

# Stomatal sensitivity of six temperate, deciduous tree species to non-hydraulic root-to-shoot signalling of partial soil drying

Jennifer L. Croker, Willard T. Witte and Robert M. Augé<sup>1</sup>

Tennessee Agricultural Experiment Station, OHL, University of Tennessee, PO Box 1071, Knoxville, TN 37901-1071, USA

Received 4 July 1997; Accepted 27 November 1997

## Abstract

The objectives of this study were to (1) characterize stomatal response of six deciduous tree species to non-hydraulic, root-sourced signals of soil drying, and (2) test whether species sensitivity to non-hydraulic signalling is allied with their drought avoidance and tolerance profiles. Saplings were grown with roots divided between two pots. Three treatments were compared: one half of the root system watered and half droughted (WD), one half of the root system watered and half severed (WS), both halves watered (WW). Drying about half of the root system caused non-hydraulic declines in stomatal conductance ( $g_s$ ) in all species, with  $g_s$  of WD plants reduced to from 40% to 60% of WS controls. Declines in stomatal conductance were closely related to declining soil matric potential ( $\psi_m$ ) between  $-0.01$  and  $-0.10$  MPa. Soil  $\psi_m$  required to cause declines in  $g_s$  of WD plants to 80% of WS controls varied from a high of  $-0.013$  to a low of  $-0.044$  MPa. Stomatal inhibition varied somewhat with leaf age in half of the species. Leaf osmotic potentials during soil drying were mostly similar among treatments. Although stomatal sensitivity to the non-hydraulic, root-sourced signal (characterized as decline in  $g_s$  per unit decline in soil  $\psi_m$ ) was not closely correlated with previously identified lethal leaf water potentials or capacity for osmotic adjustment, species having the highest stomatal sensitivity also had the least dehydration tolerance. This suggests that stomatal sensitivity to non-hydraulic root signals may be mechanistically linked to a limited extent with other

characteristics defining relative species drought tolerance.

Key words: Non-hydraulic signalling, osmotic potential, stomatal conductance, temperate deciduous trees, water relations.

## Introduction

The most sensitive indicator of a plant's overall physiological state is often stomatal behaviour (Smith and Hollinger, 1991). Stomata respond rapidly to changes in environmental conditions, including soil drying, allowing the plant to balance water loss with carbon uptake during periods of reduced soil moisture. Thus, studying how stomata respond to changes in soil water levels is integral to understanding whole plant physiology during drought.

Non-hydraulic, root-to-shoot signalling of soil drying is a relatively recent hypothesis concerning control of stomatal behaviour. Previously, it was widely believed that soil drying first affected foliage when it led to a lower shoot water status, causing direct hydraulic limitation of leaf growth and stomatal opening (Kramer, 1983). However, reduced stomatal conductance ( $g_s$ ) can occur in plants grown in drying soil in which shoot water status is held constant (Gollan *et al.*, 1986). Thus, it appears that there is a non-hydraulic mechanism enabling some plants to 'sense' and respond to soil drying (Davies and Zhang, 1991). This mechanism of sensing soil drying must logically originate in the roots and be expressed as an alteration of growth or  $g_s$  in the leaves (Gowing *et al.*, 1993).

<sup>1</sup>To whom correspondence should be addressed. Fax: +1 423 974 1947. E-mail: auge@utk.edu; URL <http://funnelweb.utcc.utk.edu/~uthor/htm/auge.htm>

Abbreviations: WW, fully-watered treatment; WD, half-dried treatment; WS, half-severed treatment;  $\psi_m$ , matric potential;  $\psi_{\pi}$ , osmotic potential; RWC, relative water content;  $g_s$ , stomatal conductance;  $\psi$ , water potential.

Such non-hydraulic signalling of drying has been observed in many herbaceous species including corn (Saab and Sharp, 1989; Zhang and Davies, 1989; Tardieu *et al.*, 1991), cowpea (Bates and Hall, 1981), sorghum (Augé *et al.*, 1995), sunflower (Neales *et al.*, 1989), rice (Baño *et al.*, 1993), and wheat (Blum *et al.*, 1991). While non-hydraulic, root-to-shoot signalling has been well documented for herbaceous species, others argue that it may not figure prominently in regulating responses to soil drought in woody species, because it would be an inefficient mechanism for rapid stomatal control due to lengthy transport time (Saliendra *et al.*, 1995). If non-hydraulic signalling does not play a role in regulating drought responses of mature woody species, it may be important in seedling and sapling development (where it has, in fact, been observed several times: Douglas-fir, Blake and Ferrell, 1977; apple, Gowing *et al.*, 1990; rose, Augé and Duan, 1991; sycamore, Khalil and Grace, 1993; and some conifer species, Jackson *et al.*, 1995). Drought resistance mechanisms such as dynamic stomatal control with soil drying are often involved in species distribution of trees, mainly because of their role in juvenile establishment and survival (Martin *et al.*, 1987).

Although the mechanism of action and the exact effects are still in question, much evidence indicates that non-hydraulic, root-to-shoot signalling is an important component of plant response to drought conditions for both herbaceous and woody plants (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 and survival (Ludlow *et al.*, 1989; Mansfield and McAinsh, 1995).

Non-hydraulic, root-sourced inhibition of stomatal opening likely varies among species, like other drought response characteristics (e.g. osmotic adjustment, deep rooting, leaf rolling). Plants classified as drought avoiders, which have dehydration-sensitive tissues (i.e. leaves die at relatively high leaf  $\psi$ ), may use non-hydraulic, root-to-shoot signalling to avoid transpirational water loss and potential leaf death during drought. Conversely, plants classified as drought tolerators, with tissues better adapted to withstand dehydration (i.e. leaves die at lower leaf  $\psi$ ), might be less sensitive to root-sourced, non-hydraulic signalling because they can withstand moderate tissue dehydration.

Most research of non-hydraulic signalling of soil drying has focused primarily on the physiological aspects of the process. The goal here was to examine the ecophysiological significance of non-hydraulic signalling in the context of drought resistance profiles of six deciduous tree species of the south-eastern United States. Specific objectives were to characterize stomatal response of six deciduous tree species to non-hydraulic root-to-shoot signals of soil drying, and to test whether species sensitivity to non-hydraulic signalling is allied with their drought avoidance

and tolerance profiles. Additionally, the effects of partial soil drying on new versus older leaves was compared.

## Materials and methods

### Plant material

Two- to three-year-old saplings of the following species were planted with the root system divided between two 11 l plastic pots (thirty individuals of each species) in the fall of 1995: red maple (*Acer rubrum* L.), flowering dogwood (*Cornus florida* L.), fringe tree (*Chionanthus virginicus* L.), carolina silverbell (*Halesia carolina* L.), tulip poplar (*Liriodendron tulipifera* L.), and sourwood (*Oxydendrum arboreum* (L.) DC.). The saplings ranged in height from about 0.5–1.5 m and were container-grown from seed obtained from local provenances. Standard nursery potting medium was used: 4 pine bark/1 sand, with 4 l dolomitic lime, 4 l 17-6-10 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 × 7.3 × 4.1 m rain shelter (two layers of 4 ml clear polyethylene stretched over bowed aluminium supports). The shelter was covered by a 50% transmission shade cloth, to promote stomatal opening.

### Treatments and environmental conditions

Each species had three split-root treatments. Water was withheld from one pot for a sustained drying period for one group of seedlings (WD). A two-control system was used as follows. In one group, one half of the root system was left intact and watered while the other half was severed (on 30 June 1996) prior to the drought episode (WS). In the second group, both halves of the root system were left intact and were fully-watered throughout the experiment (WW).

The drying episode was initiated on 11 July 1996 (hereafter referred to as day 0) by withholding water from one pot of all WD individuals; the drying period continued until 13 September 1996. 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 *PPFD* were measured every 10 min and averages recorded hourly throughout the experiment with a thermocouple and a quantum light sensor (LI-189, Li-Cor, Lincoln, Nebraska), respectively, connected to a datalogger (21 ×, Campbell Scientific, Logan, UT).

### Water status measurements

Bulk soil matric potential ( $\psi_m$ ) 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) connected through multiplexers (AM32, Campbell Scientific, Logan, UT) to a datalogger (21 ×, Campbell Scientific, Logan, UT), 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. Soil moisture characteristic curves were generated from gravimetric measurements of soil water content and psychrometric measurements of soil water potential (SC-10 thermocouple psychrometers and NT-3 nanovoltmeter thermometers, Decagon Devices, Inc., Pullman, WA, USA).

Stomatal conductance ( $g_s$ ) was measured with a diffusion porometer (AP4, Delta-T Devices, Cambridge, UK). Preliminary, pre-drying, diurnal  $g_s$  measurements of all plants

were used to determine the time of day at which  $g_s$  was reasonably constant, and during which  $g_s$  was subsequently measured throughout the drying episode (about 09:00 h to 14:00 h EST). From day -15 to day 64,  $g_s$  was measured every other day, parallel to the midvein and in the center of four leaves of each individual: the two newest, fully expanded leaves and the two next older, lateral leaves on the same shoot. Up to eight leaves were measured each day for each individual and the  $g_s$  of those terminal shoots having the most open stomates were recorded. 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. Once four plants of each species had been sampled this way, the pattern was repeated until all replicates of each 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. rubrum*, *C. florida*, *C. virginicus* etc.; day two: *C. florida*, *C. virginicus*, *H. carolina*; etc.). Replicates were blocked in this way, so that within each block, one set of WW, WD and WS replicates was always measured within about 10 min of one another, minimizing confounding effects possibly arising from environmental changes throughout the day.

