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Effects of humidity on light-induced stomatal opening: evidence for hydraulic coupling among stomata

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Abstract

A mechanism for co-ordinating behaviour of stomata within an areole during patchy stomatal conductance has recently been proposed. This mechanism depends on hydraulic interactions among stomata that are mediated by transpiration-induced changes in epidermal turgor. One testable prediction that arises from this proposed mechanism is that the strength of hydraulic coupling among stomata should be proportional to evaporative demand and, therefore, inversely proportional to humidity. When a leaf is illuminated following a period of darkness, there is typically a period of time, termed the *Spannungsphase*, during which guard cell osmotic and turgor pressure are increasing, but the pore remains closed. If hydraulic coupling is proportional to evaporative demand, then variation among stomata in the duration of the *Spannungsphase* should be lower for leaves at low humidity than for leaves at high humidity. A similar prediction emerged from a computer model based on the proposed hydraulic mechanisms. These predictions were tested by measuring individual stomatal apertures on intact transpiring leaves at low and high humidity and on vacuum-infiltrated leaf pieces (to eliminate transpiration) as PFD was increased to high values from either darkness or a low value. Results showed that the range of *Spannungsphasen* among stomata was reduced at low humidity compared to high humidities. Experiments that began at low PFD, rather than at darkness, showed no delay in stomatal opening. These results are discussed in the context of the proposed hydraulic coupling mechanism.

Key words: Stomata, humidity, light, patchy, hydraulic, turgor.

Introduction

Patchy stomatal conductance occurs when distinct areas of leaf, usually bounded by veins, exhibit widely differing stomatal conductances despite nearly identical environmental conditions. The occurrence of patchy stomatal conductance and its effects on gas-exchange data are now well documented, and the phenomenon has been the subject of several reviews (Terashima, 1992; Pospíšilová and Šantrůček, 1994; Weyers and Lawson, 1997; Beyschlag and Eckstein, 1998). However, the mechanisms for producing this remarkable behaviour have received less attention. There are two aspects of patchy stomatal conductance that must be accounted for in a mechanistic analysis. First, the behaviour of stomata within a patch must be co-ordinated; second, the average stomatal behaviour within different patches must be unco-ordinated (Mott and Buckley, 1998).

A mechanism for co-ordinating movements of stomata within an areole has recently been proposed (Haefner *et al*., 1997; Mott *et al*., 1997; Mott and Buckley, 1998). The mechanism is based on hydraulic interactions among neighbouring stomata, and it incorporates two well-established aspects of the hydraulics of stomatal functioning: (1) an increase in transpiration rate causes a decrease in epidermal turgor (Shackel and Brinckmann, 1985; Nonami and Schulze, 1989), and (2) a decrease in epidermal turgor causes the stomatal pore to open because of reduced 'backpressure' on the guard cells (Glinka, 1971; Kappen and Haeger, 1991). To understand how these interactions can result in co-ordination of stomata, it is useful to consider the simple case in which the aperture of one stoma increases while those of surrounding stomata remain initially unchanged. The increase in aperture for the 'rogue' stoma reduces the turgor of the adjacent epidermal cells because of the increased transpiration rate. This decrease in epidermal

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turgor will be transmitted to some extent among neighbouring epidermal cells and, therefore, will reduce the epidermal backpressure on neighbouring stomata. As these stomata open, they further reduce epidermal turgor, amplifying and spreading the effect. This process is likely to be terminated at the veins because they represent a source of water beyond which it would be difficult to transmit the change in turgor pressure. The increased transpiration rate through the 'rogue' stoma may also induce vapour pressure gradients within areoles, more directly affecting the epidermal cells surrounding other stomata; this effect, if significant, will also terminate at veins in heterobaric species (including *Xanthium strumarium* L., used in the present study), which have bundle sheath extensions blocking gas diffusion between areoles. Both of these effects, propagation of changes in epidermal turgor within the epidermis and changes in substomatal humidity throughout an areole, will tend to co-ordinate the movements of stomata within one areole.

