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Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*

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Abstract. Whole-canopy measurements of water flux were used to calculate stomatal conductance (g_s) and transpiration (E) for seedlings of western water birch (*Betula occidentalis* Hook.) under various soil-plant hydraulic conductances (k), evaporative driving forces (ΔN; difference in leaf-to-air molar fraction of water vapor), and soil water potentials (Ψ_S). As expected, g_s dropped in response to decreased k or Ψ_S, or increased ΔN (> 0.025). Field data showed a decrease in mid-day g_s with decreasing k from soil-to-petiole, with sapling and adult plants having lower values of both parameters than juveniles. Stomatal closure prevented E and Ψ from inducing xylem cavitation except during extreme soil drought when cavitation occurred in the main stem and probably roots as well. Although all decreases in g_s were associated with approximately constant bulk leaf water potential (Ψ_L), this does not logically exclude a feedback response between Ψ_L and g_s. To test the influence of leaf versus root water status on g_s, we manipulated water status of the leaf independently of the root by using a pressure chamber enclosing the seedling root system; pressurizing the chamber alters cell turgor and volume only in the shoot cells outside the chamber. Stomatal closure in response to increased ΔN, decreased k, and decreased Ψ_S was fully or partially reversed within 5 min of pressurizing the soil. Bulk Ψ_L remained constant before and after soil pressurizing because of the increase in E associated with stomatal opening. When ΔN was low (i.e., < 0.025), pressurizing the soil either had no effect on g_s, or caused it to decline; and bulk Ψ_L increased. Increased Ψ_L may have caused stomatal closure via increased backpressure on the stomatal apparatus from elevated epidermal turgor. The stomatal response to soil pressurizing indicated a central role of leaf cells in sensing water stress caused by high ΔN, low k, and low Ψ_S. Invoking a prominent role for feedforward signalling in short-term stomatal control may be premature.

Key words: *Betula* (water relations) – Hydraulic versus chemical signalling – Transpiration – Water relations – Water stress – Xylem cavitation

Introduction

The control of stomatal conductance (g_s) is the primary way plants regulate water flow through the soil-plant-atmosphere continuum over the short-term. An important adaptive advantage of this regulation is to prevent critically low water potential (Ψ) that would otherwise decrease fitness. Stomata appear to regulate leaf water potential (Ψ_L) as opposed to root or stem Ψ, because Ψ is lowest in leaves and tends to remain constant during water stress in many plants (*isohydric* plants; Jones 1990; Tardieu 1993). Water stress is probably sensed by passive reduction of turgor and/or cell volume as Ψ decreases in a tissue (Turner 1986). This causes the release of chemical message such as abscisic acid (ABA) which is transported to the guard cells where solute concentration is reduced and guard-cell turgor decreased (Raschke 1987). Superimposed on this hydroactive control of g_s are potential hydropassive influences resulting from changes in turgor of subsidiary and epidermal cells (Raschke 1970).

The variety of causes of water stress increases the potential for complexity in the stomatal response. The variables causing reduced Ψ_L are evident from the following relationship between Ψ_L and steady-state water flow through the soil-plant-atmosphere continuum:

$$\Psi_L = \Psi_S - [(g_t \cdot \Delta N)/(k_{S-L})] \quad \text{(Eq. 1)},$$

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Abbreviations: ABA = abscisic acid; E = transpiration rate, g_b = boundary-layer conductance to water vapor; g_s = stomatal conductance to water vapor; g_t = total conductance to water vapor; k = leaf-specific hydraulic conductance, subscripts: S–T = soil to trunk, T–P = trunk to petiole, T–L = trunk to lamina, S–P = soil to petiole, S–L = soil to lamina; ΔN = difference in molar fraction of water vapor inside the leaf and ambient air; Ψ = water potential, subscripts: S = soil, T = trunk, L = leaf

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where g_l is the area-specific leaf conductance to water vapor, ΔN is the difference in molar fraction of water vapor inside the leaf and ambient air, and k_{S-L} is the leaf area-specific hydraulic conductance from soil to leaf. Assuming a constant leaf boundary layer conductance (g_b), changes in g_l result from adjustments in g_s. Water stress (i.e., low Ψ_L) is caused by low Ψ_S (soil drought), high ΔN (atmospheric drought), and low k_{S-L}. Reduced g_s in response to each of these causes of water stress has been extensively documented, but the underlying mechanism(s) and consequences of this response are under investigation (Meinzer 1993).

How and where do stomata respond to changes in ΔN, Ψ_S, and k_{S-L} and thereby regulate Ψ_L? The simplest hypothesis is that stomata exhibit a negative feedback response to Ψ_L rather than having direct responses to ΔN, Ψ_S, and k_{S-L}. This traditional explanation (e.g., Ludlow 1980) has been discounted for both atmospheric and soil drought, because these conditions often induce stomatal closure independent of any change in bulk Ψ_L (Grantz 1990; Davies and Zhang 1991); this is the isohydric response seen in some plants (Tardieu 1993). Many investigators have attributed this phenomenon to *feedforward* control of Ψ_L; i.e., a direct stomatal response to the variables influencing Ψ_L rather than a feedback response to Ψ_L itself. In the case of atmospheric drought, a feedforward response of stomata to humidity has been postulated (Farquhar 1978). In the case of soil drought, a feedforward response to Ψ_S has been proposed that involves transport of chemical message (i.e., ABA) from roots to leaves where it induces stomatal closure independent of Ψ_L (Zhang and Davies 1991). Stomatal closure in response to reduced hydraulic conductance also occurs despite approximately constant Ψ_L; the mechanism leading to closure is similarly unclear (Teskey et al. 1983; Meinzer and Grantz 1990; Sperry et al. 1993).

It is our contention that the isohydric response of plants to these three forms of water stress does not logically eliminate the simple feedback model of stomatal conductance with leaf water status. Constant bulk leaf Ψ is the expected result of a sensitive, and therefore adaptive, feedback loop between g_s and Ψ_L. Pressure-bomb measurements of *bulk* Ψ_L mask the complex gradients of Ψ *within* the leaf (e.g., Shackel and Brinckmann 1985) to which the stomata would probably respond. This heterogeneity can explain the supposed feedforward humidity response of stomata as a feedback process at the cell and tissue level (Nonami et al. 1990). It can also explain the stomatal behaviour attributed to root signalling, and the closure of stomata when k_{S-L} is reduced (Sperry et al. 1993). The control of temperature inside a room via a thermostat setting is a familiar example of how approximate homeostasis in the average of a variable is maintained by sensing of small-scale changes in the variable itself. Unless measurements are made at appropriately small-scales in space and time, the relationship between the variable and its control will be obscure.

The root-pressure-chamber system described by Passioura and Munns (1984) provides a means of directly testing our hypothesis that g_s is dependent on Ψ_L. Pressurizing the root chamber increases the pneumatic and hydraulic pressure in the soil and root system by approximately the same amount. Therefore, turgor pressure (i.e., the pressure difference across the cell membrane) and cell volume remain approximately the same despite pressurizing. In the shoot outside the chamber, however, only the hydraulic pressure will increase by pressurizing the chamber, and cell turgor and volume will also increase. Pressurizing will increase total Ψ throughout the system (in the absence of any compensating increase in water flow), but the presumed way that plants sense Ψ, i.e., turgor and/or cell volume, is altered primarily in the shoot (Passioura and Munns 1984). Experiments with the root chamber have provided some of the strongest evidence against leaf-level control of g_s in herbaceous plants, because pressurizing the soil caused no significant difference in g_s during soil drought (Gollan et al. 1986; Schurr et al. 1992).

