

Stomatal responses to non-local changes in PFD: evidence for long-distance hydraulic interactions

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ABSTRACT

Interactions among stomata within a single areole have recently been reported, and evidence suggests that hydraulic mechanisms may be responsible for these interactions. Such interactions may play a role in patchy stomatal behaviour by coordinating stomatal behaviour within areoles. However, models suggest that longer-distance interactions may be required to produce the large-scale discoordination that is characteristic of stomatal patchiness. This study was undertaken to characterize long-distance interactions between 'artificial patches' of stomata under varying conditions of evaporative demand and soil water stress. Gas-exchange was monitored in two adjacent regions ('patches') of a wheat leaf by two independent gas mixing and analysis systems. When photon flux density (PFD) was changed in only one of these patches, stomatal conductance responded in both patches in a manner consistent with hydraulic interactions propagated by changes in xylem water potential. These data are discussed in the context of mechanisms for patchy stomatal conductance and implications for the design and analysis of gas-exchange experiments.

Key-words: hydraulic; interactions; patchy; scaling; stomata; wheat.

INTRODUCTION

The behaviour of individual stomata may be influenced by conditions elsewhere in the same plant. For example, stomata can respond to perturbations in humidity that are localized over other stomata within the same areole (Mott *et al.* 1997). In addition, stomatal closure has been observed in response to abscisic acid (ABA) generated in roots following soil water stress (Kramer 1988; Schulze *et al.* 1988; Mansfield & De Silva 1994), suggesting that stomata can respond to stimuli perceived in distant tissues. Heath & Russell (1954) also reported changes in stomatal resistance in part of a wheat leaf following changes in photon flux density (PFD) over a small region of the same leaf when

the intercellular spaces in both of these regions were flushed with CO₂-free air. These phenomena, which show that stomata cannot be treated as independent control systems responding only to their immediate local environment, have some important implications. First, gas exchange measurements made with small chambers that enclose only part of a leaf may be influenced by signals from leaf areas outside the region of experimental control, especially if those areas experience different conditions. Second, significant interactions between different regions of a leaf or plant may complicate linear up-scaling procedures such as simple extrapolation or averaging heterogeneous small-scale units (e.g. Amthor 1994; McNaughton 1994; Lloyd *et al.* 1995), which implicitly assume the small-scale units in question behave independently.

Stomatal interactions also reveal a gap in our understanding of leaf gas exchange, and may imply a novel mechanism or yet unrecognized implications of well-understood leaf properties. Recent work suggests stomata may interact hydraulically. A spatially explicit model of stomatal behaviour that incorporated local hydraulic interactions between stomata (Haefner *et al.* 1997) produced patterns of local interactions similar to those documented by Mott *et al.* (1997). Stomatal interactions may also occur at a larger scale. Under certain environmental conditions, stomatal behaviour may diverge among different regions, or 'patches' of a leaf within which stomatal conductance is relatively uniform (for review of this phenomenon, called 'patchy stomatal conductance', see Terashima 1992; Pospisilova & Santrucek 1994, 1997; Weyers & Lawson 1997; Mott & Buckley 1998; Beyschlag & Eckstein 1998). The spatially explicit model of stomatal functioning discussed above (Haefner *et al.* 1997) showed that areole-sized patches of coordinated stomatal behaviour can appear following spatially uniform perturbations of humidity over a grid of hydraulically linked stomata with randomly varying mechanical properties. However, in those simulations, the average conductance of different areole-sized patches diverged slowly, and only by virtue of differences in the patches' inherent oscillatory phases, because the model did not incorporate interactions at scales larger than an areole. Other evidence suggests that interactions may occur between patches. Sequential perturbations of Δw and PFD caused some patches to begin oscillating immediately while other patches did not begin oscillating until other patches had completed a full oscillatory cycle (Cardon *et al.* 1994);

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this suggests that the latter patches induced the former into oscillations. Additionally, it has been shown that the average conductance of patches may diverge rapidly after a perturbation (Cardon *et al.* 1994; Eckstein *et al.* 1996), in contrast to the simulations of Haefner *et al.* (1997).

It has been suggested (Haefner *et al.* 1997; Mott & Buckley 1998) that the discrepancies between these simulations and the observations of Cardon, Mott & Berry (1994) and Eckstein *et al.* (1996) could be explained by large-scale hydraulic interactions between distant stomata in the same leaf. The goal of this study was to determine whether such interactions can occur between distant patches. Specifically, we hypothesized that if we created two large 'patches' on the same leaf by enclosing two regions in separate gas-exchange chambers and changing the transpiration rate in one patch by changing PFD, this would induce changes in xylem water potential throughout the leaf, affecting stomatal conductance in the other patch. The response of stomatal conductance in the unperturbed region should be similar to responses observed following any change in the balance between water supply and demand, such as a perturbation in Δw , the pressurization of roots, or xylem cavitation. To help distinguish hydraulic signals from chemical signals propagated in the xylem, experiments were performed on leaves of a species with parallel venation (wheat, *Triticum aestivum* L.), in which xylem flow is likely to be more unidirectional than in a species with reticulate venation (if interactions occur in both directions along the leaf, xylem-borne chemical signals are probably not responsible). Similar experiments were performed by Heath & Russell (1954), but their experimental protocol precluded testing of hydraulic interactions. Schulze & Küppers (1979) also performed a related experiment, but at a different scale and using different stimuli: they enclosed one branch of a hazel bush in a chamber to maintain constant environmental conditions, while changing the evaporative gradient for the rest of the plant.