Changes in stomatal aperture have been observed following changes in evaporative demand that did not occur at the measured pore, but were experienced by neighbouring stomata (Mott *et al.*, 1997). These indirect responses imply the existence of local stomatal interactions and are consistent with the hypothesis outlined above. In addition, Haefner *et al.* (1997) reproduced the results of Mott *et al.* (1997) using a spatially explicit model of stomatal functioning based on this hypothesis, showing that hydraulic interactions could co-ordinate the movements of stomata in an areole at low humidity (high evaporative demand). They further showed that random variation among stomata in the relative influence of epidermal turgor on aperture (termed the 'mechanical advantage of the epidermis') caused differences in behaviour among areoles, leading to patchy stomatal conductance at high evaporative demand.

One testable implication of the hydraulic mechanism discussed above is that coupling among stomata should be more pronounced under conditions of greater evaporative demand, because epidermal turgor is more sensitive to changes in stomatal aperture under such conditions. This aspect of hydraulic coupling could not be tested in the previous study (Mott *et al.*, 1997) because humidity was used to perturb stomatal aperture in those experiments. In the present study, however, PFD was used to perturb stomatal apertures and coupling among stomata was studied under conditions of high and low humidity. Specifically, the effect of evaporative demand (quantified as Δw, the water vapour mole fraction gradient between leaf and air) on the lag time before stomata begin to open after an increase in PFD was examined.

When a leaf is illuminated following a period of darkness, there is typically a period of time during which guard cell osmotic and turgor pressure are increasing, but the pore remains closed. This period of time has been termed the *Spannungsphase* (Stålfelt, 1929). It occurs because guard cells must overcome the backpressure exerted by adjacent epidermal cells before the pore can open (Meidner, 1990). After the pore has opened, transpiration through the pore lowers the turgor pressure of the surrounding epidermal cells (Glinka, 1971; Meidner, 1990), accelerating subsequent increases in aperture. This second phase, during which guard cell and epidermal cell turgor pressures are changing in opposite directions and aperture is increasing rapidly, has been called the *Motorphase* (Stålfelt, 1929).

There is considerable natural variation among stomata in the duration of the *Spannungsphase* (Saxe, 1979). If the behaviour of neighbouring stomata is linked by hydraulic coupling, then variation in the *Spannungsphasen* among stomata should be greatest in the absence of transpiration (i.e. at zero Δw) because epidermal turgor is unaffected by stomatal aperture. Conversely, high values of Δw should decrease variation in the *Spannungsphase*, because transpiration from stomata with short *Spannungsphasen* will decrease epidermal turgor and thus shorten the *Spannungsphase* for other pores. In this study, this qualitative prediction was tested by quantifying the variation among stomata in the duration of the *Spannungsphase* at high and low values of Δw. To evaluate whether the proposed hydraulic-coupling mechanisms can account for the relative magnitude of observed effects of Δw on *Spannungsphase* variation, these experiments were simulated with a slightly modified version of the computer model described by Haefner *et al.* (1997). Experiments were also performed starting at a low PFD rather than at darkness to rule out the possibility that *Spannungsphase* variation is caused by a variable lag in the biochemical response of guard cells to a change in PFD. At some threshold PFD, most guard cells will have increased their osmotic and turgor pressures enough to overcome the epidermal mechanical advantage; if most stomata show no lag in the response to a subsequent increase in PFD, then there is no biochemical lag, and most variation in the *Spannungsphase* can be attributed to variation in the mechanical advantage of the epidermis.

Materials and methods

Xanthium strumarium L. plants were grown in 1.0 l pots containing a soilless media consisting of peat, perlite and vermiculite (1 : 1 : 1, by vol.). Pots were watered to excess daily with a dilute nutrient solution containing 9.1 mol m^{-3} N, 1.8 mol m^{-3} P, 2.7 K, and 11 chelated Fe (Peter's 20–20–20, Grace Sierra Horticultural Products Co., Milpitas, CA). Plants were grown in a controlled environment greenhouse with day and night temperatures of 30 °C and 20 °C, respectively. When necessary, daylength was extended to 16 h with high-pressure sodium lamps that provided a PFD of approximately 1000 μmol m^{-2} s^{-1}. Plants used for experiments were at least 6-week-old, and leaves chosen for use were fully mature but not senescing.