In this paper, we used the root-chamber system to test whether leaf-level feedback between g_s and Ψ can account for stomatal closure in response to soil drought, atmospheric drought, and reduced k_{S-T} in *Betula occidentalis*, a riparian tree of the western United States which exhibits isohydric responses to water stress. In addition, we evaluated the stomatal regulation of Ψ_L in relation to the range of Ψ known to induce cavitation in *B. occidentalis* (Sperry and Saliendra 1994). We did this because it is becoming obvious that stomatal regulation of Ψ is as important for the control of xylem cavitation as it is for the maintenance of tissue turgor and cell volume (Tyree and Sperry 1988; Jones and Sutherland 1991; Meinzer et al. 1992). Leaves of *B. occidentalis*, for example, normally approach -1.5 MPa during the day in northern Utah; this is only 0.3 MPa above the value predicted to cause complete cavitation of the petiole xylem (Sperry and Saliendra 1994).

Materials and methods

Plant material and growing conditions. Seeds of *Betula occidentalis* Hook. were collected from the Red Butte Canyon Research Natural Area adjacent to the University of Utah, Salt Lake City, UT, USA. Seeds were germinated in a greenhouse and seedlings grown under well-watered conditions in 0.02-m^3 pots. Soil mix was 4:4:4:3:2:1 topsoil (sifted clay loam), bark mulch, perlite, vermiculite, peat, sand. Plants were used when they were ca. 0.5 m tall and had ca. 0.35 m^2 leaf area (six to eight months growth). One cohort of seedlings was grown in spring and summer of 1993; a second was grown in fall and winter of 1993–94.

We also compared stomatal and hydraulic conductance between juvenile, sapling, and adult trees growing in the same riparian zone in the Red Butte Canyon site. Juveniles were less than three years old and less than 1 m tall. Saplings were between 2 and 3 m tall, and adults were full-size trees of ca. 5–10 m height.

Whole-canopy gas-exchange experiments

Experimental design. In the first of two types of experiment, we determined canopy water flux to increasing differences in molar fraction of water vapor between leaf and ambient air (ΔN) for three treatments. *Control* plants ($n = 3$) were well-watered. *Pressurized* plants ($n = 3$) were well-watered and had the potted root system enclosed in a pressure chamber that was pressurized to constant value of 0.5 MPa. *Notched* plants ($n = 3$) were well-watered and had six transverse cuts made about halfway through the stem-base from

alternating sides 0.01 m apart; this significantly reduced hydraulic conductance of the soil-to-leaf pathway (Sperry et al. 1993).

In the second type of experiment, we held ΔN as constant as possible and determined the influence of soil pressurizing on the stomatal response of individual plants to ΔN, notching, and soil drought. In the ΔN experiments ($n = 7$), we first determined g_s and transpiration (E) at low ΔN (< 0.015), and then at higher ΔN (> 0.025). During exposure to one or both ΔN settings, the soil was pressurized in increments (usually 0.25 MPa), and changes in g_s and E were monitored. In notching experiments ($n = 3$), ΔN was held constant and g_s was measured before and after notching. Soil pressure was then applied and responses in g_s and E were observed. In soil drought experiments ($n = 4$), plants were allowed to transpire at nearly constant ΔN until soil water potential began to decline. Soil-pressure responses were determined at various points during the dry-down which usually involved 3 d of gas-exchange measurements. In a variation of this experiment, we also continuously pressurized the soil to maintain zero xylem pressure in the stem during a soil dry-down. Zero stem xylem pressure was achieved by increasing the soil pressure until water rose to the cut end of a side branch of the seedling protruding through a port in the cuvette. This was the protocol used by Gollan et al. (1986).

In all experiments, g_s and E were calculated at the canopy level, and Ψ of leaves and trunk were measured periodically with a pressure chamber. At the conclusion of each experiment, we measured the leaf area-specific hydraulic conductance of the soil-to-trunk (k_{S-T}) and trunk-to-petiole (k_{T-P}) pathways. We also measured xylem embolism within the trunk.

Gas exchange methods. Seedlings were brought from the greenhouse to the laboratory on the night before the experiment. They were sealed within a split-lid pressure chamber with all but the basal 0.1 m of the shoot protruding. The shoot was sealed within a water-jacketed Plexiglas cuvette lined with Teflon film (inside dimensions: 0.48 m × 0.41 m × 0.64 m). Copper-constantan thermocouples (0.13 mm diameter) were placed on the lower surface of six leaves. Photosynthetic photon flux density of about 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was supplied with two 1000 W Na-vapour HID lamps and monitored by four gallium arsenide photodiodes. Air inside the cuvette was circulated by five fans, and temperature was controlled by circulating water through the jacketed walls of the cuvette.

Gas-exchange parameters were measured using an open system as described by Comstock and Ehleringer (1993). The concentration of CO_2 inside the cuvette was kept near ambient (ca. 350 $\mu mol \cdot mol^{-1}$). Although photosynthesis was measured, we report only water-vapor conductances and transpiration rates. Transpiration rate per leaf area (E) was calculated as

$$E = (u_c/s) \cdot [(N_0 - N_c)/(1 - N_0)] \quad \text{(Eq. 2)},$$

where u_c is the molar flow rate of air entering the cuvette; s is the leaf area in the cuvette; N_c and N_0 are the molar fractions of water vapor entering and leaving the cuvette, respectively (Von Caemmerer and Farquhar 1981). Depending on the experiment, N_c ranged from 0.00 to 0.04. Total canopy conductance to water vapor per canopy leaf area (g_t) was calculated as

$$g_t = [E \cdot (1 - \bar{N})]/\Delta N \quad \text{(Eq. 3)},$$

where $\bar{N} = (N_i + N_a)/2$; N_i and N_a are the molar fractions of water vapor inside the leaf and the surrounding air, respectively. Stomatal conductance to water vapor per leaf area (g_s) was calculated from

$$g_s = (g_b \cdot g_t)/(g_b - g_t) \quad \text{(Eq. 4)},$$

where g_b was determined to be 1.0 $mol \cdot m^{-2} \cdot s^{-1}$ for the lower leaf surface as measured directly on a model of a *B. occidentalis* shoot having Teflon-backed filter paper "leaves" (the leaves are hypostomatous). Leaf area (s) was determined with a leaf-area meter (model 3100; Li-Cor Inc., Lincoln, Neb., USA) at the end of the experiment.

Although we followed the usual practice of measuring E to deduce g_s, in terms of the plant's proximate control of water flux, it is the active control of g_s which determines E (for a given ΔN and g_b). Thus, it was appropriate to present our results in terms of g_s controlling E.

The ΔN was controlled via the humidity of the air entering the cuvette. For the humidity response curves, g_s and E determinations represent stable values obtained usually within 15–30 min of maintaining ΔN near pre-determined values (i.e., ca. 0.015, 0.025, 0.035, 0.045).