Beginning 18 July 1996 and continuing for all  $g_s$  measurement days, one leaf from each individual was collected for analysis of osmotic potential ( $\psi_\pi$ ). Leaves were immediately placed in a syringe, frozen in liquid nitrogen and stored in an ultra-low freezer (-80 °C), pending later analysis of  $\psi_\pi$  using a vapor pressure osmometer (Model 5500 XR, Wescor Inc.; Logan, UT). Syringes were removed from the freezer and allowed to thaw until no longer cold to the touch (10–15 min) before measuring  $\psi_\pi$ . The osmometer was calibrated daily with graded NaCl solutions.

On 9 September 1996, roots of all plants having soil  $\psi_m$  of -0.02 MPa or lower were excavated. The drying period ended on 13 September 1996 and the roots of remaining plants were excavated. Roots were dried at 80 °C for 1 week and then dry weights were used to determine how evenly roots were divided between the two pots and to compare the size of the root systems among treatments.

#### Experimental design and statistical analysis

A six (species) × 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. Averages were separated within each species using the General Linear Models Procedure for ANOVA (SAS, Cary, NC). ANOVA included linear contrasts of  $g_s$  among treatments for each species for each day measurements were recorded. Regression and correlation analyses were used to describe relationships between relative  $g_s$  and declining soil  $\psi_m$  for each species.

## Results

### 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

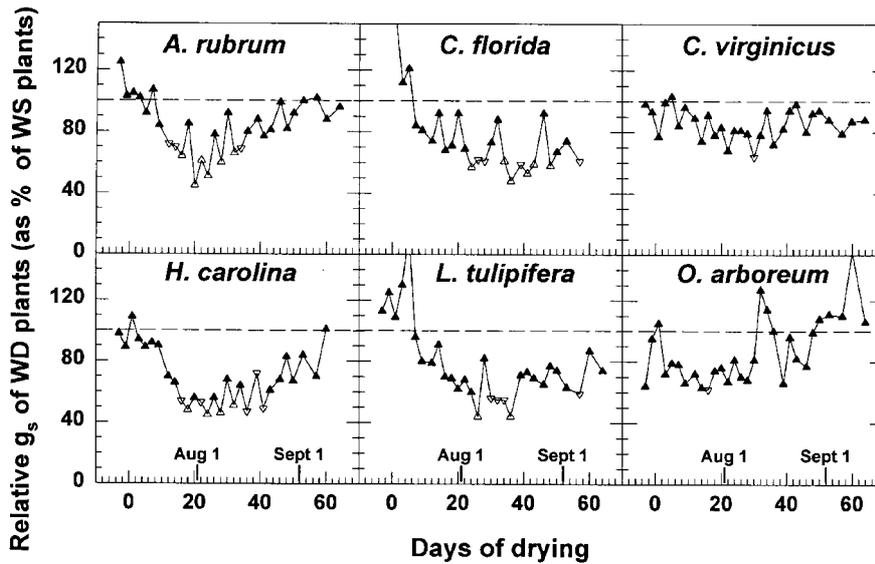
reduced relative  $g_s$  of WD trees of all species for most days during the drying episode. Largest single day declines in average relative  $g_s$  were to about 60% of WS controls in *C. virginicus* and *O. arboreum* and to about 40% of WS controls in the other four species. Relative  $g_s$  declined and mostly remained low for *C. florida*, *C. virginicus* and *L. tulipifera*, whereas relative  $g_s$  of *A. rubrum*, *H. carolina* and *O. arboreum* began to recover during the latter part of the drying period.

Stomatal conductances of WS controls were mostly similar to those of the WW controls during the drying period (Fig. 2). Relative  $g_s$  of WS was different from 100% (i.e. from relative  $g_s$  of WW trees) on only one day of the drying period for *A. rubrum*, *C. virginicus*, *L. tulipifera*, and *O. arboreum*, and was not different on any day for *H. carolina*. Relative  $g_s$  of WS trees decreased in *A. rubrum*, *C. florida* and *L. tulipifera* early in the experiment, after root excision (roots were excised 12 d prior to soil drying, on 'day -12') but essentially returned to WW levels within 2 weeks of day 0.

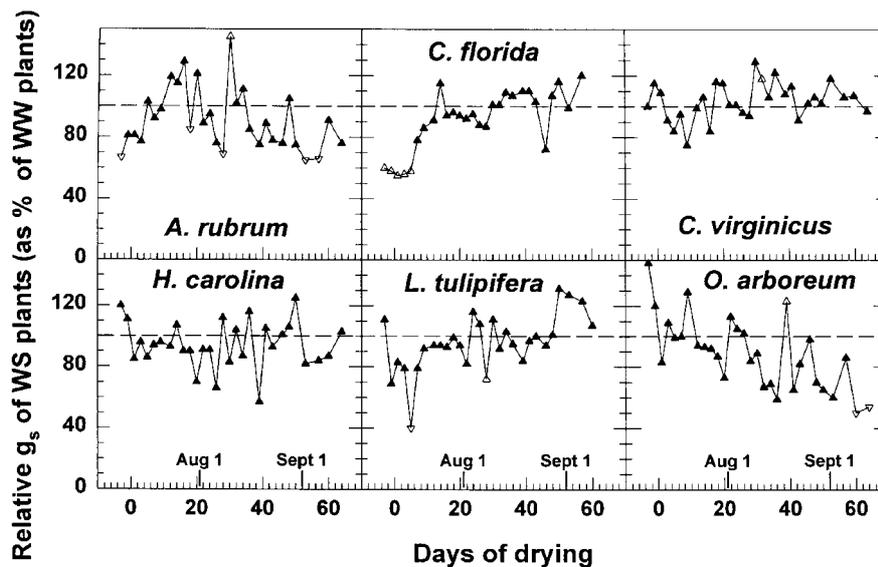
Absolute  $g_s$  fluctuated more in *A. rubrum*, *C. virginicus* and *L. tulipifera* trees than in *C. florida*, *H. carolina* and *O. arboreum* trees throughout the experiment (Fig. 3). Fluctuations were fairly consistent among treatments from day to day and thus were probably due to variations in daily environmental conditions in the rain shelter, such as humidity, irradiance and air temperature (Fig. 4).

### Stomatal conductance of WD trees versus soil matrix potential

Drying rates of WD trees differed among species and among individuals within each species (Fig. 5). Figure 6 shows relative  $g_s$  of WD trees, grouped by progressively drier soil  $\psi_m$ . Stomatal conductance began to decrease in all species before soil  $\psi_m$  decreased measurably; the moisture release plot for this soil indicated that it lost about 60% of its water before its  $\psi_m$  began to decline (Fig. 7). For all species, 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. For *C. florida*, *H. carolina* and *L. tulipifera*,  $g_s$  declined steadily as soil dried, and  $g_s$  remained inhibited below soil  $\psi_m$  of -1.0 MPa. In *H. carolina*, for example, average  $g_s$  of WD trees was 114% that of WS trees before the drying episode began. Their  $g_s$  declined to 81% of WS trees with some drying (but soil  $\psi_m$  still at -0.01 MPa). 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  $\psi_m$  had not yet declined below -0.01 MPa),  $g_s$  dropped to 64% of controls. Further drying of soil, to -0.02 MPa and below, did not result in further decreases in  $g_s$ ; relative  $g_s$  held mostly steady, at an average of 70% within the -0.02 to -0.10 MPa soil  $\psi_m$  range, 58% within the -0.1



**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:  $WD\ relative\ g_s = (WD\ absolute\ g_s) / (WS\ average\ absolute\ g_s) \times 100$ . Each individual tree's absolute or relative  $g_s$  value represents the average of four leaves. Symbols show when average  $g_s$  of WD trees ( $n=8$ ) were significantly different from WS controls (▲ NS, △  $P \leq 0.05$ , ▽  $P \leq 0.1$ ) as determined by ANOVA.



**Fig. 2.** Stomatal conductance ( $g_s$ ) during the drying period of WS trees (about half the root system severed), 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 was calculated as absolute  $g_s$  of an individual WS tree divided by the average absolute  $g_s$  of the four WW trees of that species for that day, expressed as a percentage:  $WS\ relative\ g_s = (WS\ absolute\ g_s) / (WW\ average\ absolute\ g_s) \times 100$ . 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 (▲ NS, △  $P \leq 0.05$ , ▽  $P \leq 0.1$ ).

to  $-1.0$  MPa soil  $\psi_m$  range, and 56% when soil  $\psi_m$  dropped below  $-1.0$  MPa. Relative  $g_s$  of WD trees of *C. florida* and *L. tulipifera* each declined to about 65% by the time soil had dried to within the  $-0.1$  to  $-1.0$  MPa range, and further drying did not depress  $g_s$  further. Stomatal conductances of *A. rubrum*, *C. virginicus* and *O. arboreum* also declined initially as soil dried below  $-0.01$  MPa; however,  $g_s$  recovered somewhat or fully as

soil  $\psi_m$  declined to below  $-1.0$  MPa. For all six species, maximum inhibition of  $g_s$  of WD trees occurred before soil in the one drying pot reached  $-1.0$  MPa, and further soil drying did not depress  $g_s$  further.