Observations of stomatal aperture

To observe apertures of individual stomata on the upper surface of the leaf as they responded to increases in PFD, leaves (still attached to the plant) were mounted on a modified microscope stage as described by Mott *et al.* (1997). Gas composed of 79% N_2, 21% O_2, and varying amounts of water vapour was passed over the upper surface of the leaf; the lower surface was sealed with plastic wrap to prevent gas exchange. Gas exchange measurements on leaves for which one surface was sealed showed that this procedure sometimes caused stomata on the unsealed surface to open more for a given set of conditions, but that stomata on the unsealed surface always continued to respond to environmental perturbations such as light and humidity (KA Mott, unpublished data). CO_2-free air was used in these experiments to produce large stomatal apertures to improve the accuracy of the aperture measurement.

A PFD of approximately 1000 μmol m^{-2} s^{-1} was provided to the upper surface of the leaf by a 500 W xenon bulb using two 0.5 cm fibre optic bundles. To observe stomatal apertures, it was necessary to reduce this illumination on the upper surface of the leaf for approximately 15 s, and apply light to the bottom of the leaf through the condenser of the microscope. This light was filtered to remove wavelengths below 700 nm, making it invisible to the human eye and presumably also to the stomata since there is no known response of guard cells to phytochrome (Taiz and Zeiger, 1998). However, the video camera used to take digital photomicrographs was sensitive to wavelengths above 700 nm, and images of the stomata could be clearly seen on the video monitor and could be captured digitally. The application of light above 700 nm to the underside of the leaf had no measurable effect on stomatal apertures, and this procedure allowed measurement of stomatal apertures in darkness at the beginning of some experiments (Fig. 1).

Digital photomicrographs of part of an areole approximately half-way between the midrib and the edge of the leaf were taken at 15 min intervals using a video digitizing board in a PC. Areoles contained approximately 10 stomata, and the apertures of 5–8 stomata within the same areole were measured using digital image analysis software (see Mott *et al.*, 1997, for details).

To examine the nature of *Spannungsphase* variation in the absence of hydraulic coupling, similar experiments were carried out with leaves that had been infiltrated with 10 mM MES (pH 6.15). Infiltrating a section of leaf should bring all cells in the leaf to the same water potential (essentially zero) and provide all cells with a continuous immediate local supply of nearly pure water. To infiltrate leaves, they were cut into square pieces approximately 1 cm across. These leaf pieces were immersed in the buffer solution and placed in a large syringe. The leaf pieces were then vacuum infiltrated by sealing the needle end of the syringe and pulling on the plunger until the leaf pieces looked uniformly dark green.

Modelling

The computer model was similar to that developed by Haefner *et al.* (1997) with the following modifications. A hydraulic compartment representing the minor veins that surround each individual areole was added to the model. The water potential of this compartment was determined by the inflow of water from the major veins (which remained at a constant water potential of zero) and the outflow of water into the marginal epidermal cells of the areole. Hydraulic resistances for these two flows were chosen to yield model behaviour that was qualitatively similar to observed behaviour with regard to the stability of stomatal conductance and water potentials, and the relative magnitudes of epidermal and guard cell turgor pressures, under different regimes of evaporative demand. Sensitivity analysis (not shown) verified that the qualitative results described below were not critically sensitive to these heuristically estimated parameters.