Field measurements of g_s and E

Measurements of g_s and E were performed in the field using a steady-state porometer (model LI-1600 m; Li-Cor Inc.). Depending on the size of the shoot, 10 to 20 leaves were marked in each shoot for porometry. Porometric measurements were conducted on sunny days from 1000 to 1500 hours Mountain Standard Time when photosynthetic photon flux density exceeded 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In-situ leaf temperature, humidity, and boundary-layer conductance may differ from those obtained during porometric measurements (McDermitt 1990). Using g_s obtained from porometry and $g_b = 1.0$ $mol \cdot m^{-2} \cdot s^{-1}$ (corresponding to a wind speed of 2 $m \cdot s^{-1}$) and in-situ measurements of relative humidity, leaf and air temperature, we estimate the E measured by porometry exceeded actual values by 26%. We report the corrected values (e.g., Fig. 7).

Leaf, stem, and soil Ψ

Leaves were sampled by enclosing a transpiring leaf in a small plastic bag, rapidly excising the petiole at the mouth of the bag and sealing the bag immediately to avoid post-excision decline in Ψ. Leaf water potential (Ψ_L) was measured with a commercial pressure chamber (PMS Inc., Corvallis, Ore, USA). For trunk water-potential measurements (Ψ_T), leaves along the base of the main axis were covered with aluminum foil at least 1 d before measurements were taken to promote Ψ equilibration with the subtending stem.

In the field, Ψ_S was estimated from predawn Ψ_L measurements. In the laboratory, Ψ_S at the conclusion of the experiment was assumed equal to the pressure intercept of the linear relationship between flow rate and pressure obtained for the measurement of k_{S-T} (Passioura and Munns 1984; see below).

Hydraulic conductance and xylem embolism

Hydraulic conductance is defined as the flow rate of liquid water divided by the pressure difference across a defined flow path. Generally we expressed this per leaf area supplied by the flow path (*leaf-specific* conductance, k). We use both *direct* and *indirect* methods to determine k.

Direct measurement of the leaf-specific hydraulic conductance of the soil-to-trunk flow path (k_{S-T}) employed the pressure-flux technique (Markhart and Smit 1990). After gas-exchange measurements were concluded, the shoot was detached, and the soil-root system in the pressure chamber was pressurized initially at 0.6 MPa, then, successively at 0.4 and 0.2 MPa. Flow rates from the cut stem were measured after stabilizing at each pressure. This took 1–1.5 h at 0.6 MPa, but only 20 min at the two lower pressures. Flow rates were measured by collecting xylem sap from the protruding stump with a pre-weighted vial filled with absorbent paper. The flow rates per canopy leaf area were plotted as a function of the applied pressure, and k_{S-T} was estimated as the slope of the linear regression (e.g., Fig. 1A). The pressure intercept gave an estimate of the soil water potential (Passioura and Munns 1984). In the soil-drought experiments, we measured k_{S-T} before and after watering the soil.

We used a similar approach for direct measurement of the trunk-to-petiole hydraulic conductance (k_{T-P}). The base of a stem of a defoliated shoot was connected to a tubing filled with a weak HCl solution. This tubing led to a reservoir on an analytical balance (model A200S; Sartorius Crop., Bohemia, N.Y., USA). The defoliated shoot was sealed in a long (ca. 2 m) cylindrical vacuum chamber

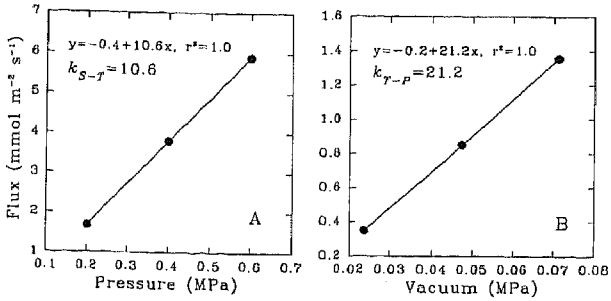

Fig. 1A, B. The relationship between flux (per leaf area) and pressure (**A**), or vacuum (**B**) applied on detopped soil-root system and defoliated shoot, respectively, of a well-watered *B. occidentalis* seedling. The slope of the linear regression gives the *leaf-specific* hydraulic conductance (mmol·m^{-2}·s^{-1}·MPa^{-1}) of the soil-to-trunk (k_{S-T}; **A**) and trunk-to-petiole (k_{T-P}; **B**) pathways

with the base protruding. Vacuum of 0.024, 0.047 and 0.071 MPa was applied sequentially, and flow rate through the shoot measured at each pressure difference. Measurements were automated by interfacing a computer with the balance. Steady-state flow rates at a given pressure were attained within 5–15 min. A linear relationship was obtained between pressure and flow rate with generally a slightly non-zero flow-rate intercept. The slope of the regression line gave the k_{T-P} for the shoot (e.g., Fig. 1B). We attributed the non-zero intercept to osmotic uptake of water by the shoot. If a zero intercept had been consistently obtained, we could have calculated k_{T-P} from a single flow-rate measurement as is usually done when the conductance of short (0.1–0.3 m) stem segments is measured (i.e., as in the embolism measurements on stem segments, see below). Total leaf specific plant conductance of the soil-to-petiole flow path (k_{S-P}) was calculated from the root and shoot hydraulic resistance in series,

$$k_{S-P} = (k_{S-T} \cdot k_{T-P})/(k_{S-T} + k_{T-P}) \quad \text{(Eq. 5)}.$$

Indirect estimates of the total leaf specific conductance of the soil-to-lamina flow path (k_{S-L}) were calculated from E and the difference between Ψ_S and Ψ_L at the time of E measurement:

$$k_{S-L} = E/(\Psi_S - \Psi_L) \quad \text{(Eq. 6)}.$$

In the field, E was measured near mid-day when it was most stable. Partitioning of k_{S-L} into two components, soil-to-trunk (k_{S-T}) and trunk-to-leaf (k_{T-L}), was accomplished by using the equations:

$$k_{S-T} = E/(\Psi_S - \Psi_T) \quad \text{(Eq. 7)}$$

$$k_{T-L} = E/(\Psi_T - \Psi_L) \quad \text{(Eq. 8)}.$$

We also combined indirect measurements of k_{S-T} (Eq. 7) with direct measurements of the trunk-to-petiole hydraulic conductance of the same plant (k_{T-P}) to estimate the conductance of the soil-to-petiole flow path for plants in the field (k_{S-P}, Eq. 5).

Xylem embolism was quantified by the percentage the initial hydraulic conductance of short (ca. 0.1 m) stem segments was below a final maximum value obtained after repeated high-pressure (i.e., 100 kPa) flushes of measuring solution. This pressure treatment caused air in embolised vessels to dissolve (Sperry et al. 1988). Measurements were made on four to ten segments cut from the main axis underwater (to avoid causing additional air-blockage). The technique is described elsewhere (Sperry et al. 1988).