In this study, stomatal responses in two regions of a wheat leaf were monitored by two independent gas-exchange systems following perturbations of PFD in one region. Perturbations consisted of a drop from high PFD to darkness, achievement of steady-state gas exchange, and a return to high PFD. Experiments were performed at high and low Δw in well-watered and water-stressed plants, and perturbations were applied to either the proximal or distal half of each leaf in separate experiments. When PFD was perturbed in only one region of a leaf, stomatal conductance in another region of the same leaf responded in a manner consistent with hydraulically mediated interactions.

MATERIALS AND METHODS

Plant material

Wheat (*Triticum aestivum* L. cv. Beery 10 provided by the Utah State University Crop Physiology Laboratory) was grown in a temperature-controlled greenhouse, in a substrate consisting of equal parts of perlite, vermiculite and

peat moss. Supplemental lighting from sodium lamps was used when necessary to maintain a day length of 16 h. Plants were kept in individual 1.8 L pots and watered to excess daily with a nutrient solution containing 9.1 mmol L⁻¹ N, 1.8 mmol L⁻¹ P, 2.7 mmol L⁻¹ K and 11 μ mol L⁻¹ chelated Fe (Peter's 20-20-20, Grace Sierra Horticultural Products Co., Milpitas, California, USA). Day and night temperatures were 30 and 20 °C, respectively. For each experiment, a fully expanded, non-senescent flag leaf was chosen from a plant that had not undergone anthesis, and that had not previously been used for gas-exchange experiments. Some plants (referred to as 'non-stressed') were watered daily before gas exchange measurements, while others ('stressed' plants) were not watered for 72–96 h before measurements.

Gas exchange

A flag leaf was placed within two adjacent, identical gas-exchange chambers such that each chamber contained approximately one-half of the leaf (8–16 cm²). The environmental conditions in the chambers were controlled independently of each other by a pair of identical but completely independent gas mixing and analysis systems (one of these systems was described in Mott, Cardon & Berry 1993). Both chambers received light from the same halogen light source, and the light was blocked for one chamber when necessary. Preliminary experiments verified that this procedure did not influence light levels in the other chamber. PFD at the leaf surface was between 800 and 1000 μ mol m⁻² s⁻¹, while the rest of the plant was kept in near darkness (approximately 10 μ mol m⁻² s⁻¹). Pyrex trays containing 2–3 inches of water were placed between the light sources and the chambers to absorb infrared radiation. Leaf temperature was maintained at 25.0 \pm 0.5 °C. The gas mixture in the chamber contained 350 μ mol mol⁻¹ CO₂, 210 mmol mol⁻¹ O₂, and an amount of water vapour that was varied to control Δw (the water vapour mole fraction gradient between the leaf and the chamber gas mixture), all in nitrogen.

To begin each experiment, PFD in both chambers was increased from darkness in three increments of approximately 300 μ mol m⁻² s⁻¹, with the first step occurring between 0630 and 0730 h. Δw was maintained at either 10.0 or 20.0 \pm 1.0 mmol mol⁻¹ (hereafter low Δw and high Δw , respectively). On many occasions, conductance in one or both chambers reached a very high initial maximum in the morning, and subsequently declined to a lower steady-state value ('steady-state' was defined as beginning after 50 min during which the stomatal conductance, transpiration and assimilation changed by less than 10%). Once this steady state was reached, it persisted until at least 1600 h, after which conductance showed an inherent diurnal decline despite constant environmental conditions. However, at high Δw in stressed plants, persistent small-amplitude oscillations prevented the achievement of a true steady state (Figs 1d and 2d). For these experiments, steady state was defined as following 50 min during which the 'conductance mode' (the value about which conductance oscillated) changed by less than 10%.