A function describing the response of stomata to light was added to the model by modifying Equation 8 of Haefner *et al.* (1997) to yield the following function for steady-state guard cell osmotic pressure ($\pi_{g,ss}$):

$$\pi_{g,ss} = \pi_{g,min} + (\pi_{g,max} - \pi_{g,min}) \left(\frac{P_e}{\pi_e}\right)\left(\frac{L}{L + k_L}\right) \qquad (1)$$

Equation 1 differs from Equation 8 in Haefner *et al.* (1997) by the presence of the last term, which represents a hyperbolic response of steady-state guard cell osmotic pressure to increasing PFD; $\pi_{g,min}$ and $\pi_{g,max}$ are the minimum and maximum possible values for guard cell osmotic pressure, respectively; P_e and π_e are epidermal turgor and osmotic pressure, respectively (both positive in our convention); L is PFD; and k_L is a parameter scaling the slope of the hyperbolic light response curve. Osmotic and turgor pressures are in MPa, and L and k_L have arbitrary units because they are used in a rational expression.

Outline of experiments

(I) *Microscopic observation of several neighbouring stomatal apertures following a step increase in PFD to 1000 μmol m^{-2} s^{-1}*: Experiments were performed from an initial PFD of (a) darkness or (b) 100 μmol m^{-2} s^{-1}, for each of the following: (1) attached leaves at high humidity ($\Delta w = 6$ mbar bar^{-1}), (2) attached leaves at low humidity ($\Delta w = 24$ mbar bar^{-1}), and (3) detached leaves that were cut into pieces and vacuum infiltrated with buffer solution. A total of six distinct experiments were thus performed. Experiments with attached leaves were repeated three times; experiments with infiltrated leaf pieces were repeated two times.

(II) *Model simulations of the experiments described in I*: All experiments were simulated using the model presented by Haefner *et al.* (1997), with the modifications described above. A single areole was simulated by 9 stomata in a 3×3 grid. Each stoma was randomly assigned a different value for mechanical advantage (Haefner *et al.*, 1997) from a triangular probability density function ranging from 1.6 to 2.0 with mode 1.8. A single set of random values was used for all model runs (with $\Delta w = 0$, 5 and 20; these values have arbitrary units; see Haefner *et al.*, 1997) within a given set of parallel simulations (dark-to-high light, or low-to-high light). Most data collected in the experiments corresponded directly to output from the analogous simulations, with exceptions. (1) Experiments with infiltrated detached leaves were simulated by fixing the evaporative gradient, Δw, to zero. (2) Low PFD was simulated by choosing a value for PFD that caused all stomata to be either exactly at, or beyond, the end of the *Spannungsphase*; since stomatal responses were always expressed as changes in aperture following full illumination, the plotted time-courses for all stomata in the simulated low-to-high PFD treatment begin at the same point.

Results

Stomatal apertures for infiltrated leaves

To examine the variation in *Spannungsphase* among stomata in the absence of hydraulic coupling, epidermal

turgor was fixed by infiltrating leaves with buffer as described in the Materials and methods section. These leaves were then placed under the microscope and exposed to either darkness or low PFD for 1 h, after which the PFD was increased in a single step to 1000 μmol m^{-2} s^{-1}. At least two replicate experiments were performed for each PFD treatment, with similar results between replicates.

Figure 1 shows results from one replicate of the dark-to-high PFD treatment (data from both replicates are shown in Fig. 3). Stomata appeared closed following the 1 h of darkness that preceded the increase in PFD. Stomatal apertures increased following the change to 1000 μmol m^{-2} s^{-1}, and stable apertures were reached after approximately 2 h (Fig. 1a). However, some stomata showed long delays (up to 75 min) before any increase in aperture was observed, while other stomata began to open almost immediately following the increase in PFD. The magnitude of the final change in aperture also varied among stomata.