Relative  $g_s$  was positively correlated with soil  $\psi_m$  between  $-0.01$  and  $-0.10$  MPa for all species except *O. arboreum* (Fig. 8). Drier soil (below  $-0.10$  MPa) occasionally resulted in somewhat more inhibition of  $g_s$ , but

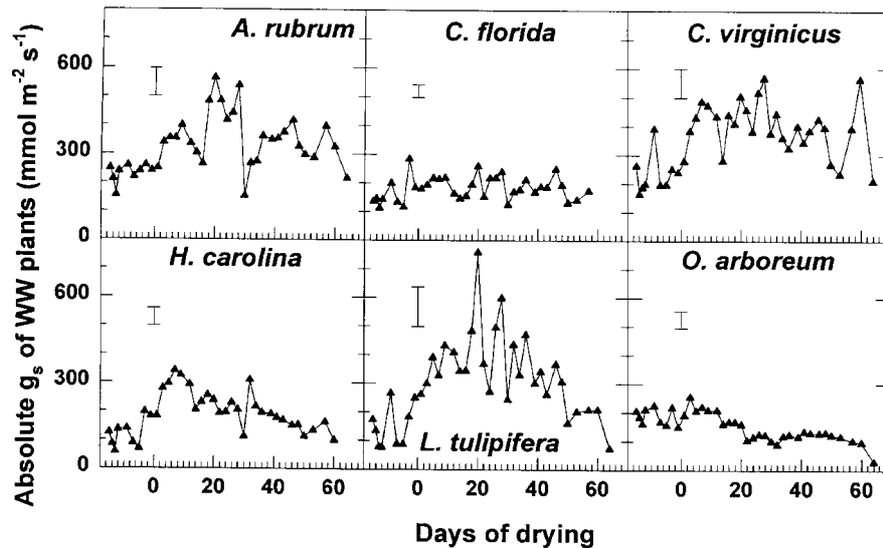


Fig. 3. Absolute stomatal conductance ( $g_s$ ) of fully-watered (WW) trees (both halves of the root system watered regularly) during the experiment. Symbols represent daily tree averages ( $n=4$ ), four leaves per tree. Bars represent standard error of the means for each species. Water was withheld from half-dried (WD) trees beginning day 0 (11 July 1996).

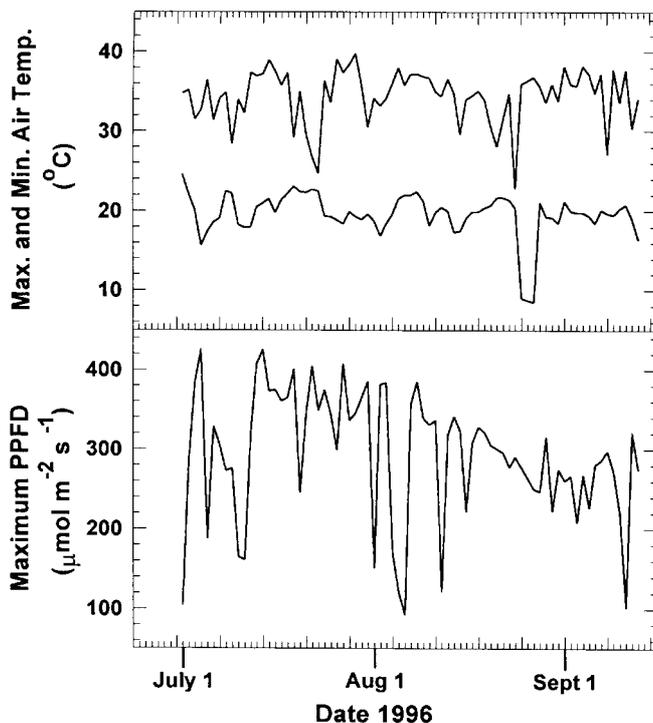


Fig. 4. Maximum and minimum air temperatures and maximum PPFD (hourly averages of measurements of each at 10 min intervals) in the rain shelter during the experiment.

only in *A. rubrum* and *C. florida*, and below  $-0.10$  MPa relative  $g_s$  and soil  $\psi_m$  were not correlated. Rates of decline in relative  $g_s$  as a function of soil  $\psi_m$  (i.e. slopes of the regressions depicted in Fig. 8) varied among species, with *L. tulipifera* (largest slope) having about twice the decline as *H. carolina* (smallest slope) in  $g_s$  per unit decline

in soil  $\psi_m$ . As another way of describing and comparing stomatal sensitivity among species, the soil  $\psi_m$  at 20% inhibition of  $g_s$  was calculated from the regressions shown in Fig. 8: i.e. soil  $\psi_m$  required to bring average  $g_s$  of WD trees to 80% of WS controls (Table 1). 80% was chosen because it probably represents the first real non-hydraulically-regulated declines in  $g_s$ ; above 80% relative  $g_s$ , stomates may just have been showing biological variation in  $g_s$  unrelated to drought. *O. arboreum* responded to the least soil drying, reaching 80% of WS  $g_s$  at an average soil  $\psi_m$  that was 0.031 MPa higher than *A. rubrum*, which required the most soil drying to reach 80% of WS  $g_s$ .

#### Root dry weights and leaf osmotic potential

Root dry weights were measured for both halves of the root system of all individuals, to estimate relative water gathering capacity among treatments (Fig. 9). Average root dry weights of the watered side of WS trees were similar ( $P \leq 0.05$ ) to average root dry weights of the watered side of WD trees for all species.

Leaf  $\psi_\pi$  was similar among treatments for most days of the experiment, suggesting that foliar water status was similar among treatments during the drying episode (Fig. 10). Leaf  $\psi_\pi$  of WD trees remained mostly constant as soil dried (Fig. 11), indicating that declines in soil  $\psi_m$  within the drying pot did not result in any change in leaf  $\psi_\pi$  of WD trees.

#### Leaf age comparisons

Inhibition of  $g_s$  of WD trees varied with leaf age in *A. rubrum*, *H. carolina* and *O. arboreum* (Fig. 12). For

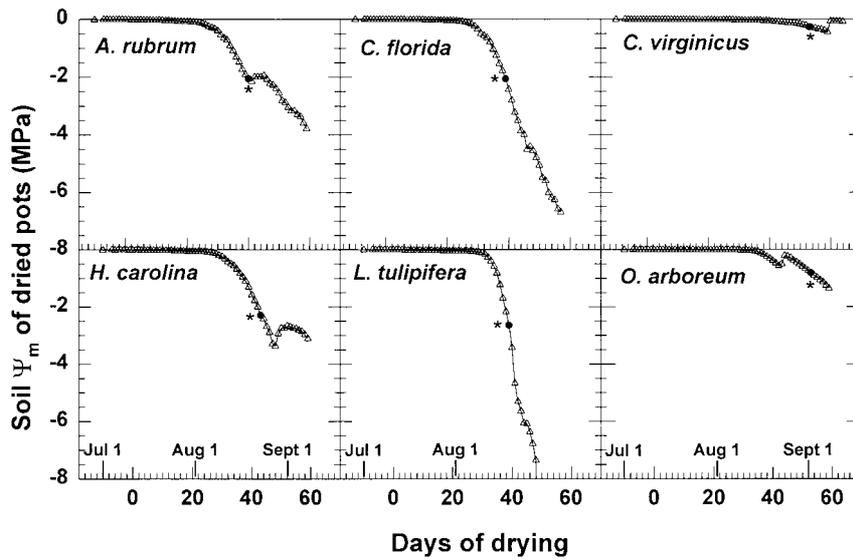


Fig. 5. Average soil  $\psi_m$  in dried pots of WD trees during the drying episode. Symbols represent daily averages of eight WD trees and of measurements recorded every 4 h. Water was withheld from WD trees beginning day 0. Symbols (\*) indicate the first day soil  $\psi_m$  of dried WD pots was different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