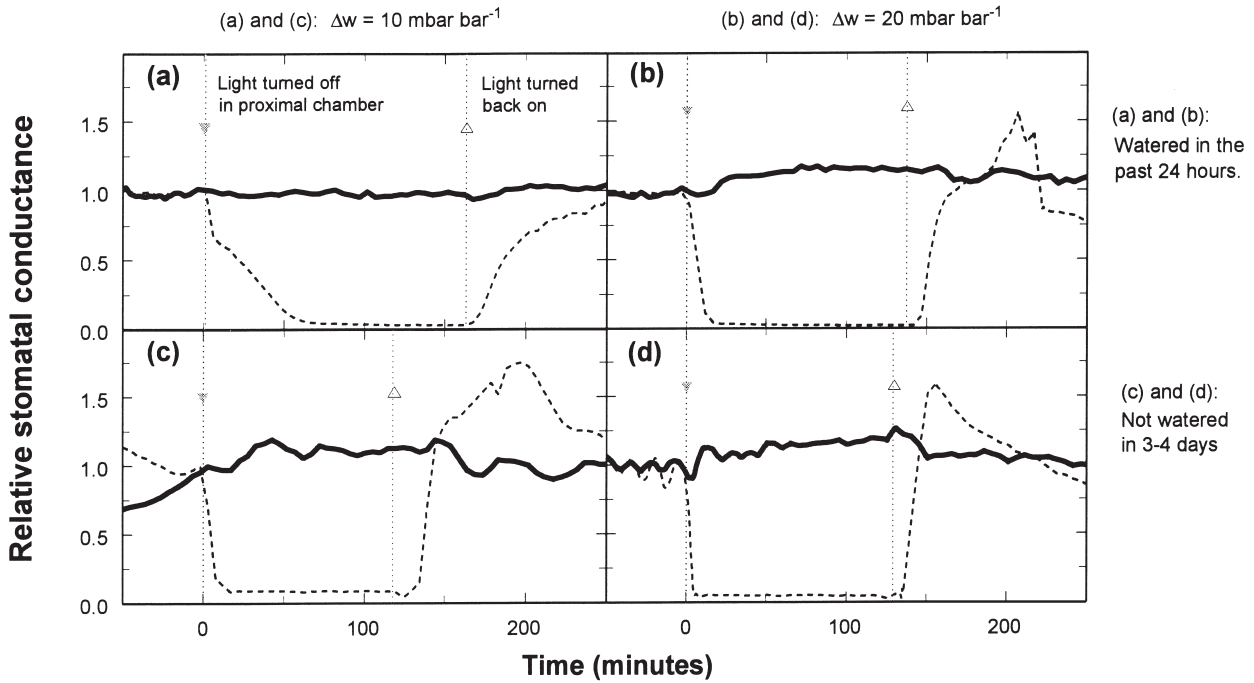


Figure 1. Responses in g_s after changing PFD over the proximal half of a wheat leaf; each panel presents sample traces from a single leaf. PFD was reduced to zero in the proximal region at 0 min (downward grey arrows), and returned to $900 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the times indicated by the second dotted line (open upward arrows). Experiments were performed at low Δw (10 mbar bar^{-1}) and high Δw (20 mbar bar^{-1}) on non-stressed (recently watered) and stressed (not watered for 72–96 h before the experiments) plants. (a) Low Δw , non-stressed; (b) high Δw , non-stressed; (c) low Δw , stressed; (d) high Δw , stressed. Solid traces: distal half of leaf, where light levels were held constant; dashed traces: proximal half of the leaf, where light was turned off, and then on again. Relative stomatal conductance is stomatal conductance divided by its average value for 10 min before time = zero.