Results

Response to atmospheric drought and reduced hydraulic conductance. The stomatal response of control plants

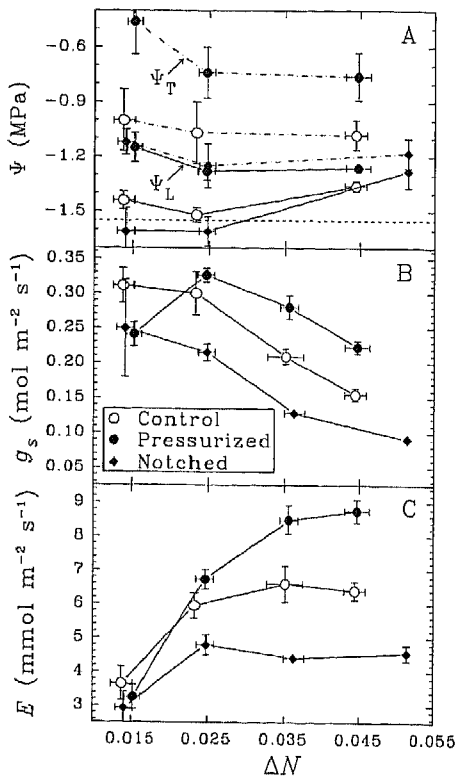

Fig. 2A–C. Leaf (Ψ_L; *solid lines*) and stem (Ψ_T; *dash-dotted lines*) water potential (**A**), g_s (**B**), and E (**C**) versus ΔN for control (*open circles*), pressurized (*solid circles*), and notched (*solid diamonds*) *B. occidentalis* plants. Data are means ± SE, $n = 3$. *Dashed line* at − 1.55 MPa in (**A**) corresponds to the highest Ψ predicted to induce cavitation in petiole xylem based on Sperry and Saliendra (1994)

($n = 3$) to increasing ΔN is shown in Fig. 2B (open circles). The stomatal conductance (g_s) was stable and maximum at ΔN below 0.025; above this, g_s declined steadily. During the decline in g_s, E and bulk Ψ_L remained approximately constant (Fig. 2A, C; open circles with solid line). Bulk Ψ_L remained near values predicted to initiate cavitation in the petiole xylem (Fig. 2A; dashed line at − 1.55 MPa; Sperry and Saliendra 1994). Stem embolism averaged 1% (± 1, $n = 3$) and average k_{S-P} (direct measurement) was 7.0 ± 0.1 mmol·m^{-2}·s^{-1}·MPa^{-1} ($n = 3$).

Notched plants ($n = 3$) had a k_{S-P} that was 34% below controls on average (4.6 ± 0.7 versus 7.0 ± 0.1 mmol·m^{-2}·s^{-1}·MPa^{-1}, $n = 3$; direct measurement). This was associated with a 20–40% reduction in g_s at all ΔN (Fig. 2B; diamonds). Maximum E was 35% below controls (Fig. 2C; diamonds). Bulk Ψ_L (Fig. 2A; diamonds with solid line) and stem embolism were not significantly different from controls.

When the entire ΔN versus g_s curve was done at 0.5 MPa pressure in the root chamber, g_s and E were lower than in controls when ΔN was below ca. 0.025 (Fig. 2B, C; filled circles). At higher ΔN, however, both parameters exceeded controls. Bulk Ψ_L was significantly higher than controls (Fig. 2A; filled circles with solid line). Shoot embolism (4 ± 3%, $n = 3$)

and k_{S-P} (8.0 ± 0.4 mmol·m^{-2}·s^{-1}·MPa^{-1}, $n = 3$; direct measurement) were both similar to controls. This indicated that pressurizing and depressurizing the root system did not cause embolism by injection of air into the vascular system, or by air coming out of solution during depressurizing (i.e., the "bends" effect).

It was surprising that the plants in Fig. 2 (control, pressurized, notched), showed little or no decrease in Ψ_L or Ψ_T despite their large increase in E as ΔN increased from 0.015 to 0.025. There was also a tendency in control and notched plants for Ψ_L to increase despite constant E as we raised ΔN above 0.025. These disproportional changes in E and Ψ may reflect either variable hydraulic conductance, or deviation from steady-state flow (Passioura and Munns 1984), or gradients in Ψ within the leaf due to patchy stomatal closure at high ΔN (Mott et al. 1993). Significant differences in CO_2 assimilation at relatively constant mesophyll CO_2 partial pressure, whether the plant was untreated, notched, or pressurized (data not shown), indicated that patchy stomatal closure may have occurred at high ΔN.

Indirect measurements of k_{T-L} made at ΔN above 0.025 were not significantly different from direct measurement of k_{T-P} (15.3 ± 4.8 versus 20.6 ± 3.2 mmol·m^{-2}·s^{-1}·MPa^{-1}, $n = 4$). In contrast, indirect estimates of k_{S-T} were much lower than direct ones (8.3 ± 0.7 versus 14.3 ± 2.6 mmol·m^{-2}·s^{-1}·MPa^{-1}, $n = 5$). Direct measurements of k_{S-P} for the plants in Fig. 2 overestimated Ψ_L by 0.30 to 0.65 MPa (using Eq. 1 and $\Psi_S = 0$) at maximum ΔN when steady-state flow was most likely to exist. This was probably larger than the normal petiole-lamina Ψ difference (i.e. using k_{S-P} in Eq. 1 gives petiole rather than lamina pressure). The discrepancy probably resulted from erroneously high direct measurements of k_{S-T}. These may have arisen from refilling of air spaces in soil, root cortex, and xylem by positive pressures used during the measurement.

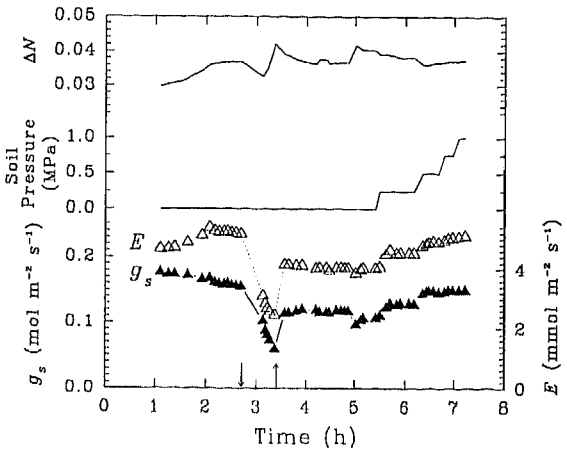

Fig. 3. Time course of g_s, E, ΔN, and soil hydrostatic pressure for a well-watered *B. occidentalis* plant. The ΔN was held between 0.03 and 0.04 throughout the experiment. After stable g_s and E were obtained, the sodium-vapor lights were turned off (*down arrow*) and the stem was notched. After the lights were turned on (*up arrow*), g_s and E stabilized below their pre-notch values. Stepped increases in soil pressure corresponded to an increase in g_s and E to pre-notch values

The lower g_s and E in notched versus control plants shown in Fig. 2 was consistent with the response of individual plant to notching (Fig. 3). Three plants were notched after reaching a steady transpiration rate at ΔN of 0.030 to 0.040. To notch the stems we had to interrupt the gas-exchange measurements (e.g., turn off the Na-vapour lights and open the cuvette). When measurements resumed, all notched plants had lower g_s (25–38%) and E (12–34%) relative to their initial values (e.g., Fig. 3). In all cases, reduced E and g_s were not associated with a change in bulk Ψ_L (data not shown, but see Fig. 2A notched versus control Ψ_L). When the soil was pressurized after the stem was notched, the stomatal closure induced by notching was reversed (Fig. 3). The stomatal response occurred within 5 min of pressurizing the soil.