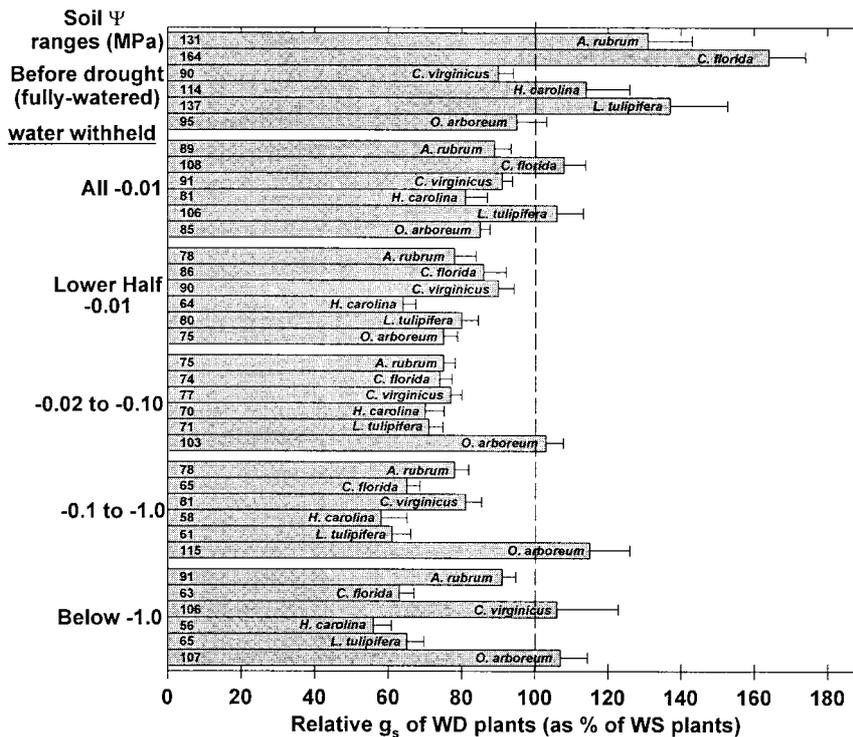


Fig. 6. Relative  $g_s$  (see Fig. 1 legend for explanation) of WD trees as a function of soil moisture. Each soil moisture grouping shows average  $g_s$  of all WD individuals within a particular soil  $\psi_m$  range, for each species. The first group shows  $g_s$  before water was withheld from WD 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  $\psi_m$  to decline (soil  $\psi_m$  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  $\psi_m$  was within different  $\psi_m$  ranges:  $-0.02$  to  $-0.10$ ,  $-0.1$  to  $-1.0$ , and below  $-1.0$  MPa. Values within shaded bars give bar lengths;  $n = 3-120$ . Lines at right of each bar represent standard errors.

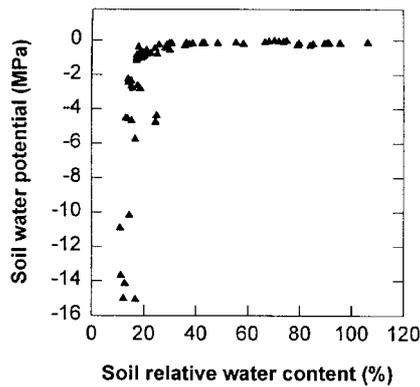


Fig. 7. Soil moisture release plot for the experimental soil, generated with gravimetric measurements of soil water content and psychrometric measurements of soil water potential. Each point represents one pair of soil water content and water potential measurements.

*H. carolina* and *O. arboreum*, the first and second newest, fully expanded ('newer') leaves had much lower relative  $g_s$  compared to the third and fourth newest, fully expanded ('older') leaves, for most of the drying episode. The magnitude of the leaf age difference was similar for *A. rubrum* and *H. carolina*; however, the difference did not occur until later in the experiment for *A. rubrum*. There did not appear to be large differences in  $g_s$  between the newer and older leaves for the WD treatment of *C. virginicus*, *L. tulipifera* or *C. florida*. However, even for *C. florida* and *C. virginicus*, relative  $g_s$  of the newer leaves tended to be lower than the older leaves of WD trees for many days of the drying period; the average

Table 1. Comparison among species of stomatal sensitivity to non-hydraulic root signals of soil drying, with foliar dehydration tolerance

Stomatal sensitivity to non-hydraulic root signals of soil drying was characterized as soil  $\psi_m$  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  $\psi_m$  (slopes of the regressions depicted in Fig. 8). Foliar dehydration tolerance was characterized in another experiment with other trees as lethal leaf  $\psi$  ( $n=10-16$ , see Augé *et al.* 1998, Fig. 2). Values of soil  $\psi_m$  at 80%  $g_s$  were calculated from regressions of relative  $g_s$  against soil  $\psi_m$  (Fig. 8). Relative  $g_s$  ( $g_s$  of WD trees relative to WS trees) is defined in Fig. 1. Similar letters following lethal leaf  $\psi$  values indicate statistically similar averages ( $P \leq 0.05$ ).

Species	Soil $\psi_m$ at 80% $g_s$ (MPa)	Slope (% $g_s$ /0.01 MPa)	Lethal leaf $\psi$ (MPa)
<i>L. tulipifera</i>	-0.033	13.1	-2.38 a
<i>O. arboreum</i>	-0.013	10.2	-3.98 bc
<i>H. carolina</i>	-0.027	6.7	-4.11 bcd
<i>A. rubrum</i>	-0.044	10.6	-4.43 cd
<i>C. florida</i>	-0.032	7.0	-4.88 de
<i>C. virginicus</i>	-0.026	7.9	-5.63 ef

relative  $g_s$  of all days past day 10 was 85% for *C. florida* and 92% for *C. virginicus*. Absolute  $g_s$  of newer leaves also tended to be lower than absolute  $g_s$  of older leaves, for all species, with *A. rubrum*, *H. carolina*, and *O. arboreum* having the largest differences (data not shown).

Stomatal conductance of different-aged leaves of WD trees responded differently to various soil  $\psi_m$  ranges (Fig. 13). Before the drying episode began, relative  $g_s$  of WD trees was similar in the newer and older leaves, for all species. With some drying (but soil  $\psi_m$  still at

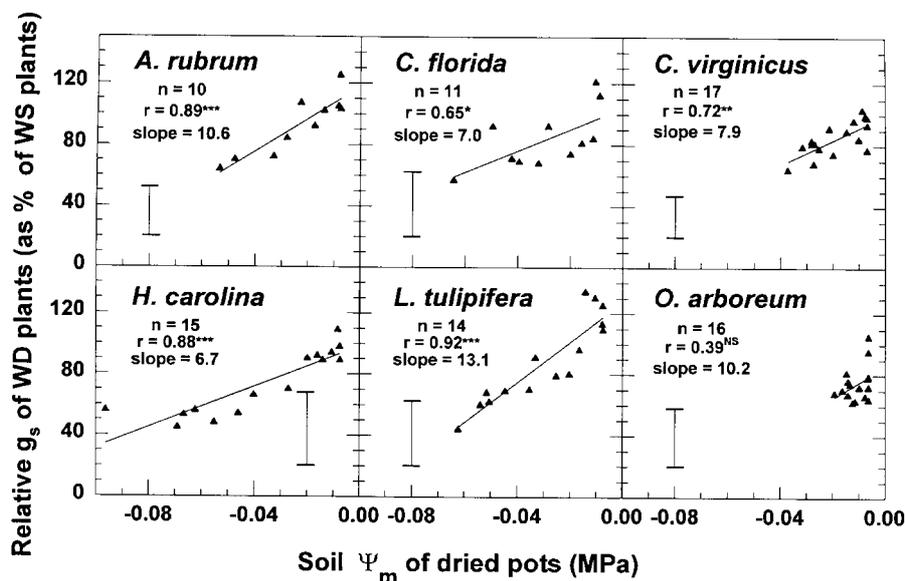
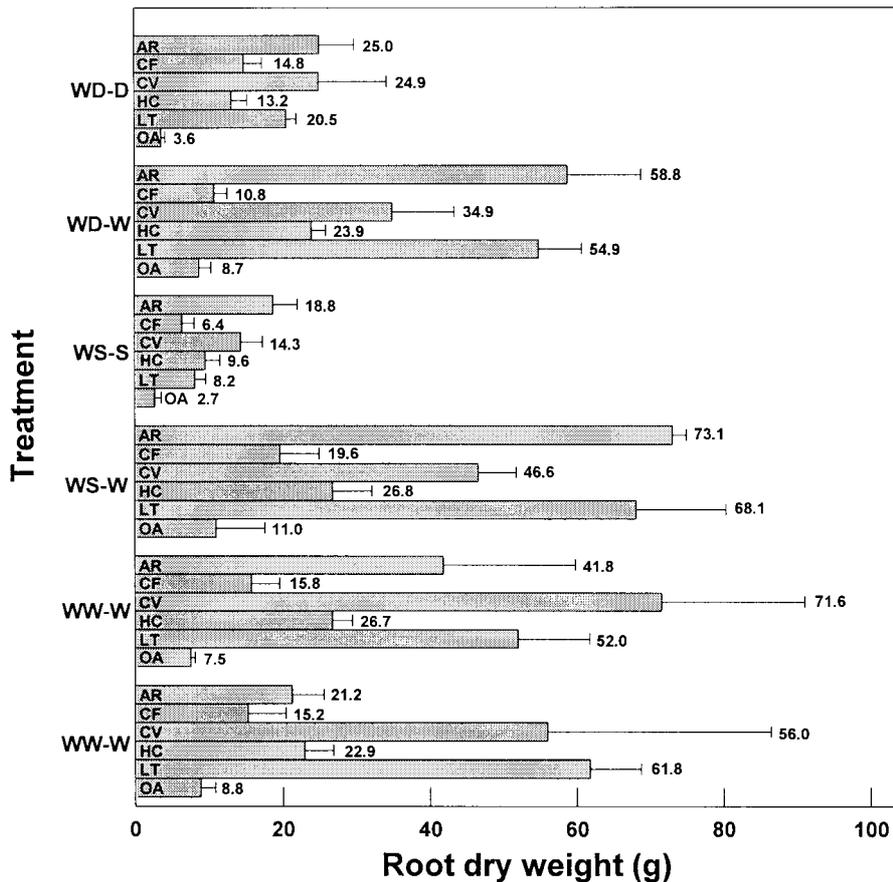


Fig. 8. Relationship between relative  $g_s$  (see Fig. 1 legend) and soil  $\psi_m$  of the one dried pot of WD trees. Symbols represent daily averages, for both  $g_s$  and soil  $\psi_m$ , of eight WD trees for all days after day 0 that soil  $\psi_m$  averages fell within the range depicted. Relative  $g_s$  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 ( $r$ ) and slopes are given for each species. Asterisks indicate significance of correlations (\*\*\*)  $P \leq 0.0001$ , (\*\*)  $P \leq 0.001$  and (\*)  $P \leq 0.05$ . Vertical lines represent standard error of the means for each species.