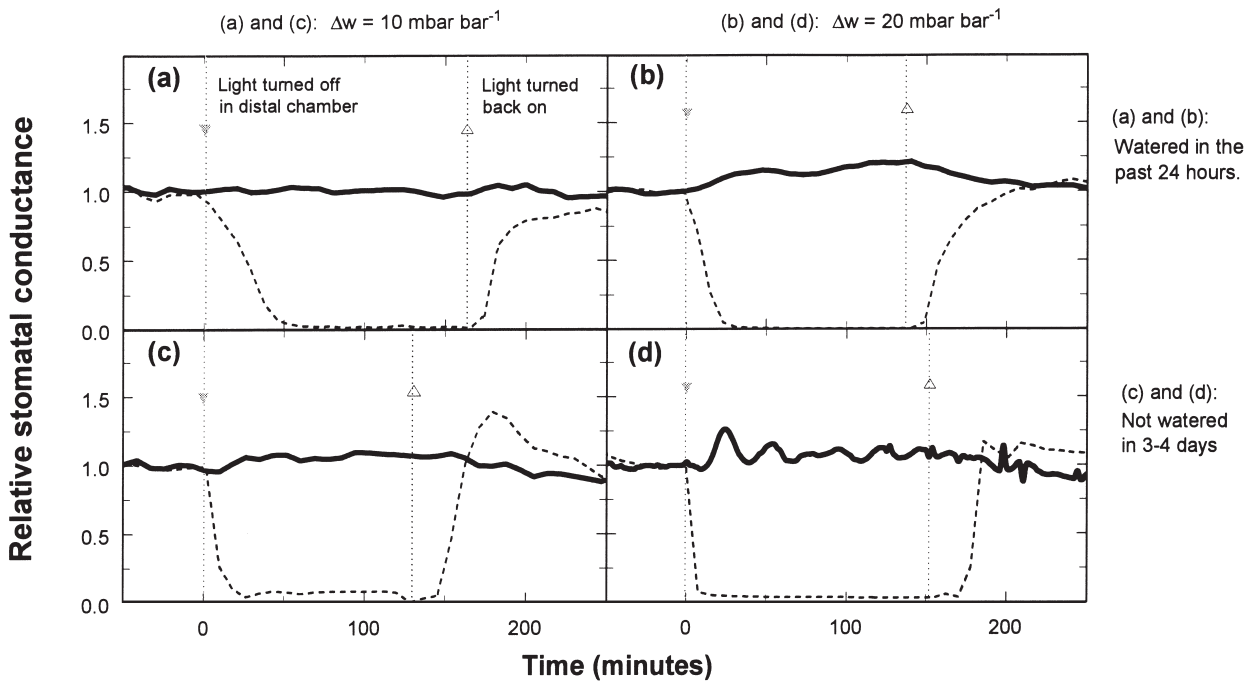


Figure 2. Responses of g_s after changing PFD over the distal half of a wheat leaf; each panel presents sample traces from a single leaf. PFD was reduced to zero in the distal region at 0 min (downward grey arrows), and returned to $900 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the times indicated by the second dotted line (open upward arrows). (a) low Δw , non-stressed; (b) high Δw , non-stressed; (c) low Δw , stressed; (d) high Δw , stressed. Solid traces: proximal half of leaf; dashed traces: distal half of the leaf.

After reaching steady state, the supply of light to one chamber was blocked, reducing the PFD in that chamber to zero. The value of Δw was held constant in both chambers before and after this perturbation. The leaf temperature in the perturbed chamber declined to between 23.5 and 24.0 °C after the removal of light, but was returned to 25.0 ± 0.5 °C as quickly as possible (usually within 1 min). After stomatal conductance (g_s) had reached steady state again in both chambers (approximately 100–160 min), PFD in the darkened chamber was returned to its original value in a single step. Gas exchange was monitored until steady state was achieved or until 1600 h. All experimental treatments for Δw and water stress were repeated at least four times: in two replicates PFD was perturbed in the chamber containing the proximal half of the leaf (the region nearer to the culm), and in the other two replicates PFD was perturbed for the distal leaf half (the region farther from the culm, nearer to the leaf tip).

Water potential measurements

Pre-dawn water potential was measured with three thermocouple psychrometers (JRD Merrill Supply, Logan, Utah, USA). Each instrument was calibrated with six solutions of known osmotic potential between 0 and –20 bar, and water potential was linearly related to microvolt output ($r^2 \geq 0.99$ for each instrument). Using a hole punch, one leaf sample was collected from each of three randomly selected leaves (on different culms than the leaf that was chosen for gas-exchange measurements) at approximately 0700 h, and these samples were allowed to equilibrate in the psychrometric chambers for 5–8 h before readings were taken. Repeated calibration verified that equilibration time had no consistent influence on water potential readings between 5 and 8 h.

RESULTS

Effects of water stress treatment

Pre-dawn water potential was lower in stressed plants than in non-stressed plants ($P = 0.04$, one-tailed t test), and the morning steady-state stomatal conductance within a given leaf region (proximal or distal) and Δw level (high or low)

was lower in stressed than non-stressed plants in all cases ($P < 0.03$ in all cases, one-tailed t test; Table 1). Together these results verify that withholding water for 72–96 h before experiments had a significant physiological influence. As described previously, water-stressed plants at high Δw often did not achieve a true steady-state in the morning, but oscillated around a steady mode.

Gas exchange

After the supply of light was blocked from one half of the leaf, stomatal conductance in that chamber always declined to approximately zero, and after the light supply was resumed, g_s always returned to approximately its initial value (sample traces from experiments on individual leaves are presented in Figs 1 & 2). In most cases (Figs 1b–d and 2b–d), these changes were also associated with some response in stomatal conductance in the other leaf region, which experienced no direct changes in environmental conditions. These responses were generally more pronounced when the proximal leaf region was perturbed (Fig. 1) than when the distal leaf region was perturbed (Fig. 2).

In one of the four experimental treatments (non-stressed plants at low Δw), blocking light to half of the leaf failed to produce a significant response in g_s in the unperturbed region (Figs 1a & 2a). However, all other treatments elicited some response from stomata in the unperturbed region. At high Δw in non-stressed plants, blocking the light supply to half of the leaf resulted in a brief decline in conductance in the other half of the same leaf, followed by a slight increase in the subsequent steady-state value of conductance in the unperturbed region (Figs 1b & 2b). After reversing the perturbation (resuming the light supply by removing the light barrier that covered the perturbed region), g_s in the unperturbed region initially declined and then oscillated towards equilibrium at approximately its morning steady-state value.