The higher g_s and E in pressurized versus control plants at high ΔN (i.e., > 0.025; Fig. 2B, C) was consistent with the response of single plants to pressurizing over the same ΔN range (e.g., Fig. 4). In seven plants tested from both seedling cohorts, g_s and E increased within 5 min of pressurizing to 0.25 or 0.5 MPa and stabilized after 15–20 min (Fig. 4A, pressurizing at 4.5 h, $\Delta N = 0.04$).

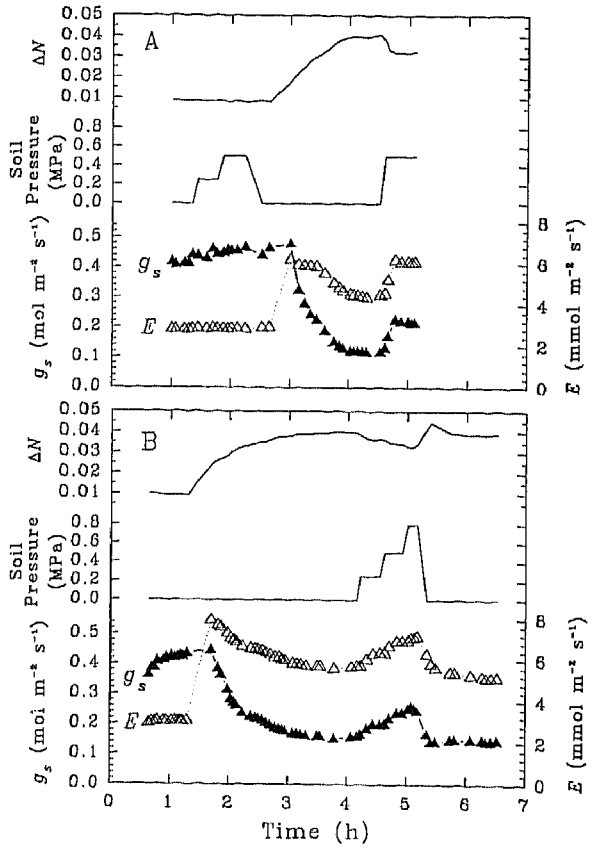

Fig. 4A, B. Time course g_s, E, ΔN, and soil hydrostatic pressure for two well-watered *B. occidentalis* plants (**A, B**). Initial ΔN was near 0.01 to establish maximum g_s (Fig. 2A). The ΔN was then increased to near 0.04 to induce stomatal closure. **A** Two-step increase of soil pressure to 0.5 MPa had no effect on g_s or E at $\Delta N = 0.01$. One-step increase in soil pressure to 0.5 MPa caused a significant and immediate increase in g_s and E at ΔN near 0.04. **B** Three-step increase of soil pressure to 0.8 MPa at ΔN near 0.04 saturated the g_s response at a value below the maximum obtained at $\Delta N = 0.01$

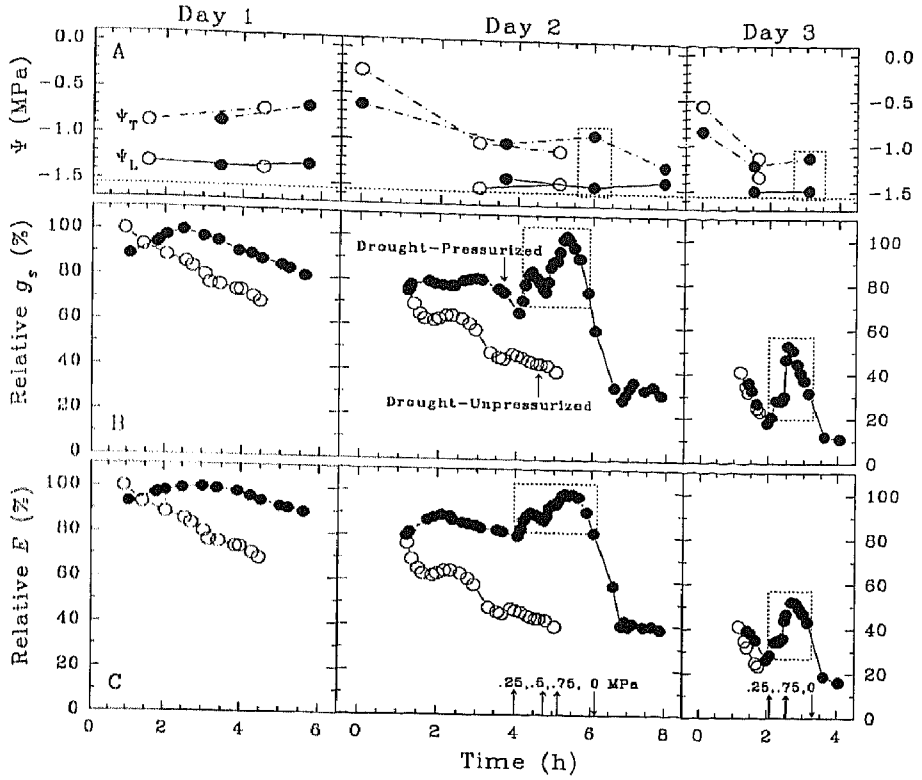

Fig. 5A–C. Time course of Ψ_L (*solid lines*; A), Ψ_T (*dash-dotted lines*; A), g_s (**B**), and E (**C**) as soil water was depleted over 3 d. The g_s and E are expressed as percentage of maximum obtained on Day 1. The ΔN was held between 0.029 and 0.037 throughout. Data for two *B. occidentalis* plants are shown; a control (*open circles*) and a pressurized (*filled circles*) plant subjected to episodes of increased soil pressure (*dashed boxes*). Numbers above arrows on the x-axis indicate the amount of increase (*up arrows*) or decrease (*down arrows*) in soil pressure (MPa). *Dashed line* in A indicates maximum Ψ predicted to cause petiole cavitation (Sperry and Saliendra 1994)

Subsequent increases in pressure to 0.8 MPa saturated the g_s response at a value below the maximum obtained at low ΔN (Fig. 4B). For example, in Fig. 4, the maximum g_s obtained at $\Delta N = 0.01$ was ca. 0.45 mol·m^{-2}·s^{-1} while the maximum g_s obtained during pressurizing at $\Delta N = 0.04$ was near 0.25 mol·m^{-2}·s^{-1}.

The lower g_s and E in pressurized versus control plants at low ΔN (< 0.025) shown in Fig. 2 (filled versus open circles) was not consistent between cohorts of seedlings. Seedlings raised in the greenhouse during winter (as opposed to summer for those in Fig. 2), exhibited no stomatal response when soil was pressurized to 0.5 MPa and ΔN was 0.010 (Fig. 4A; pressurizing the soil at 1.5 h).

Response to soil drought. In the four plants subjected to soil drought, there was a significant decline in both g_s and E, and no change in bulk Ψ_L which remained within a tenth of an MPa of the predicted cavitation pressure for petiole xylem (Fig. 5). Soil water potential at the conclusion of the 2–3 d drought period was between −0.20 and −0.68 MPa. The ΔN was held as constant as possible between 0.038 and 0.047.