**Fig. 9.** 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. AR, *Acer rubrum*; CF, *Cornus florida*; CV, *Chionanthus virginicus*; HC, *Halesia carolina*; LT, *Liriodendron tulipifera*; OA, *Oxydendrum arboreum*.

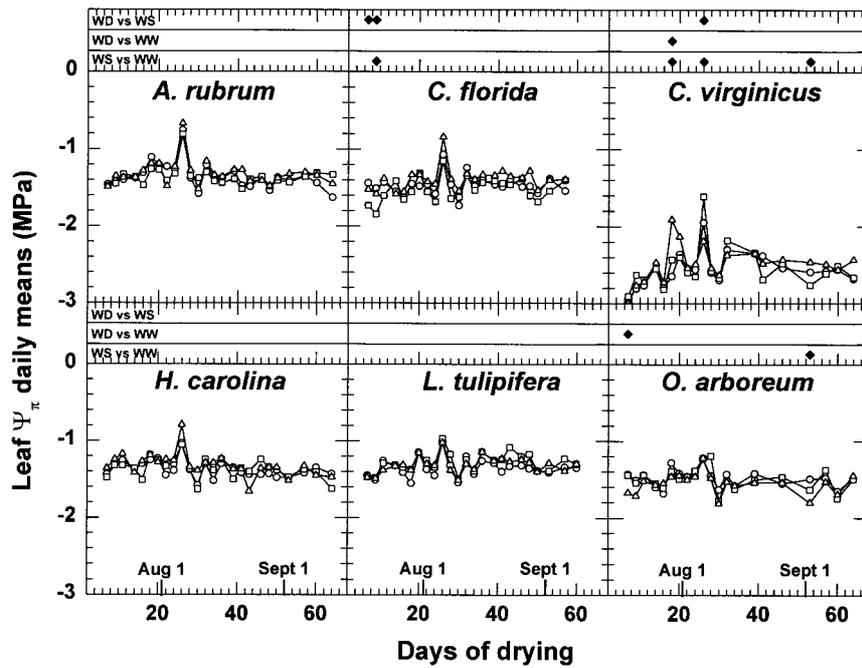
$-0.01$  MPa),  $g_s$  of newer leaves was more sensitive than older leaves to the non-hydraulic signal (i.e. newer leaf  $g_s$  declined more than older leaf  $g_s$ ), in *O. arboreum*, *C. virginicus* and *H. carolina*. Once soil  $\psi_m$  dried to between  $-0.02$  and  $-0.10$  MPa, newer leaves showed more inhibition of  $g_s$  than older leaves for *A. rubrum*, *H. carolina* and *O. arboreum*. These leaf age differences became even more pronounced at soil  $\psi_m$  of  $-0.10$  to  $-1.0$  MPa for these three species, and leaf age differences continued at soil  $\psi_m$  below  $-1.0$  MPa. Relative  $g_s$  of the newer leaves of *O. arboreum* went down quickly and remained inhibited; conversely, relative  $g_s$  of the older leaves increased as soil dried. Stomatal response to the partial soil drying of root systems of WD trees did not vary with leaf age in *C. florida* or *L. tulipifera*.

## Discussion

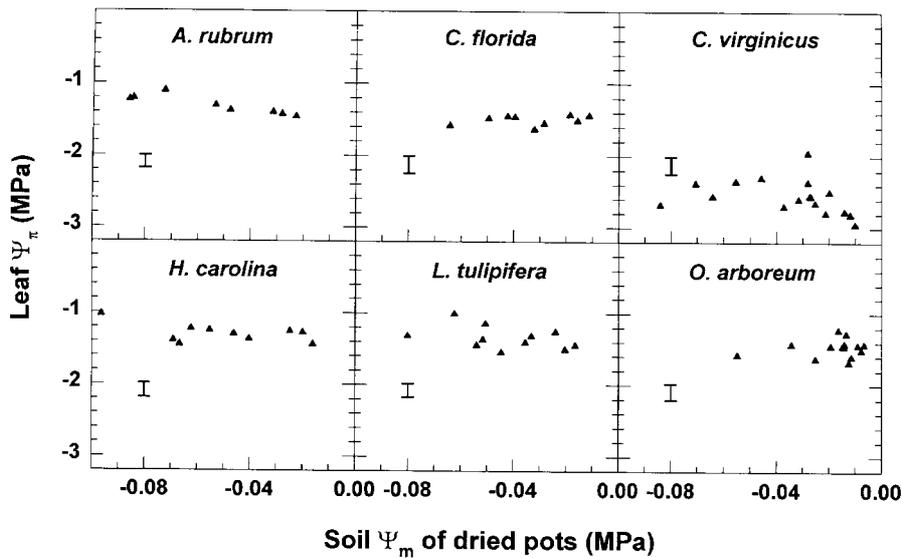
About one-half of the root system of WD trees was fully-watered throughout the drying period, and the water status (measured here as leaf  $\psi_\pi$ ) of WD trees was similar to that of both half-severed and fully-watered controls.

The similar leaf  $\psi_\pi$  across treatments suggests that the decline in  $g_s$  of half-dried plants was probably not the direct result of a hydraulic signal (i.e. a decline in leaf water status). Leaf  $\psi_\pi$  has been used as a reliable indicator of leaf water status and is often as sensitive or a more sensitive measure of plant water status than leaf  $\psi$  (Gallardo *et al.*, 1994).

Much stronger evidence for the non-hydraulic nature of the signal was the behaviour of WD relative to WS foliage. Although leaf  $\psi_\pi$  was mostly consistent among treatments during the experiment, total leaf  $\psi$  and its components can fluctuate extensively and quickly as environmental conditions change (Jones, 1990). It is possible that reductions observed in  $g_s$  of WD trees relative to WW trees could have been due to unmeasured or unmeasurable changes in leaf water status. Therefore, having only a WW control receiving about twice as much water as WD plants would have had two disadvantages: (1) instruments were possibly not sensitive enough to measure small fluctuations in leaf water status, and (2) measurements of water status were occasional and invasive, not continuous, thus overlooking temporary differ-



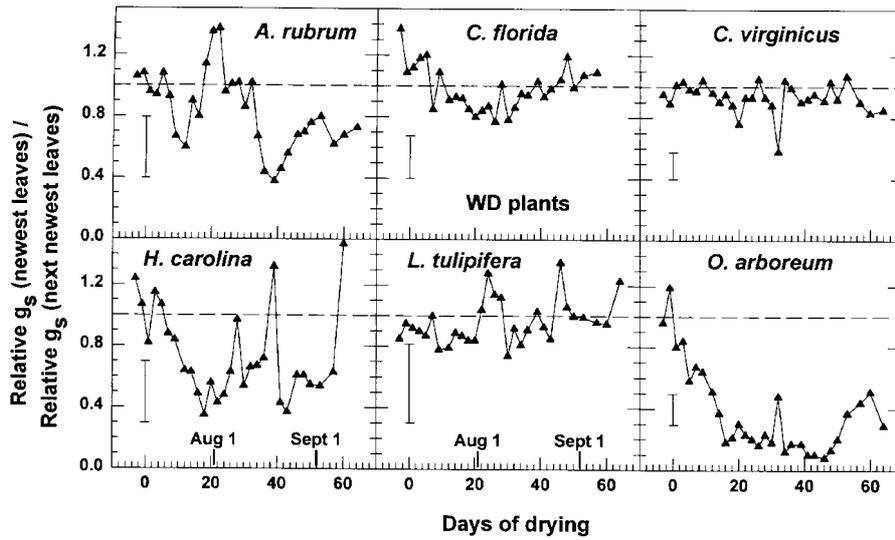
**Fig. 10.** Leaf  $\psi_{\pi}$  during the experiment for trees of all treatments. Symbols within graphs represent daily averages ( $n=8$  for WD,  $n=4$  for WS and WW). Symbols above graphs indicate significant differences between treatments on a given day (linear contrasts;  $P \leq 0.05$ ). Water was withheld from WD trees beginning day 0.