A similar pattern of stomatal behaviour was observed at low Δw in stressed plants (Figs 1c & 2c). After blocking the light supply to half of the leaf, g_s in the unperturbed region first dropped and then briefly oscillated, finally reaching a slightly higher value (Figs 1c & 2c). However, in experiments where the proximal region was perturbed, this increase in g_s can not be conclusively attributed to the PFD

Leaf region	Proximal region		Distal region	
	Low Δw	High Δw	Low Δw	High Δw
Unstressed	1.27 ± 0.66	0.56 ± 0.18	1.05 ± 0.31	0.61 ± 0.20
Stressed	0.36 ± 0.22	0.16 ± 0.05	0.26 ± 0.11	0.18 ± 0.03
P	0.029	0.012	0.004	0.012

Table 1. Morning steady-state stomatal conductance.

The average value of stomatal conductance (g_s , mol m⁻² s⁻¹) was calculated during the 30 min prior to perturbing PFD in one chamber. Values for P are for one-tailed t tests (assuming unequal variances) between unstressed and stressed plants at each value of Δw for each region

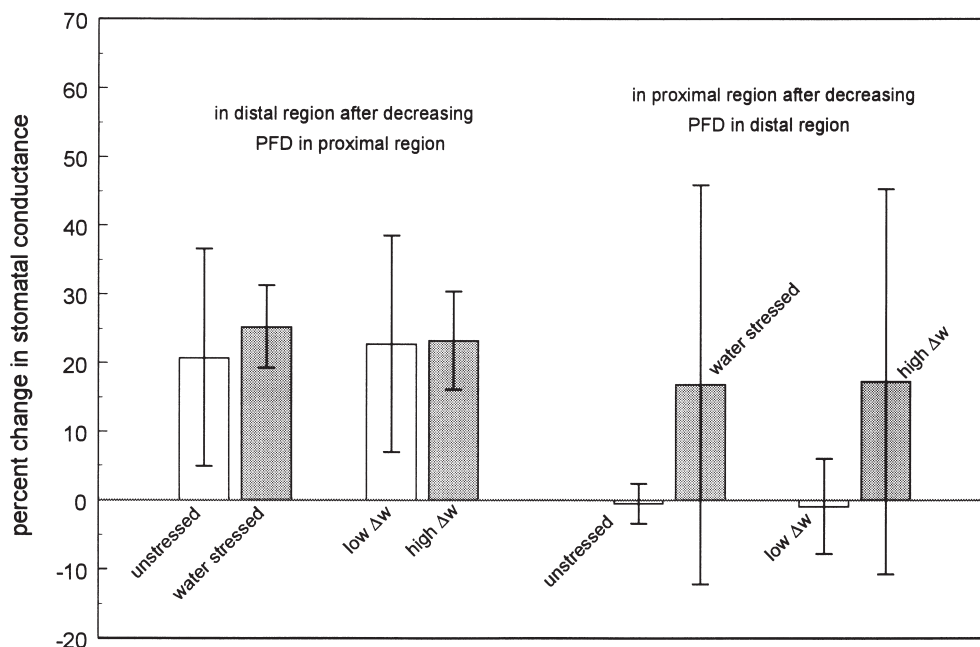


Figure 3. Percentage change in g_s in one leaf region after reducing PFD to zero in the other region. The change was calculated as the difference in relative g_s averaged for 30 min before the perturbation and between 75 and 105 min after the perturbation. For each treatment (Δw or water stress), results were pooled from experiments at both levels of the other treatment. Bars are mean \pm SD.

perturbation, because a true steady state was never achieved in the morning in stressed plants at low Δw . When the light supply was resumed in the perturbed region, g_s in the unperturbed region first increased slightly and then exhibited damped oscillations around a lower value.

When PFD was reduced to zero in the proximal half of a water-stressed leaf at high Δw , the immediate response of g_s in the distal leaf region was masked by persistent small-amplitude oscillations in g_s (Fig. 1d). Nevertheless, the value around which g_s oscillated in the unperturbed region increased within 10–20 min after the light perturbation, and this value decreased again when the supply of light to the perturbed region was resumed. When the distal leaf region was darkened in water-stressed leaves at high Δw , a transient wrong-way response and reversible increase in g_s was observed in the proximal region (Fig. 2d).