Two of these plants were pressurized after significant stomatal closure had occurred. In both cases, g_s and E began increasing within 5 min of pressurizing (Fig. 5B, C; dashed boxes). Unlike the stable responses to pressure in well-watered soil (Figs. 3, 4), g_s and E typically peaked after 15 min before declining (Fig. 5B, C; dashed boxes). We attributed the decline to rapidly decreasing soil water content and hydraulic conductance caused by the increase in E during pressurizing. In the early stages of drought we could completely reverse the stomatal closure by pressurizing (Fig. 5B, Day 2; dashed box). Later in the drought, pressurizing to 0.75 MPa caused a significant increase in g_s, but not to initial values (Fig. 5B, Day 3; dashed box). Depressurizing was associated with a precipituous drop in g_s and E values that were lower than pre-pressurized values.

Unlike bulk Ψ_L, Ψ_T dropped during drought (e.g., Fig. 5A, Day 1–3; from −0.85 to −1.25 MPa) and increased during pressure cycles (Fig. 5A, Day 2, 3; dashed boxes). The drop in Ψ_T was associated with an average of 34 ± 14% embolism in stem xylem for the three most severely droughted plant (final Ψ_S = −0.45, −0.63, −0.68 MPa). The remaining plant was only droughted to final Ψ_S of −0.20 MPa and its stem xylem was 2.4 ± 3.6% embolised. The occurrence of cavitation in the stem xylem during drought despite its apparent avoidance in petioles was consistent with our earlier finding that the xylem of the main axis was more vulnerable to cavitation than that of minor twigs and petioles in this species (Sperry and Saliendra 1994).

All four droughted plants had considerably lower k_{S-P} than controls (0.9 ± 0.2 versus 7.0 ± 0.1 mmol·m^{-2}·s^{-1}·MPa^{-1}, respectively; direct measurement). In the three most-droughted plants, both k_{S-T} and k_{T-P} components decreased. In the least-droughted plant, only k_{S-T} was reduced below controls in keeping with the absence of stem embolism in this plant that would have reduced its k_{T-P}. These data suggested that k dropped first in the soil-root system during drought, and later progressed into the stem. Rewatering the soil caused k_{S-T} to increase from 1.0 ± 0.2 to 7.4 ± 1.1 mmol·m^{-2}·s^{-1}·MPa^{-1}, which approached control values (8.3 ± 0.7 mmol·m^{-2}·s^{-1}·MPa^{-1}). The increase was probably because of increased soil hydraulic conductance and better soil-root contact as well as refilling of embolized vessels in the root by positive pressures used during k_{S-T} measurement (Fig. 1A). We

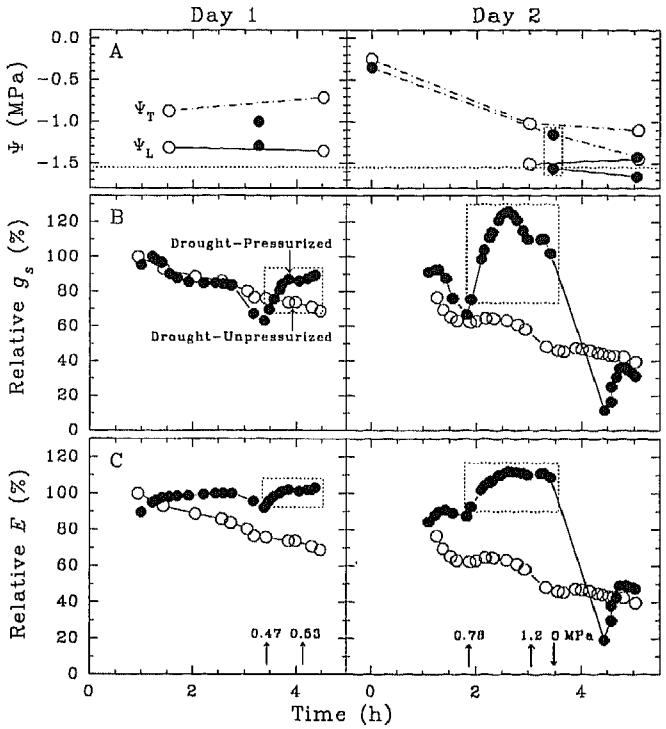

Fig. 6A–C. Time course Ψ_L (*solid lines*; **A**), Ψ_T (*dash-dotted lines*, A), g_s (**B**), and E (**C**) as soil water was depleted over 2 d. The g_s and E are expressed as percentage of maximum obtained on Day 1. The ΔN was held between 0.024 and 0.034 throughout. Data for two *B. occidentalis* plants are shown; a control (*open symbols*; same data as in Fig. 5), and a pressurized (*filled symbols*) plant subjected to episodes of increased soil pressure (*dashed boxes*). Increases in soil pressure (MPa; numbers above arrows on the x-axis) were made to keep $\Psi_T = 0$ on a cut side branch. This was possible up to a soil pressure of 0.78 MPa (Day 2). After this, we could not reach $\Psi_T = 0$ despite 1.2 MPa of soil pressure. During this time Ψ_T was -1.18 MPa as measured in a bagged leaf (**A**). *Dashed line* in A corresponds to maximum Ψ causing petiole cavitation (Sperry and Saliendra 1994)

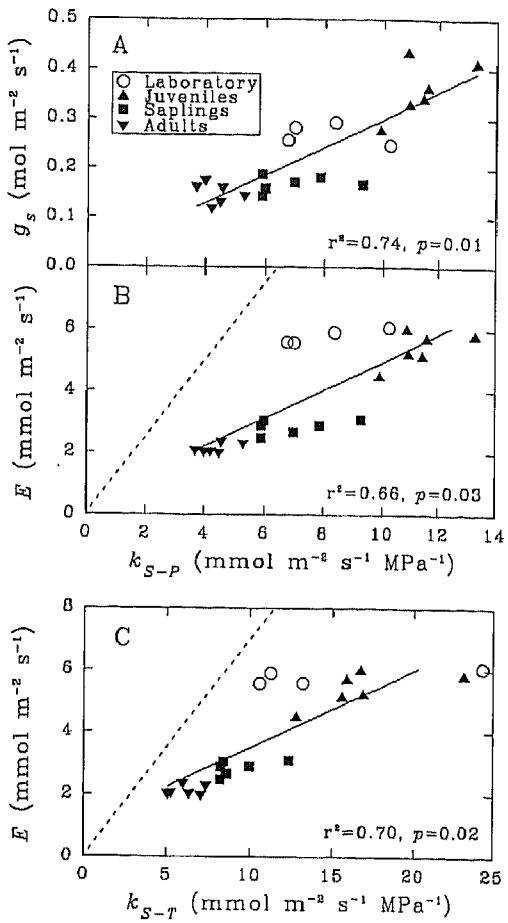

Fig. 7A–C. Plots of g_s (**A**) and E (**B**) versus k_{S-P} for juveniles, saplings, and trees of *B. occidentalis* in the field (*filled symbols*). Laboratory data (*open circles*) for seedlings are also shown. *Dashed line* in **B** corresponds to minimum E predicted to cause cavitation in petiole xylem (Sperry and Saliendra 1994). **C** Plot of E versus k_{S-P} for juveniles, saplings, and trees in the field; and seedlings in the laboratory. *Dashed line* corresponds to minimum E predicted to cause $\geqslant 15\%$ cavitation in stem xylem (Sperry and Saliendra 1994)

suspect root cavitation exceeded stem cavitation during the drought based on the greater vulnerability of root xylem to cavitation versus stem xylem in this species (Sperry and Saliendra 1994).