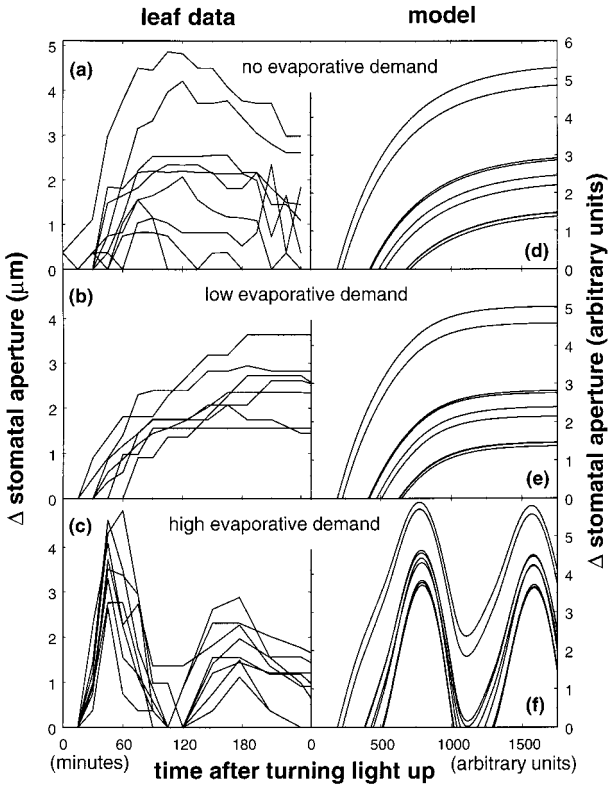

Fig. 1. The effect of an increase in PFD from darkness to 1000 μmol m^{-2} s^{-1} on stomatal apertures. Each line is a time-course of the *change* in aperture of a single stomata from its value before PFD was increased. Panels a, b, and c are data for five to eight adjoining stomata within an areole of *X. strumarium*; panels d, e, and f are for nine stomata comprising one areole in computer model (see text). Panels a and d are for a Δw value of zero (infiltrated leaf pieces for panel a); panels b and e are for a low value of Δw (6 mmol mol^{-1} for the leaf data, 6 arbitrary units for the model data); panels c and f are for high value of Δw value (24 mmol mol^{-1} for the leaf data, 24 arbitrary units for the model data).

These experiments were repeated for infiltrated leaves that were initially held under low PFD (100 μmol m^{-2} s^{-1}) rather than darkness. In these 'low-to-high' PFD treatments, some stomata were slightly open while others remained closed after 1 h at low PFD, but apertures were steady in all cases. When PFD was increased, all stomata opened (Fig. 2a, only one replicate is shown). However, in these experiments, there was little or none of the delay that was observed in the darkness to high PFD experiments (compare Figs 1a and 2a). The amount of variation in the total increase in aperture was also smaller than in the dark to high PFD experiments.

Stomatal apertures for intact transpiring leaves

To measure variability in the *Spannungsphase* under low and high degrees of hydraulic coupling, the response of stomata to an increase in PFD from darkness or from low PFD (100 μmol m^{-2} s^{-1}) was measured in intact transpiring leaves at low and high values for Δw. In these experiments leaves were held at room light (about 15 μmol m^{-2} s^{-1}) for about 1 h and then in darkness or