The effect on steady-state relative conductance in the unperturbed region by changing PFD in the other region was quantified by averaging g_s in the unperturbed region for 30 min before the perturbation and between 75 and 105 min after the perturbation (Fig. 3). When the distal region was perturbed, stomatal conductance usually increased in the proximal region (stressed plants [both Δw treatments pooled together], $P = 0.330$; unstressed plants [both Δw treatments], $P = 0.744$; low Δw [stressed and unstressed plants pooled together], $P = 0.538$; high Δw [stressed and unstressed plants], $P = 0.306$; two-tailed t test). Conductance increased in the distal region when the proximal region was perturbed (for stressed plants, $P = 0.004$; unstressed plants, $P = 0.080$; low Δw , $P = 0.064$; high Δw , $P = 0.008$; one-tailed t test). However, the change in con-

ductance did not differ significantly between treatments (high versus low Δw , unstressed versus stressed, proximal versus distal region perturbed) (three-way ANOVA, $P > 0.10$ for each treatment). These results are summarized in Fig. 3.

DISCUSSION

Mechanisms of long-distance interactions

The results presented above show that stomata in one region of a wheat leaf can respond to changes in PFD over another region of the same leaf, despite constant environmental conditions in the former region. Some signal must be therefore transmitted from the perturbed region to the unperturbed region. This signal may be chemical, electrical or hydraulic in nature. Although experiments were not performed to directly detect or measure any signals, the data can be used to make inferences about the relative plausibility of each hypothetical signal. Hydraulic signals involve fairly well-understood physical principles, and will be discussed in detail first. Other possibilities will be briefly discussed afterwards.

A hydraulic mechanism

The following hydraulic mechanism, based on known hydromechanical properties of leaves, may suffice to explain the long-distance stomatal interactions reported here. When the supply of light to one half of the leaf is blocked, stomata in that region close, causing an immedi-

ate reduction in the whole-leaf rate of water loss without directly affecting the rate of water supply to the leaf. This causes an increase in xylem water potential throughout the leaf. Leaf tissues, including those in the unperturbed region (still experiencing full PFD and still transpiring), respond to this change by taking up water. Once a new equilibrium is achieved, tissues in the unperturbed region will have a higher water potential.

Although it is clear that some stomatal responses to changes in water potential in either leaves or roots are not a direct response to water potential *per se* but perhaps instead to ABA (e.g. Cornish & Zeevaert 1984; Neales *et al.* 1989) or some other signal (Trejo & Davies 1991), it is also clear that stomata can respond directly to a change in local water potential. Saliendra, Sperry & Comstock (1995) isolated the effect of water potential from that of non-hydraulic root signals by applying changes in pneumatic pressure in roots to produce an immediate change in leaf water potential, and observed a positive relationship between steady-state g_s and root hydrostatic pressure.

Malone and co-workers (Malone & Stankovic 1991; Malone 1992; Boari & Malone 1993) have shown that transient increases in water potential in response to localized wounding can cause extremely rapid increases in epidermal turgor (and leaf thickness) at points distant from the wound site; similar responses were also observed when tissue water potential was locally increased with a pressure bomb (Malone & Stankovic 1991). Raschke (1970) subjected the water supply of detached corn leaves to step decreases or increases in water potential by applying or removing a vacuum to the leaf base, and observed transient and steady-state stomatal movements throughout the leaf. It is therefore clear that local changes in leaf water potential can be propagated throughout the leaf and can influence epidermal water relations and stomatal behaviour at distant sites. What was not clear prior to the present study, however, was the nature and magnitude of responses of distant stomata to changes in transpiration rate caused by changes in stomatal conductance in one part of a leaf. Because changes in stomatal conductance are not purely hydraulic, we wished to characterize empirically the interactions between two patches with different stomatal conductances and compare these interactions with the established hydraulic effects described above.

The data shown in Figures 1 and 2 appear to be consistent with the hydraulic mechanism outlined above in several ways. First, steady-state stomatal conductance (g_s) responded in a manner consistent with the result of Saliendra *et al.* (1995), i.e. it increased in a given region after PFD was reduced to zero in the other region, and then decreased again when the perturbed region was re-illuminated (Figs 1 & 2). Second, the relative magnitude of stomatal responses to distant perturbations in PFD was greater in high- Δw trials than low- Δw trials, and greater for water-stressed plants than for unstressed plants (Fig. 3). The proposed hydraulic mechanism predicts this result, because an increase in water potential should have a greater relative effect in tissues that are substantially limited by water

supply (either at the source, as in soil water stress, or the sink, as in high Δw). Note, however, that one would expect to see the same trends if the signalling mechanism were some chemical produced and released in response to stress; all one can conclude is that these trends are consistent with hydraulics.

Third, stomatal responses in the proximal leaf region to changes in PFD over the distal region (Fig. 2) were qualitatively similar to, but generally less pronounced than responses observed in the distal region after perturbing the proximal region (Fig. 3). This is consistent with a hydraulic mechanism because by virtue of its position the proximal leaf region experiences higher xylem water potential and thus enjoys a more favourable overall water status; this contention is supported by the fact that when PFD was uniform across the leaf, stomatal conductance was generally higher in the proximal region (Table 1). Proximal stomata should therefore be less sensitive to any hydraulic perturbations, including those induced by changes in PFD or Δw elsewhere in the leaf. Consistent with this prediction, smaller and slower stomatal responses to a change in Δw were observed in the proximal leaf region during preliminary experiments where both high- and low- Δw trials were attempted in a single day (data not shown).