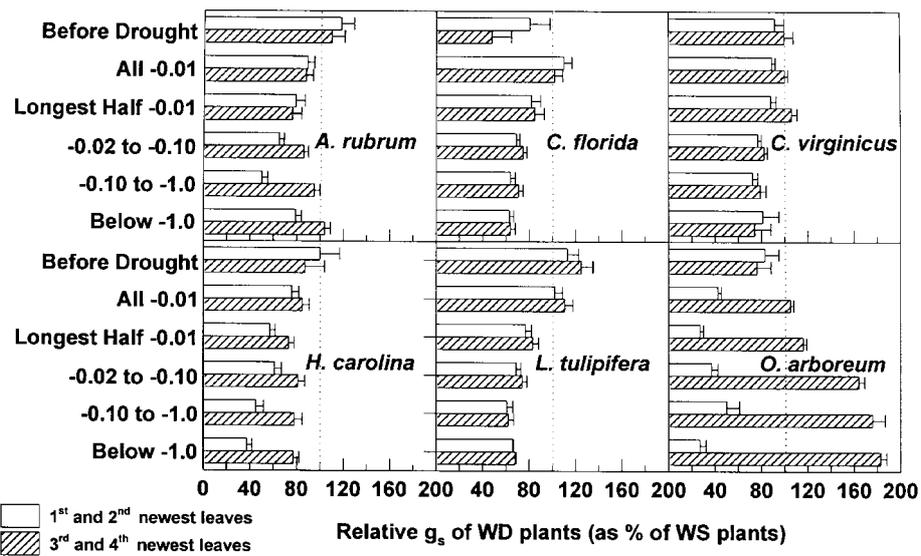
**Fig. 11.** Relationship between soil  $\psi_m$  and leaf  $\psi_{\pi}$  of WD trees, as soil  $\psi_m$  first began to decline during the drying period. Symbols represent daily averages for both leaf  $\psi_{\pi}$  ( $n=8$ ) and soil  $\psi_m$  ( $n=8$ ). The soil  $\psi_m$  range of  $-0.01$  to  $-0.10$  MPa is depicted, as it is within this range that most inhibition of  $g_s$  occurred in WD plants of most species, and because declines in  $g_s$  were related to soil  $\psi_m$  within this range (Fig. 7). Leaf  $\psi_{\pi}$  remained similarly unaffected by declining soil  $\psi_m$  below  $-0.10$  MPa (data not shown). Vertical lines represent standard error of the means for each species.

ences in leaf water status between WD and WW plants. The purpose of the WS individuals was to control for possible undetected effects on leaf  $\psi$  caused by reducing the water supply of the WD plants by half, relative to WW plants. Half-dried and WS trees had similar amounts of roots receiving water regularly, so decreases in  $g_s$  of

WD trees relative to WS could not have been due to a direct hydraulic signal. Further, severing half of the root system caused  $g_s$  to decline somewhat in plants of most 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.



**Fig. 12.** Ratio of relative  $g_s$  of the two newest, fully expanded leaves and the two next oldest leaves (third and fourth newest, fully expanded leaves), in half-dried (WD) trees during the drying period. Relative  $g_s$  of each of the eight WD trees was computed as described in Fig. 1: its absolute  $g_s$  for that day (average of four leaves) divided by the average absolute  $g_s$  of the four WS trees of that species for that day, multiplied by 100: WD relative  $g_s = (\text{WD absolute } g_s) / (\text{WS average absolute } g_s) \times 100$ . For this plot, these relative  $g_s$  values were separated by leaf age within each species, and the average of the first and second newest leaves was divided by the average of the third and fourth newest leaves); each symbol represents the daily averages ( $n=8$ ) for each species. Vertical lines show standard errors of the means for each species.



**Fig. 13.** Relative  $g_s$  of WD trees at increasing levels of soil drying; depicted are the average of the first and second newest, fully expanded leaves (newer leaves) and the average of the third and fourth newest, fully expanded leaves (older leaves). Daily relative  $g_s$  of newer leaves for each of the eight WD trees of each species was calculated as the average of the absolute  $g_s$  of these two leaves for each individual WD tree divided by the average absolute  $g_s$  of these two newer leaves for the four WS trees of that species for that day, expressed as a percentage: WD relative  $g_s^{\text{newer}} = (\text{WD absolute } g_s^{\text{newer}}) / (\text{WS average absolute } g_s^{\text{newer}}) \times 100$ . The calculation was similar for older leaves. Soil moisture groupings are as explained in Fig. 6;  $n=3-120$ . Lines at right of each bar represent standard errors.

Although non-hydraulic signalling of soil drying is now fairly well documented for some woody species, its importance in trees has been questioned (Saliendra *et al.*, 1995). Carbon gain appears to be maximized in woody species at the expense of water loss, while water is conserved in herbaceous species at the expense of carbon gain (Knapp and Smith, 1990). From this observation, one might

conclude that herbaceous species would be more likely than woody plants to use a non-hydraulic signal to control  $g_s$ . Trees generally have relatively low photosynthetic and transpiration rates, deep and extensive root systems and large amounts of tissue for water storage. These factors may lessen the impact of water stress; therefore, the physiological status of mature trees may

not be as dependent on environmental variation as herbaceous plants (Knapp and Smith, 1990). However, due to the low boundary layer resistance, canopy transpiration of trees is expected to be regulated more by stomatal responses than herbaceous canopies which typically have much higher boundary layer resistances (Schulze, 1986). As proposed above, mature trees may exhibit relative physiological homeostasis during environmental changes; however, small, juvenile, woody plants would be expected to have physiological responses more closely tied to environmental variation, as expected with herbaceous species. Much of the work on water stress in trees has been done with seedlings and saplings (Kwon and Pallardy, 1989; Abrams and Kubiske, 1990). Also, younger trees have controlled stomatal opening more during drying compared to older trees (apple, Jones *et al.*, 1983). Additionally, drought-induced damage is often more pronounced in younger than in older trees (Hursh and Haasis, 1931). Because of their smaller stature, tree seedlings and saplings may both rely on a root-sourced, non-hydraulic mechanism for regulating stomata, and be more susceptible to drought than older, larger trees.

Maximum inhibition of daily average  $g_s$  ranged from 60% of controls for *C. virginicus* and *O. arboreum* to about 40% of controls for the other four species, with individual trees showing lower values. These values are similar to maximal stomatal inhibition observed in other woody species, in response to soil drying. In apple, transpiration (controlled by stomatal opening) of half-dried plants was reduced to 30% of controls (Gowing *et al.*, 1990). Similarly, half-dried sycamore trees had  $g_s$  that dropped to 26% of control seedlings (Khalil and Grace, 1993).

Stomatal inhibition was first observed at relatively high soil  $\psi_m$  (i.e. inhibition of  $g_s$  began while soil  $\psi_m$  still measured  $-0.01$  MPa, the value returned by the soil  $\psi_m$  sensors for fully moistened soil). The moisture release data for this soil shows that when its  $\psi_m$  reached  $-0.10$  MPa, the soil had lost about two-thirds of its water. Soil  $\psi_m$  above  $-0.10$  MPa initiated stomatal inhibition of 20% or more in all species. At soil  $\psi_m$  above  $-0.10$ , inhibition of  $g_s$  for these tree species was closely related to declining soil  $\psi_m$ . Demonstrating that the magnitude of  $g_s$  declines appears to be a function of the magnitude of soil  $\psi_m$  declines adds considerable weight to the assertion that partially drying a root system can invoke a non-hydraulic, root-to-shoot signalling mechanism for regulation of stomatal behaviour during drought.

Following initial drought-induced declines in  $g_s$  of WD plants, continued soil drying caused  $g_s$  of *C. florida*, *C. virginicus* and *L. tulipifera* to remain inhibited while  $g_s$  of *A. rubrum*, *H. carolina* and *O. arboreum* increased (though  $g_s$  values were still lower than WS controls on most days). For *C. florida*, *C. virginicus* and *L. tulipifera*, the sustained inhibition of  $g_s$  throughout the experiment may indicate

that the signal continued to move from very dry roots to shoots or that stomata did not recover once the signal diminished. The eventual increase in  $g_s$  observed for the other three species may have been due to a reduced loading of root xylem and/or root-to-shoot transport of the non-hydraulic signal once soil drying became severe enough that those roots were no longer contributing much to the transpiration stream. A similar initial decline in  $g_s$ , followed by an increase as soil dried further, was observed before in sycamore seedlings (Khalil and Grace, 1993). In a split-root system, the roots in fully-watered soil will continue to grow while the roots in dry soil would not be expected to grow as much and may possibly even die as soil drying becomes severe. Thus, the effect of the split-root system treatment (i.e. half of roots in drying soil resulting in production of a non-hydraulic signal) may be expected to decline as root growth in the wet pot exceeds growth in the dry pot (Davies *et al.*, 1986).

Soil  $\psi_m$  increased near the end of the experiment for some individuals of *A. rubrum*, *C. florida*, *H. carolina*, *L. tulipifera*, and *O. arboreum*. The most likely explanation of this occurrence is that the roots in drying soil were rehydrated by the roots in wet soil. Although reverse transport of water from roots to soil has been disputed (Kirkham, 1980; Molz and Peterson, 1976), rehydration of roots in drying soil has been observed by several others in split-root experiments (cowpea, Ebel *et al.*, 1996; maize, Augé *et al.*, 1994; Saab and Sharp, 1989; sorghum, Ebel *et al.*, 1994; Xu and Bland, 1993).

