot had dried to the extent that its Ψ_m changed (i.e. before soil Ψ_m declined below control values of -0.01 MPa). In most genera stomatal inhibition was extensive by the time soil around roughly half of the root system dried to -0.10 MPa. Surface forest soils frequently undergo drying episodes of this and greater magnitudes, even in relatively moist regions and even in wet years. Hence, long before soil drying is extensive enough to cause dehydration of leaves, root-sourced nonhydraulic signals can cause substantial stomatal closure.

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