Finally, a transient stomatal response in the direction opposite to the final response was observed whenever PFD was changed elsewhere in the same leaf (Figs 1 & 2). This can also be explained by the proposed hydraulic mechanism in conjunction with known properties of stomatal function. The transient response is essentially similar to the first two phases (closure and opening) of the well-known 'Iwanoff effect' that occurs immediately after excising a leaf. Rufelt (1963) provided evidence that changes in leaf water status following excision directly affected the transpiration rate independently of stomatal movements, but Meidner (1965) showed that stomata controlled these transients by directly observing their movements following excision. However, Darwin & Pertz (1911) are credited for first suggesting the current mechanistic explanation for transient responses in stomatal conductance to hydraulic perturbations. The first effect of an increase in tissue water potential should be a rapid and equal increase in the turgor pressures of both epidermal and guard cells. However, this will cause stomatal aperture to decrease because of the mechanical advantage of epidermal turgor (Sharpe, Wu & Spence 1987; Franks *et al.* 1995). Stomatal aperture does not increase until guard cells actively take in osmotic solutes. Thus, an increase in water potential should induce a transient decrease in stomatal conductance followed by a gradual increase to a higher steady state. When PFD is increased from darkness in the perturbed region, the opposite sequence of events occurs: g_s in the unperturbed region first rises, then declines to a lower steady state. This pattern of conductance is also often observed when Δw is perturbed (e.g. Kappen, Andresen & Losch 1987; Kappen & Haeger 1991).

It is noteworthy, however, that Saliendra *et al.* (1995) did not observe a wrong-way response in g_s when leaf water

potential was increased by pressurizing roots. These authors were measuring gas exchange on whole seedlings, requiring a gas exchange system with large volume and thus large turnover time; the resulting temporal insensitivity to subtle transients may have masked any small wrong-way responses. However, this seems unlikely because they did observe a wrong-way response to perturbations of Δw . The greater magnitude of stomatal responses to local changes in transpiration rate in *Betula* may reflect a more distal position of epidermal cells in the transpiration stream, relative to the mesophyll cells that first perceive increases in xylem water potential. In wheat, with its dense vasculature (every longitudinal row of stomata is no more than two narrow cells from a row of epidermal cells in contact with a vein), any increase in xylem water potential may more rapidly and strongly influence epidermal turgor, causing a greater transient wrong-way response. This speculation could be tested by repeating the experiments of Saliendra *et al.* (1995) with a more sensitive gas-exchange design (a smaller chamber and a single leaf), perhaps on several different species with well-characterized leaf hydraulic anatomy.

Non-hydraulic mechanisms

It is possible that a chemical signal mediates the long-distance stomatal interactions reported here. Perhaps the best candidate for a chemical signal is abscisic acid (ABA), because evidence suggests that ABA may mediate interactions between water-stressed roots and stomata (Davies *et al.* 1987; Neales *et al.* 1989; Mansfield & De Silva 1994), because ABA is known to be xylem-mobile (Raschke 1987), and because ABA has reliable and well-documented effects on stomatal behaviour (g_s decreases when the ABA content of leaf tissue increases, whether the increase is caused by local synthesis, transport from distant sites of synthesis, or exogenous application; Raschke 1987). However, it is unlikely that ABA is responsible for the interactions described in this paper, for three reasons: (1) a transient wrong-way response was observed in the unperturbed region whenever PFD was perturbed elsewhere in the leaf, but stomatal closure in response to ABA is not preceded by transient stomatal opening (Raschke 1987); (2) the observed responses were rapidly reversible, whereas stomatal responses to ABA tend to be reversible only over long time periods, if at all; and (3) to produce the observed stomatal opening effects, it is necessary to postulate that a steady but small flow of ABA is always occurring in both directions down the leaf, and that lowering PFD in one region stops the outflow of ABA from that region, allowing stomata in the other region to open somewhat; this is a fairly complicated assumption based on phenomena that have never been documented, and that seem physiologically unlikely if not implausible.

Although there are other possible chemical signals (e.g. auxin or calcium, both of which influence stomatal responses to ABA and may themselves directly affect stomatal aperture; Snaith & Mansfield 1982; Atkinson 1991),

there are two arguments against a central role of any chemical signal as a mediator of the observed interactions. First, xylem water flows in the direction of proximal-to-distal in transpiring monocot leaves, so only phloem can transport a chemical signal in the opposite direction. It seems unlikely that phloem could distribute a chemical signal to distant stomata as quickly as xylem. Distal-to-proximal interactions should therefore exhibit a longer lag time between the perturbation and the long-distance response, but the responses of stomata in the proximal region to distal perturbations were no slower than distal responses to proximal perturbations (compare Figs 1 & 2). Second, calculations of the maximum xylem flow velocity based on observed transpiration rates (from gas exchange), xylem cross-sectional areas within the leaf (estimated by microscopy for the wheat leaves used in this study; data not shown), and the known distance between the adjacent chambers, suggest that any xylem-borne chemical signal would require at least 3 min to travel from the most distal tissues in the proximal region to the most proximal tissues in the distal region. Responses to perturbations in one region were observed in the other region within 10–15 s.