The chief purpose in characterizing and comparing the stomatal sensitivity of these six species to non-hydraulic, root-to-shoot signalling of soil drying was to explore the ecophysiological significance of this drought response: is relative species sensitivity to the non-hydraulic signal(s) allied with other physiological tendencies that characterize species drought avoidance or tolerance? Specifically, do species typically characterized as drought avoiders have greater non-hydraulically-regulated stomatal sensitivity to soil drying than drought tolerators? The varying drought avoidance and tolerance of species reflect an amalgam of morphological and physiological characters, but relative avoidance/tolerance has been previously operationally defined by the actual dehydration tolerance of tissues, usually leaves (Ludlow, 1989). Foliar dehydration tolerance, in turn, is measured as the lethal leaf  $\psi$  or relative water content: the extent of water loss a plant can withstand before most of its foliage dies (Sinclair and Ludlow, 1986; Chapman and Augé, 1994). It is postulated that those species having the highest lethal leaf  $\psi$  would also display the largest overall non-hydraulically-induced declines in  $g_s$  and the largest declines in  $g_s$  per unit drop in soil  $\psi_m$ , and that they would require the least soil drying to inhibit  $g_s$ .

Stomatal sensitivity to non-hydraulic signals of soil

drying was characterized in this study in three ways. The lowest points in Fig. 1 depict the maximum declines in daily average relative  $g_s$  of WD trees. Soil  $\psi_m$  required to depress relative  $g_s$  by 20% are a measure of how much soil drying was necessary to initiate inhibition of  $g_s$ . Perhaps the best gauge of stomatal sensitivity to the non-hydraulic drought signal is the slope of the relative  $g_s$ /soil  $\psi_m$  regression, which indicates how quickly  $g_s$  declines as soil  $\psi_m$  changes (Table 1; Fig. 8). Slopes were not significantly correlated with lethal leaf  $\psi$ ; correlation analysis yielded a correlation coefficient of 0.71, with  $P$  of 0.12. However, *L. tulipifera* had the least dehydration tolerance (lethal leaf  $\psi = -2.38$  MPa; Augé *et al.*, 1998) as well as the most sensitive stomates (slope = 13.1). *O. arboreum* also had relatively high lethal leaf  $\psi$ , which corresponded with a high stomatal sensitivity (slope = 10.2). *C. florida* and *C. virginicus* each had low lethal leaf  $\psi$  as well as fairly low sensitivity (small slopes). It is possible, then, that examination of a larger group of species with perhaps a broader range of species dehydration tolerance would reveal that foliar dehydration tolerance is in fact linked with stomatal sensitivity to root-sourced, non-hydraulic signals of soil drying.

*L. tulipifera*, *A. rubrum*, *O. arboreum* and *H. carolina*, all mesic hardwoods often found in moist, bottomland habitats, were previously determined to have relatively dehydration sensitive leaves (i.e. high lethal leaf  $\psi$ , Table 1). In a previous study, *A. rubrum* was one of the most drought-sensitive trees among six woody species, showing rapid stomatal closure at relatively high leaf  $\psi$  (Davies and Kozlowski, 1977). Also, stomates of *L. tulipifera* were found to be relatively sensitive to a variety of environmental stresses, including drought (Roberts, 1990). As drought avoiders (i.e. plants with tissues that are sensitive to dehydration, tending to have characteristics that enable them to avoid dehydration, such as leaf rolling, deep roots, alterations in leaf morphology, etc.), these species might be expected to respond most quickly to and to the largest extent to a non-hydraulic, root-sourced signal of soil drying, as has been observed with some herbaceous species (e.g. cowpea versus maize, Ebel *et al.*, 1996). *L. tulipifera* did have the most sensitive stomates among species (as discussed above) followed by *A. rubrum*; however, *A. rubrum* required the most soil drying to inhibit  $g_s$  to 80% of WS controls, followed by *L. tulipifera*. This seems to indicate that although stomates of these two species are fairly sensitive to non-hydraulic signalling, the signalling is initiated at drier soil moisture levels compared to other species.

In a study of several deciduous tree species during a drought period (e.g. *Acer saccharum*, *Juglans* spp., *Quercus* spp.), *C. florida* was the first to show stomatal inhibition, and its stomates were closed for the highest percentage of days during the growing season among

species (Hinckley *et al.*, 1979). Others have also found that *C. florida* is usually not very tolerant to drought (Bahari *et al.*, 1985). However, this species has a relatively low lethal leaf  $\psi$  (Augé *et al.*, 1998; Fig. 2), suggesting that it may not have well-developed drought-avoidance mechanisms. *C. florida* is known to be a shallow-rooted tree in natural settings (Pallardy, 1981). *C. florida* was one of the more sensitive species to non-hydraulic signalling in terms of overall percentage reduction in relative  $g_s$  of WD plants and the stomates did not recover near the end of the experiment as observed for some of the other species. However, it was one of the least sensitive species based on how quickly  $g_s$  declined as soil  $\psi_m$  declined.

*C. virginicus* was determined to be relatively dehydration-tolerant among several deciduous tree species (Augé *et al.*, 1998); however, across a broader spectrum of plants, its lethal  $\psi$  defines it as intermediate in dehydration tolerance (Ludlow, 1989). Even though *C. virginicus* is relatively dehydration tolerant, its stomates responded after fairly little soil drying. This species has also been found to have low constitutive leaf  $\psi_\pi$  and to show large osmotic adjustment during drought (Augé *et al.*, 1998; Fig. 5), which may explain why it had relatively low rates of  $g_s$  decline with declining soil  $\psi_m$ .

Stomatal inhibition of WD plants during the drying period was higher in younger than in older leaves of *H. carolina*, *O. arboreum* and *A. rubrum*, while stomatal inhibition did not vary much with leaf age in *L. tulipifera*, *C. virginicus* and *C. florida* during the drying period. In several herbaceous species, the youngest leaves of drought-stressed plants have been observed to have higher ABA levels and larger declines in  $g_s$  compared with older leaves (e.g. wheat, Atkinson *et al.*, 1989; lupin, Correia and Pereira, 1995; *Xanthium*, Raschke and Zeevaart, 1976; *Ricinus* and *Xanthium*, Zeevaart and Boyer, 1984). Conversely,  $g_s$  of cotton in water-stressed plants was reduced more for older leaves compared to youngest leaves, although the measured ABA accumulations were negatively related to the reduction in  $g_s$  (i.e. ABA concentration was higher in youngest leaves; Ackerson, 1980; Jordon *et al.*, 1975). Maximum leaf conductance probably does not change much in leaves of deciduous hardwoods during the period between complete leaf maturation and initial senescence; however, maximum leaf conductances do vary in developing foliage (Hinckley *et al.*, 1981). For example, immature *Quercus alba* leaves had relatively high cuticular conductances due presumably to incomplete development of the stomatal mechanism and vascular elements in ring porous species (Hinckley *et al.*, 1981). The youngest leaves measured in the present study were fully expanded, mature leaves.

In conclusion, stomatal inhibition in saplings exposed to partial soil drying was not due to direct hydraulic limitation but to some non-hydraulic, root-to-shoot sig-

nalling. The extent of inhibition was similar to previously reported values for other woody species and was larger than much of the inhibition reported for several herbaceous species. Very little soil drying was required to initiate stomatal closure and declines in  $g_s$  were closely related to declines in soil  $\psi_m$  at soil  $\psi_m$  above  $-0.10$  MPa. Species varied in sensitivity of stomatal opening and extent of stomatal inhibition due to non-hydraulic signalling, as was expected based on the native habitats of the species studied. Also, stomatal inhibition varied with leaf age in some species but not in others. Comparison of non-hydraulic signalling characteristics with previously determined relative drought resistance profiles of these species indicated that the non-hydraulic signalling mechanism was allied to a limited extent with other drought strategies of species classified as drought avoiders.

### Acknowledgements

This manuscript is based upon work supported by the US Department of Energy, *National Institute for Global Environment Change, Southeast Region*, under Award No. DE-FC03-90ER61010. We are grateful to Phil Flanagan and Mark Arena for helping with construction and maintenance of the rain shelter, to Ann Stodola and Shawn Duan for assistance with  $g_s$  and leaf  $\psi_m$  measurements, and to Dr Arnold Saxton for statistical advice.