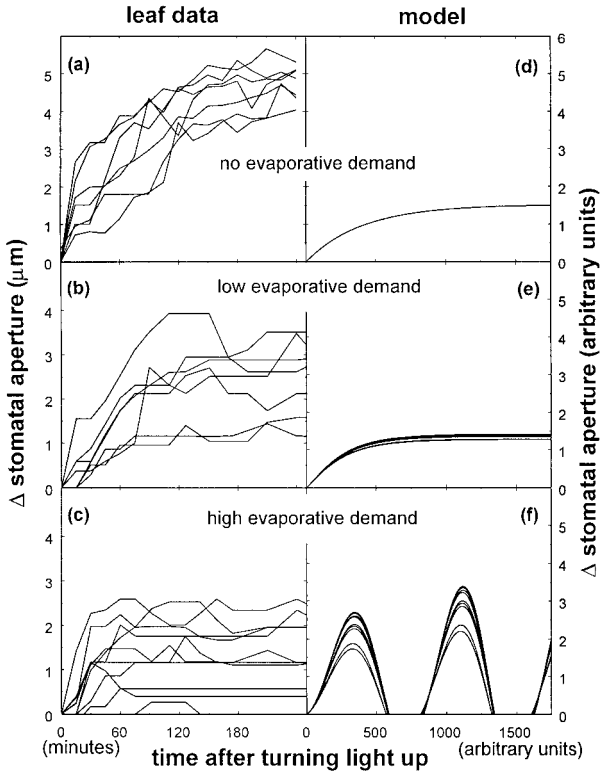

Fig. 2. The effect of an increase in PFD from 100 to 1000 μmol m^{-2} s^{-1} on stomatal apertures. Each line is a time-course of the change in aperture of a single stomata from its value before PFD was increased. Panels a, b, and c are data for five to eight adjoining stomata within an areole of *X. strumarium*; panels d, e, and f are for nine stomata comprising one areole in computer model (see text). Panels a and d are for a Δw value of zero (infiltrated leaf pieces for panel a); panels b and e are for a low value of Δw (6 mmol mol^{-1} for the leaf data, 6 arbitrary units for the model data); panels c and f are for high value of Δw value (24 mmol mol^{-1} for the leaf data, 24 arbitrary units for the model data).

low PFD for about 1 h before increasing PFD to 1000 μmol m^{-2} s^{-1}. At least three replicate experiments were performed at Δw values of 6 or 24 μmol mol^{-1}; data for one replicate are shown in Fig. 1, but data from all experiments are shown in Fig. 3.

After the 1 h of darkness at either high or low Δw, stomata appeared closed. At the low value of Δw, stomatal apertures increased following the increase in PFD, but the lag time before the aperture first increased (the *Spannungsphase*) varied among stomata (Fig. 1b). In the data shown, this delay lasted between 15 and 60 min. In three replicate experiments not shown in Fig. 1, the delays ranged from 15 to 60 min, 30 to 75 min, and 15 to 105 min. In all cases, the rates of opening after the delay were not appreciably different among the stomata.

At the high value of Δw, stomatal aperture increased in response to the increase in PFD, but in this case there was little or no delay in opening (Fig. 1c). For the experiment shown in Fig. 1c, all stomata began to open within 15 min of the increase in PFD. For two replicate experiments not shown, all stomata began to open within 45 min, and for neither experiment was there more than 15 min difference between the shortest and longest delays among stomata. In the experiments at high Δw, the initial rate of opening was higher than for experiments at low Δw, and apertures always oscillated somewhat. This behaviour ranged from sustained oscillations (as shown in Fig. 1c) to smaller transient decreases in conductance in the replicate experiments.

Similar experiments were performed with an increase in PFD from 100 to 1000 μmol m^{-2} s^{-1}. Most stomata were slightly open after 1 h at low PFD, and apertures were steady with time. In all replicate experiments at both high and low Δw, all apertures began to increase almost immediately following the increase in PFD (Fig. 2b, c). There was not a large difference in the rate of opening between the two values of Δw, but the increase in aperture was larger at low Δw, and therefore stable apertures were reached more quickly at high Δw.

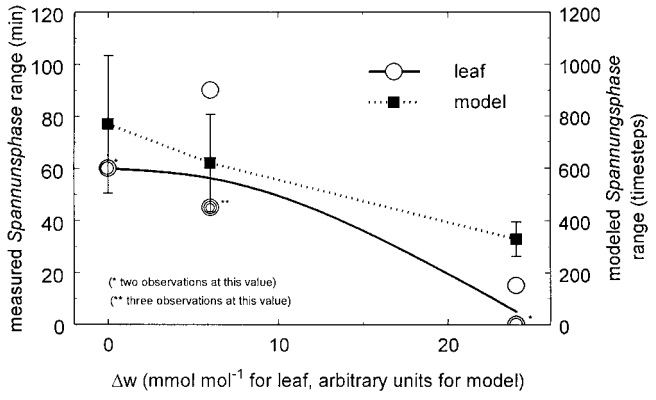

Fig. 3. The range in the duration of the *Spannungsphase* as a function of Δw. Data on the ordinate axis represent the difference in the duration of the *Spannungsphase* between the first and last stoma to open. For leaf data, each point represents the range of *Spannungsphasen* among 5–8 stomata measured in a single experiment; because few experiments (three or four) were performed at each value of Δw, all data are presented rather than their mean and s.d. Nested points (marked with asterices) represent multiple experiments that yielded the same result. For model data, each point represents the mean and s.d. (among 10 model runs, each with 9 stomata) for the range of *Spannungsphasen*.