This study did not test for electrical signals (action potentials), although these may also be involved in the long-distance stomatal interactions described above. Indeed, action potentials do not suffer from the shortcomings that make chemical signals so unlikely. There is no *a priori* reason to assume that action potentials should travel less readily or rapidly in opposite directions down the leaf, and the speed of propagation of action potentials is more than adequate to produce the observed responses (e.g. Fromm & Bauer 1994; Zawadski, Dziubinska & Davies 1995). Further experiments should thus be performed to test conclusively for electrical signals. Nevertheless, the hydraulic mechanism advanced early in this section seems most parsimonious, because it is based on well-understood aspects of leaf function and it appears to explain most or all of the observed results.

Other reports of long-distance stomatal interactions

Heath & Russell (1954) performed an experiment wherein two regions of a wheat leaf were observed after only one region experienced a change in PFD. In their experiments, the two regions (each 0.6 cm long, 1.85 cm wide, and 1.6 cm apart from each other) were enclosed in separate leaf chambers, CO₂-free air was forced into the leaf (and out through adjacent stomata not within the chamber), and stomatal resistance was measured with a porometer. In contrast to our own results, Heath & Russell (1954) observed that stomatal resistance declined in the unperturbed chamber when PFD was increased in the other chamber. However, their experimental protocol may have prevented the hydraulic signals to which we have attributed the long-distance stomatal interactions reported in the present study. By forcing CO₂-free air through the leaf and adjusting its pressure to maintain a constant flow rate of this air through

the leaf as stomatal resistance changed, Heath & Russell (1954) made the rate of water loss from these leaf tissues independent of stomatal resistance. Thus, the two 'patches' in their study had the same, constant transpiration rate (although 'water loss rate' may be more appropriate because of the unusual path of this flux in their experiments), independent of stomatal resistance and PFD, and local changes in PFD could not have produced the hydraulic signals discussed in the present study. There are several other differences between the work of Heath & Russell (1954) and the present study: (1) our chambers enclosed roughly 15 times more leaf area than theirs, allowing approximately 15 times the change in transpiration rate and thus water potential possible with the earlier authors' apparatus, (2) our experiments were performed at much higher PFDs ($900 \mu\text{E m}^{-2} \text{s}^{-1}$ versus a maximum of approximately $80\text{--}120 \mu\text{E m}^{-2} \text{s}^{-1}$), (3) we performed experiments at ambient CO_2 levels (350 ppm), and (4) Heath & Russell's CO_2 -flushing protocol would have eliminated any effect of changing stomatal conductance on transpiration, so their experiment could not have produced the hydraulic signals that we sought to characterize in the present study.

Schulze & Kupperts (1979) reported much larger-scale hydraulic interactions between leaves with different transpiration rates. In their experiments, a hazel bush (*Corylus avellana* L.) was enclosed in a controlled-environment chamber and subjected to a cycle of increases and decreases in Δw , while one branch was enclosed in a sub-chamber at constant Δw . The transpiration rate in the main part of the plant (which experienced changing Δw) first increased when evaporative demand was increased, but then declined to zero. As Δw was decreased in two steps later in the day, transpiration in the main part of the plant gradually increased again. Meanwhile, stomatal conductance in the sub-chamber (which experienced constant Δw) gradually increased over a period of several hours during which the rest of the plant was not transpiring at all, and then decreased when the rest of the plant began to transpire again. These results are largely consistent with the results of the present study: conductance in the unperturbed region was negatively correlated with transpiration rate in the perturbed region. Conductance in the sub-chamber also roughly followed the trend of leaf water potential in the main chamber, although water potential was not measured while the main chamber was at zero transpiration rate. Notably, there was no response in conductance in the sub-chamber during the hour in which the main chamber transpiration rate increased before dropping to zero. We suggest that this may be due to a lag caused by hydraulic buffering in the plant or soil. One might expect the kinetics of long-distance hydraulic interactions to be different when they occur through the entire hydraulic structure of a woody plant, rather than when operating between two adjacent regions of a monocot leaf, as in the present study.

In conclusion, the data presented in this study demonstrate that changes in stomatal conductance or transpiration in one portion of a wheat leaf can affect stomatal conductance in other portions of the same leaf. The inter-

actions are consistent with hydraulic signals and may contribute to large-scale spatial patterns of conductance within a leaf.

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