### References

- Abrams MD, Kubiske ME. 1990. Photosynthesis and water relations during drought in *Acer rubrum* L. genotypes from contrasting sites in central Pennsylvania. *Functional Ecology* **4**, 727–33.
- Ackerson RC. 1980. Stomatal response of cotton to water stress and abscisic acid as affected by water stress history. *Plant Physiology* **65**, 455–9.
- Atkinson CJ, Davies WJ, Mansfield TA. 1989. Changes in stomatal conductance in intact aging wheat leaves in response to abscisic acid. *Journal of Experimental Botany* **40**, 1021–8.
- Augé RM, Duan X. 1991. Mycorrhizal fungi and non hydraulic root signals of soil drying. *Plant Physiology* **97**, 821–4.
- Augé RM, Duan X, Croker JL, Witte WT, Green CD. 1998. Foliar dehydration tolerance of twelve deciduous tree species. *Journal of Experimental Botany* **49**, 753–759.
- Augé RM, Duan X, Ebel C, Stodola AJW. 1994. Non hydraulic signaling of soil drying in mycorrhizal maize. *Planta* **193**, 74–82.
- Augé RM, Stodola AJW, Ebel RC, Duan X. 1995. Leaf elongation and water relations of mycorrhizal sorghum in response to partial soil drying: two *Glomus* species at varying phosphorus fertilization. *Journal of Experimental Botany* **46**, 297–307.
- Bahari ZA, Pallardy SG, Parker WC. 1985. Photosynthesis, water relations, and drought adaptation in six woody species of oak-hickory forest in central Missouri. *Forest Science* **31**, 557–69.
- Baño A, Dörffing K, Bettin D, Hahn H. 1993. Abscisic acid and cytokinins as possible root-to-shoot signals in xylem sap of rice plants in drying soil. *Australian Journal of Plant Physiology* **20**, 109–15.
- Bates LM, Hall AE. 1981. Stomatal closure with soil water depletion not associated with changes in bulk leaf water status. *Oecologia* **50**, 52–65.
- Blake J, Ferrell WK. 1977. The association between soil and xylem water potential, leaf resistance and abscisic acid content in droughted seedlings of Douglas-fir (*Pseudotsuga menziesii*). *Plant Physiology* **39**, 106–9.
- Blum A, Johnson JW, Ramseur EL, Tollener EW. 1991. The effect of a drying top soil and a possible non-hydraulic root signal on wheat growth and yield. *Journal of Experimental Botany* **42**, 1225–31.
- Chapman DS, Augé RM. 1994. Physiological mechanisms of drought resistance of four native ornamental perennials. *Journal of the American Society of Horticultural Science* **119**, 299–306.
- Correia MJ, Pereira JS. 1995. Abscisic acid in apoplastic sap can account for the restriction in leaf conductance of white lupins during moderate soil drying and after rewatering. *Plant, Cell and Environment* **17**, 845–52.
- Davies WJ, Kozłowski TT. 1977. Variations among woody plants in stomatal conductance and photosynthesis during and after drought. *Plant and Soil* **45**, 435–44.
- Davies WJ, Metcalf J, Lodge TA, da Costa AR. 1986. Plant growth substances and the regulation of growth under drought. *Australian Journal of Plant Physiology* **13**, 105–25.
- Davies WJ, Tardieu F, Trejo CL. 1994. How do chemical signals work in plants that grow in drying soil? *Plant Physiology* **104**, 309–14.
- Davies WJ, Zhang J. 1991. Root signals and the regulation of growth and development of plants in drying soil. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**, 55–76.
- Ebel RC, Stodola AJW, Duan X, Augé RM. 1994. Non-hydraulic root-to-shoot signalling in mycorrhizal and non-mycorrhizal sorghum exposed to partial soil drying or root severing. *New Phytologist* **127**, 495–505.
- Ebel RC, Welbaum GE, Gunatilaka M, Nelson T, Augé RM. 1996. Arbuscular mycorrhizal symbiosis and non hydraulic signaling of soil drying in *Vigna unguiculata* (L.) Walp. *Mycorrhiza* **6**, 119–27.
- Gallardo M, Turner NC, Ludwig C. 1994. Water relations, gas exchange and abscisic acid content of *Lupinus cosentinii* leaves in response to drying different portions of the root system. *Journal of Experimental Botany* **45**, 909–18.
- Gollan T, Passioura JB, Munns R. 1986. Soil water status affects stomatal conductance of fully turgid wheat and sunflower leaves. *Australian Journal of Plant Physiology* **13**, 459–64.
- Gowing DJG, Davies WJ, Jones HG. 1990. A positive root-sourced signal as an indicator of soil drying in apple, *Malus × domestica* Borkh. *Journal of Experimental Botany* **41**, 1535–40.
- Gowing DJG, Jones HG, Davies WJ. 1993. Xylem-transported abscisic acid – the relative importance of its mass and its concentration in the control of stomatal aperture. *Plant, Cell and Environment* **16**, 453–9.
- Hinckley TM, Dougherty PM, Lassoie JP, Roberts JE, Teskey RO. 1979. A severe drought: impact on tree growth, phenology, net photosynthetic rate and water relations. *The American Midland Naturalist* **102**, 307–16.
- Hinckley TM, Teskey RO, Duhme F, Richter H. 1981. Temperate hardwood forests. In: Kozłowski TT, ed. *Water deficits and plant growth*, VI. New York: Academic Press, Inc. 153–207.
- Hursh CR, Haasis FW. 1931. Effects of 1925 summer drought on southern Appalachian hardwoods. *Ecology* **12**, 380–6.

- Jackson GE, Irvine J, Grace J, Khalil AMM.** 1995. Abscisic acid concentrations and fluxes in droughted conifer saplings. *Plant, Cell and Environment* **18**, 13–22.
- Jones HG.** 1990. Physiological aspects of the control of water status in horticultural crops. *HortScience* **25**, 19–26.
- Jones RJ, Luton MT, Higgs KH, Hamer PJC.** 1983. Experimental control of water status in an apple orchard. *Journal of Horticultural Science* **58**, 301–16.
- Jordan WR, Brown KW, Thomas JC.** 1975. Leaf age as a determinant in stomatal control of water loss from cotton during water stress. *Plant Physiology* **56**, 595–9.
- Khalil AAM, Grace J.** 1993. Does xylem sap ABA control the stomatal behaviour of water-stressed sycamore (*Acer pseudo-platanus* L.) seedlings? *Journal of Experimental Botany* **44**, 1127–34.
- Kirkham MB.** 1980. Movement of cadmium and water in split-root wheat plants. *Soil Science* **129**, 339–44.
- Knapp AK, Smith WK.** 1990. Stomatal and photosynthetic responses to variable sunlight. *Physiologia Plantarum* **78**, 160–5.
- Kramer PJ.** 1983. *Water relations of plants*. New York: Academic Press.
- Kwon KW, Pallardy SG.** 1989. Temporal changes in tissue water relations of seedlings of *Quercus acutissima*, *Q. alba*, and *Q. stellata* subjected to chronic water stress. *Canadian Journal of Forest Research* **19**, 622–6.
- Ludlow MM.** 1989. Strategies in response to water stress. In: Kreeb HK, Richter H, Hinckley TM, eds. *Structural and functional response to environmental stresses: water shortage*. The Hague, Netherlands: SPB Academic Press, 269–81.
- Ludlow MM, Sommer KJ, Flower DJ, Ferraris R, So HB.** 1989. Influence of root signals resulting from soil dehydration and high soil strength on the growth of crop plants. *Current Topics in Plant Biochemistry and Physiology* **8**, 81–99.
- Mansfield TA, McAinsh MR.** 1995. Hormones as regulators of water balance. In: Davies PJ, ed. *Plant hormones*. The Hague, Netherlands: Kluwer Academic Publishers, 598–616.
- Martin U, Pallardy SG, Bahari ZA.** 1987. Dehydration tolerance of leaf tissues of six woody angiosperm species. *Physiologia Plantarum* **69**, 182–6.
- Molz FJ, Peterson CM.** 1976. Water transport from roots to soil. *Agronomy Journal* **68**, 901–4.
- Neales TF, Masia A, Zhang J, Davies WJ.** 1989. The effects of partially drying part of the root system of *Helianthus annuus* on the abscisic acid content of the roots, xylem sap and leaves. *Journal of Experimental Botany* **40**, 1113–20.
- Pallardy SG.** 1981. Closely related woody plants. In: Kozlowski TT, ed. *Water deficits and plant growth*, VI. New York: Academic Press, Inc. 511–48.
- Raschke K, Zeevaart AD.** 1976. Abscisic acid content, transpiration, and stomatal conductance as related to leaf age in plants of *Xanthium strumarium* L. *Plant Physiology* **58**, 169–74.
- Roberts BR.** 1990. Physiological response of yellow-poplar seedlings to simulated acid rain, ozone fumigation, and drought. *Forest Ecology and Management* **31**, 215–24.
- Saab IN, Sharp RE.** 1989. Non-hydraulic signals from maize roots in drying soil: inhibition of leaf elongation but not stomatal conductance. *Planta* **179**, 466–74.
- Saliendra NZ, Sperry JS, Comstock JP.** 1995. Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. *Planta* **196**, 357–66.
- Schulze ED.** 1986. Whole-plant responses to drought. *Australian Journal of Plant Physiology* **13**, 127–41.
- Sinclair TR, Ludlow MM.** 1986. Influence of soil water supply on the plant water balance of four tropical grain legumes. *Australian Journal of Plant Physiology* **13**, 329–41.
- Smith WK, Hollinger DY.** 1991. Measuring stomatal behaviour. In: Lassoie JP, Hinckley TM, eds. *Techniques and approaches in forest tree ecophysiology*. Boca Raton: CRC Press Inc.
- Tardieu F, Katerji N, Benthénod O, Zhang J, Davies WJ.** 1991. Maize stomatal conductance in the field: its relationship with soil and plant water potentials, mechanical constraints and ABA concentration in the xylem sap. *Plant, Cell and Environment* **14**, 121–6.
- Xu X, Bland WL.** 1993. Reverse water flow in sorghum roots. *Agronomy Journal* **85**, 384–8.
- Zeevaart JAD, Boyer GL.** 1984. Accumulation and transport of abscisic acid and its metabolites in *Ricinus* and *Xanthium*. *Plant Physiology* **74**, 934–9.
- Zhang J, Davies WJ.** 1989. Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. *Plant, Cell and Environment* **12**, 73–81.