Modelling

When the experiments described above were simulated with the model of Haefner *et al.* (1997), qualitatively similar results were observed in most cases. At low and moderate evaporative demand (Figs 1d, e: 2d, e), stomatal apertures increased hyperbolically with time, and the final change in aperture varied widely among stomata within an areole. The rate of opening increased with evaporative demand. At high evaporative demand (Figs 1c, f: 2c, f), stomatal apertures oscillated after PFD was increased to saturating levels from either darkness or low PFD (oscillations result from a large gain in the feedback loop governing the approach to hydraulic equilibrium between epidermis and guard cells; see Cowan, 1972, or Rand *et al.*, 1981, for more detail). Although the numerical values of these oscillating apertures varied among stomata within a single areole, all stomata were entrained to the same oscillatory phase. Oscillations were not observed for the low-to-high PFD transitions.

In both experimental data and model simulations of zero-to-high PFD transitions, the variability in the *Spannungsphase* always decreased with increasing evaporative demand (Figs 1, 2). However, the relative magnitude of this decrease in variability was much smaller in the simulations than in experimental data. To quantify this discrepancy more completely, a series of 10 model runs, each with a different set of spatially randomized mechanical advantages in each run, was performed for each Δw value. For each set of 10 runs, the mean and standard deviation for the range among stomata in the duration of the *Spannungsphase* was calculated and plotted in Fig. 3. The ranges in *Spannungsphase* duration observed in real leaves (the leaf data was extracted from the data shown in Figs 1 and 2 and from replicates that are not shown) were plotted with open symbols in Fig. 3 for comparison. For leaves and simulations, the range in *Spannungsphase* duration was greatest at zero evaporative demand, and decreased monotonically with Δw. (The presence of one stoma that took 105 min to open is responsible for the outlier at low Δw in Fig. 3.) Going from zero to high evaporative demand, the mean range of *Spannungsphase* duration decreased from 60 min to 5 min in leaves (a decrease of 92%), but the range decreased from roughly 780 to 330 timesteps in the model (a decrease of 58%).

Discussion

In this study data are presented showing that there is substantial variation among stomata within an areole in the duration of the *Spannungsphase* for vacuum-infiltrated leaves and for intact leaves at low Δw. These data are consistent with the observations of Saxe (1979) on *Commelina communis* L., in which the time between illumination and opening varied between 15 min and several hours, even among neighbouring stomata. Those experiments were performed at a Δw of approximately 11 μmol m^{-1}. In contrast, variation in the *Spannungsphase* was considerably reduced at high Δw, as was its average duration. One explanation for this result is that when one or more stomata in an areole make the transition from *Spannungsphase* to *Motorphase* (i.e. they begin to open) following an increase in PFD, the resulting transpiration from these stomata begins to draw down the epidermal turgor pressure of the entire areole. This reduces the backpressure of the epidermis on nearby stomata that have not yet opened, making it easier for them to open and thus shortening the *Spannungsphase* for those stomata. These hydraulic interactions therefore serve to co-ordinate the movements of neighbouring stomata and thereby reduce variation in the average duration of the *Spannungsphase*. Since these interactions result from a transpiration-induced reduction in epidermal turgor, it follows that the degree of coupling should be proportional to transpiration rate and thus to Δw. Variation in the *Spannungsphase* should therefore be lower at high Δw than at low Δw, consistent with the data in Fig. 1.