When we attempted to continuously maintain $\Psi_S = 0$ during soil drought according to the procedure in Gollan et al. (1986), we were able to prevent stomatal closure relative to non-pressurized droughted plants (Fig. 6). However, the elevated E increased the rate of soil drying and made it difficult to maintain balancing pressure at the cut surface of the lateral branch. After 3 h of pressurizing we could no longer keep $\Psi = 0$ in the branch despite a pressure of 1.2 MPa and we depressurized the chamber. This caused g_s and E to drop considerably. Bulk Ψ_L remained between -1.29 and -1.66 MPa.

Hydraulic conductance versus stomatal conductance in the field. The k_{S-P} (Eq. 5, but using indirectly measured k_{S-T}) of plants in the field was dependent on developmental stage with juveniles having on average 1.9 times the k_{S-P} of adults and saplings combined (Fig. 7, filled symbols). The k_{S-P} values of laboratory seedlings (Fig. 7, open circles) were intermediate. Under conditions of similar ΔN (0.020 to 0.032), k_{S-P} and k_{S-T} were positively correlated with mid-day g_s and E (Fig. 7A–C). This was consistent with there being no major differences between pre-dawn and mid-day water potentials across all plants in the laboratory or field (data not shown).

The dashed lines in Fig. 7B,C corresponded to E required to induce more than 15% loss of hydraulic conductance via cavitation in petiole (Fig. 7B) and stem (Fig. 7C) xylem based on Sperry and Saliendra (1994). At higher E, cavitation would increase sharply according to their data. Stems showed a smaller safety-margin than petioles, and adults (i.e., plants with lower k_{S-P}) showed a smaller safety-margin than juveniles.

The higher k_{S-P} in juvenile versus adult (saplings included) plants resulted from almost proportional increases in both k_{S-T} and k_{T-P} components (Fig. 8). In terms of resistances, the soil-to-trunk resistance was 69 and 73% of the total soil-to-petiole resistance in juveniles and adults, respectively. The k_{T-P} divided by the length of

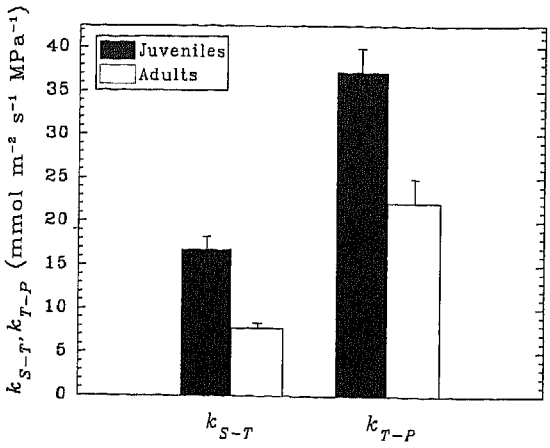

Fig. 8. Values of k_{S-T} (indirect measurement) and k_{T-P} (direct measurement) in juveniles versus adults (saplings + trees) of *B. occidentalis*

the flow path was not significantly different across age categories; nor was the leaf area per stem transverse area (data not shown). Therefore, the increase in shoot conductance of juveniles was because of their smaller size (i.e., shorter length of flow-path) relative to adults. We did not analyze the root system and cannot say to what extent the increase in k_{S-T} in juveniles resulted from shorter path lengths versus inherent differences in root mass versus leaf area or xylem conductance per unit length of roots.

Discussion

The results supported our hypothesis that leaf water status has a major influence on stomatal closure in response to decreased k_{S-P}, decreased Ψ_S, and increased ΔN. When k_{S-P} was decreased below control values by stem notching, there was an associated decrease in g_s at all ΔN relative to controls (Fig. 2B, C). This decrease in g_s was reversible by pressurizing the soil (Fig. 3). The closure of stomata in response to increased ΔN was partially prevented or reversed by pressurizing the soil (Figs. 2, 4). Pressurizing the soil also prevented or reversed stomatal closure in response to soil drought (Figs. 5, 6). Because soil pressurizing will not alter root cell turgor or volume to as large a degree (if at all) as it will shoot cell turgor and volume, it follows that the stomatal response to all manipulations was linked to changes in shoot rather than root cell water status (Passioura and Munns 1984). In addition, the response of stomata to changes in soil pressure occurred within minutes. It is unlikely that this response time could be achieved via a root-sourced chemical signal acting at the guard cells.

Superficially, our results present a paradox. On the one hand, the soil-pressurization experiments demonstrated that changing Ψ_L influenced g_s. On the other hand, bulk Ψ_L varied only by a few tenths of an MPa regardless of treatment (except for pressurizing at low ΔN, Fig. 2A). We can resolve the paradox with the reasonable assumption that the feedback between Ψ_L and g_s is too sensitive to be reflected by whole-leaf averages of Ψ. The increase in Ψ_L caused by pressurizing was not observed, because it immediately (i.e., within minutes) induced stomatal opening and increased E leading to a drop in Ψ_L. To see a correlation between g_s and Ψ would require continuous measurements of Ψ in the population of cells involved in the sensing-signalling function. These fine-scale measurements are far beyond the capabilities of the pressure chamber.

According to this traditional feedback mechanism, g_s would be maximum for E up to the lowest value causing Ψ_L to reach the set-point. Under natural circumstances, this would occur when the soil was wet and ΔN was low and/or k_{S-P} was high (see Turner 1974; Ludlow 1980). Under these conditions, pressurizing the soil in the root chamber should induce no *active* increase in guard cell turgor, because it would already be at its maximum. Indeed, in the cohort of seedlings raised during winter we saw no response to pressurizing at low ΔN (Fig. 4A). On the other hand, in the cohort raised during summer we saw a *decrease* in g_s and E, along with an increase in Ψ_L (Fig. 2). It is possible that in this second cohort, the increase in Ψ_L resulting from pressurizing at maximum g_s caused epidermal turgor to increase sufficiently to hydropassively close stomata (Raschke 1970). Closure in response to root pressurizing was also observed under non-stressful conditions for *Capsicum annuum* and attributed to hydropassive effects (Janes and Gee 1973). We do not know why this response was not seen in the first seedling group. Perhaps the different seasons of growth caused differences in epidermal turgor pressures and/or wall structure in the two cohorts.

Although we could completely reverse stomatal closure from reduced k_{S-P} (Fig. 3) and reduced Ψ_S (at least in early stages of drought, Figs. 5, 6) by pressurizing the soil, this was not the case for stomatal closure from increased ΔN. We could get g_s to increase, but not to its maximum level measured at low ΔN despite continued increases in soil pressure (Fig. 4B). Perhaps local drop in Ψ at the site of evaporation within the leaf contributed to stomatal closure, and the increase in Ψ caused by pressurizing the soil was only weakly propagated to this extreme distal end of the flow path owing to low hydraulic conductance of the mesophyll (and increased evaporation). The response of stomata to manipulations of Ψ_L will depend on where Ψ is sensed in the leaf.

Why did we find dramatic stomatal responses to soil pressurizing when previous studies found none with the same experimental approach (Gollan et al. 1986; Schurr et al. 1992)? The simplest explanation is that woody plants like *B. occidentalis* are less dependent on root sensing of water stress than the herbaceous species used exclusively in the other studies. This is supported by recent work with seedlings of *Psuedostuga menziesii* and *Alnus oregona* which exhibited rapid stomatal opening in response to pressurizing dry soil just as did *B. occidentalis* (Fuchs and Livingston 1994). It is logical that large woody species would lack a chemical root signal, because long transport time would make root-signalling ineffective for short-term stomatal regulation (Schulze 1991). However, we have observed stomatal opening in response to soil pressurizing in the small desert shrub *Hymenoclea salsola* (data not shown). It is possible that the high humidities used in

earlier experiments (e.g., ΔN of ca. 0.01, Gollan et al. 1986; Schurr et al. 1992) minimized the active stomatal response to soil pressure, and increased the possibility of complications from hydropassive closure. More work needs to be done comparing root-versus leaf-level signalling in herbaceous versus woody plants.

Our results with *B. occidentalis* urge caution in interpreting the large and growing literature on root signalling. As we have seen in *B. occidentalis*, isohydric behaviour itself cannot be used as evidence for root versus leaf signalling, and yet this has formed the foundation for invoking root control (Davies and Zhang 1991). While it is well established that roots produce ABA in response to stress (Davies and Zhang 1991), leaf tissues do as well (Pierce and Raschke 1980), and it is far from clear whether ABA from roots accounts for observed stomatal behaviour. Recent models of root signalling have been forced to incorporate an influence of leaf water status of imported ABA in the leaf (Tardieu and Davies 1993). Tardieu (1993) has also shown that normal short-term stomatal behaviour is expected when root-sourced ABA is entirely excluded from a model. It is possible that the difficulties in modelling short-term stomatal responses via root-sourced hormones arises because they play no dominant role.

The leaf-based signalling process we observed in *B. occidentalis* by no means excludes root water status influencing g_s. Changes in k_{S-P} or Ψ_S from *any* cause (root pruning, splitting root systems, root shrinkage, stem notching, defoliation, soil drying, soil compaction, flooding, etc.) will almost immediately change leaf Ψ (Eq. 1) regardless of the velocity of water flow (i.e., velocity of a pressure wave through xylem could approach the speed of sound in water in tissues with low capacitance; Malone 1993). This *hydraulic signal* is a simple and rapid form of root-to-shoot communication that can initiate stomatal responses or other leaf-level changes (e.g., Malone 1993; Chazen and Newman 1994). Referring to a root signal transported at the relatively sluggish velocity of the transpiration stream as a *feedforward* response to soil water status (Schulze 1993) is misleading, because a chemical signal will necessarily arrive at the leaf *after* the hydraulic one has influenced leaf water status.

Ironically, it is a *non-isohydric* response to soil drought that may require a chemical signal from the root. If short-term stomatal regulation is a leaf-level feedback process, adjustment of the Ψ_L set-point to a lower value during prolonged drought requires information of root water status that is necessarily independent of significant changes in bulk Ψ_L. Perhaps chronic exposure of leaf tissue to incoming ABA from the roots induces osmotic adjustment and a shift in the set-point. However, non-isohydric responses to water stress could also result from imperfect feedback regulation of Ψ_L; for example, ABA release within the leaf causing insufficient closure of stomata to maintain constant bulk Ψ_L.

In *B. occidentalis*, stomatal regulation of Ψ_L in response to manipulations of k_{S-P}, Ψ_S, and ΔN maintained Ψ_L close to the value predicted to cause cavitation in petiole xylem (e.g., Figs. 2A, 5A, 6A). Interestingly, loss of turgor in adult *B. occidentalis* leaves occurred near -1.5 MPa; approximately the same Ψ predicted to cause cavitation in petiole xylem (Sperry and Saliendra 1994). This suggests that the Ψ_L set-point for stomatal regulation could correspond with incipient loss of turgor. At least one study has shown a correspondence between turgor loss, ABA release within leaf tissue, and stomatal closure (Pierce and Raschke 1980). The small margin of safety between Ψ_L and complete cavitation in *B. occidentalis* (ca. 0.3 MPa) may explain its isohydric behaviour to water stress. Any adjustment of the set-point to a lower Ψ (e.g., by osmotic adjustment) would be mal-adaptive, because it would eliminate water transport. Non-isohydric species, on the other hand, must have relatively large safety margins from complete cavitation to accommodate large decreases in Ψ_L during stress.

During severe soil drought, cavitation in the stem occurred despite constant Ψ_L and the presumed avoidance of petiole cavitation. Root cavitation probably occurred as well, although this was not directly measured. Because Ψ decreases distally, this implies the proximal main stem and root xylem is more vulnerable to cavitation than the distal twig and petiole xylem as was found for adults of this species (Sperry and Saliendra 1994). The occurrence of cavitation during soil drought presents a strong a-priori argument for the adaptive value of sensing Ψ in the leaf. If Ψ was not sensed in the leaf, cavitation occurring in the stem and root during drought stress would cause a drop in shoot Ψ that would trigger more cavitation and lower Ψ until all transport ceased and the shoot died.

Could the drought-induced cavitation we observed be adaptive? Certainly, partial cavitation would have reduced E because it reduced k_{S-P} while the stomata continued to maintain constant bulk Ψ_L. Reduced E would prolong water availability in the soil, and perhaps optimize its extraction by minimizing the drop in soil hydraulic conductance to the root surface. According to Jones and Sutherland (1991), partial cavitation during drought may also optimize (maximize) g_s for a given Ψ_S. The localization of the cavitation in the root system and proximal stem xylem contradicts Zimmermann's segmentation hypothesis (1983), wherein cavitation should occur first in distal parts of the plant during stress to preserve the hydraulic integrity of the stem. Perhaps the more proximal xylem may be better positioned to re-fill via elevated Ψ in the event the drought was survived. In addition, the changes in Ψ_L caused by cavitation in the root or stem would be less abrupt than if it occurred in the leaf itself. This would allow greater response time for stomatal closure to prevent further drop in Ψ that could trigger positive feedback with cavitation (Tyree and Sperry 1988). Such "runaway" cavitation in *B. occidentalis* was in fact occasionally seen when stomata failed to respond in time to an abrupt reduction k caused by stem notching (Sperry et al. 1993).

The notching experiments (e.g., Fig. 3) indicated that the correlation between k_{S-P} and g_s (Fig. 7A) was mediated through the influence of k_{S-P} on Ψ_L. The higher k_{S-P} in juveniles versus adults may be a general tendency because it resulted partly from size differences. The link between hydraulic and stomatal conductances in this context may explain why juveniles tend to have lower water-use efficiencies than adults of the same species under similar environmental conditions (Donovan and Ehleringer 1992).

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