The low-to-high PFD experiments were designed to differentiate between two possible explanations for variability in the *Spannungsphase*. One explanation, and that which underlies our interpretation of the data in Fig. 1 (panels a–c), is that the epidermal mechanical advantage varies among stomata within an areole. If this is the case, more stomata (perhaps all) should open immediately in response to full illumination from low PFD than from darkness, because at low PFD the influence of epidermal turgor has already been largely negated by a prior increase in guard cell turgor. Alternatively, stomata may exhibit variable lags in the biochemical response time of guard cells to an increase in PFD. If so, variability should appear in opening responses from low PFD as well as from darkness. The results in Fig. 2 (panels a–c) appear to reject the latter explanation and support the former, because when PFD was increased after allowing stomata to stabilize at low PFD, all stomata responded immediately.

The hydraulic coupling hypothesis was further tested by simulating these experiments in a spatially explicit model of stomatal functioning (Haefner *et al.*, 1997) that incorporates only hydraulic mechanisms for local stomatal interactions (and thus excludes chemical signals) and assumes random spatial variability in the mechanical advantage rather than in biochemical PFD response characteristics. The model predicts that the variation in the *Spannungsphase* is reduced at low humidities when compared with the variation observed at lower evaporative demands (Fig. 1, panels d–f). Although these results support the notion that hydraulic coupling can reduce the variation in the *Spannungsphase*, the effect observed in the model appears to be smaller than that observed in real leaves (Fig. 3). Direct rigorous comparison of these results is not possible because the data for real leaves are in discrete 15 min intervals while the model produces effectively continuous time-courses. Nevertheless, the discrepancy between the model's predictions and data from real leaves suggests that hydraulic coupling, as embodied in this model, can not produce the observed magnitude of decreases in *Spannungsphase* variation as evaporative demand is increased. Possible explanations for this discrepancy include (1) this model may not adequately describe the hydraulic interactions that exist in real leaves, and (2) chemical signals may be preferentially dispersed throughout the areole under conditions of high evaporative demand.

This study was conducted as part of a larger endeavour to explain patchy stomatal conductance mechanistically, which requires two (possibly distinct) mechanisms: one for co-ordinating stomatal behaviour within an areole, and one for unco-ordinating behaviour among areoles. Specifically, the experiments described here were conducted to test the hypothesis that hydraulic interactions propagated by changes in epidermal turgor pressure can co-ordinate stomatal behaviour within an areole and should increase in strength with Δw (variability in the duration of the *Spannungsphase* served as a convenient measure of stomatal unco-ordination that could be observed while independently controlling Δw). The results presented here support this hypothesis, leading to the prediction that high Δw may also increase the prevalence of patchiness following an increase in PFD. Consistent with this prediction, it has been reported that high Δw or water stress was required for PFD-induced patchiness n three of seven species tested (Eckstein *et al.*, 1996). Recent work in this laboratory (TN Buckley and KA Mott, unpublished data) also suggests that for a single species, patchy stomatal conductance following an increase in PFD from darkness is more prevalent at high values of Δw than at low values. Additionally, the data shown in Fig. 2 indicate that all stomata responded immediately to an increase from low-to-high PFD, negating unco-ordination at any scale and thus making stomatal patchiness less likely to occur. This is consistent with data suggesting that patchiness is rare following low-to-high PFD transitions at any Δw (TN Buckley and KA Mott, unpublished data).

The mechanisms controlling local stomatal interactions and patchiness are important because these phenomena complicate predictive modelling of plant gas exchange. Dynamics that operate between scales (such as stomatal interactions and patchiness) make it difficult to translate mechanistic predictions of individual stomatal responses to larger scales such as a canopy, or even a leaf. It is necessary to understand the mechanisms by which individual stomata interact with each other, and to characterize the divergence of individual stomatal behaviour from the modes represented by small-scale mechanistic models. In the context of those objectives, this study provides substantial evidence for hydraulic coupling as a mechanism for stomatal interactions and characterizes variation among stomata in the kinetics of their responses to PFD, showing that this may result from variability in the mechanical advantage of the epidermis.

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