Plant response to atmospheric humidity

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Abstract. Plants growing in environments differing in prevailing humidity exhibit variations in traits associated with regulation of water loss, particularly cuticular and stomatal properties. Expansive growth is also typically reduced by low humidity. Nevertheless, there is little evidence in plants for a specific sensor for humidity, analogous to the blue light or phytochrome photoreceptors. The detailed mechanism of the stomatal response to humidity remains unknown. Available data suggest mediation by fluxes of water vapour, with evaporation rate assuming the role of sensor. This implies that stomata respond to the driving force for diffusional water loss, leaf-air vapour pressure difference. Induction of metabolic stomatal response to humidity may involve signal metabolites, such as abscisic acid, that are present in the transpiration stream. These materials may accumulate in the vicinity of guard cells according to the magnitude and location of cuticular transpiration, both of which could change with humidity. Such a mechanism remains hypothetical, but is suggested to account for feedforward responses in which transpiration decreases with increasing evaporative demand, and for the apparent insensitivity of stomatal aperture in isolated epidermis to epidermal water status. Other responses of plants to humidity may involve similar indirect response mechanisms, in the absence of specific humidity sensors.

Key-words: cuticle; humidity; sensory transduction; stomata; transpiration; vapour pressure difference; water use.

Introduction

Plants exhibit a multitude of apparent responses to atmospheric humidity. These include rapid responses such as stomatal movements and explosive dehiscence of seed pods, developmental changes such as epicuticular wax deposition, and evolutionary adaptations such as cuticle thickness and stomatal sensitivity to humidity. It is a common observation that plants grow best when evaporative demand is relatively low. Yet few of these responses can be uniquely attributed to humidity and none can be shown to be mediated by a humidity sensor.

A recurring theme in plant biology is the difficult and often arbitrary choice of an appropriate measure of humidity to relate to a specific plant process. Experimental studies remain correlative and generally inconclusive. Theoretical studies are dependent on interpretation of experimental data regarding pathway and mode of water transport. In this regard, at least, progress in plant biology has not lagged behind progress in other fields. Insect physiologists, for example, enjoy the great experimental advantage of using isolated humidity sensors and associated neurons to obtain quantitative electrophysiological data. Yet it remains unclear whether insects that navigate toward sources of humidity sense relative humidity (RH) or another measure of humidity (Altner & Loftus, 1985). Similarly, widespread use is made of the parameter, RH, as a human comfort index, though its approximation of the true parameters of human energy balance is widely recognized. Rigorous definition in plants of the humidity parameter that stimulates a specific physiological response would greatly facilitate characterization of the response mechanism.

The following discussion will consider what is known and what might be speculated regarding direct responses of plants to humidity. The physical nature of humidity signals and possible perception and transduction mechanisms will be considered, followed by a detailed analysis of the stomatal response to humidity. The stomatal response remains the most thoroughly investigated and yet the most enigmatic of the putative humidity responses. As stomatal response is well integrated with other plant functions (Zeiger, 1983), and reflects a wide range of developmental responses, this approach may encourage a broad view of 'sensory' perception of humidity by plants. Recent reviews on related subjects include those of Boyer (1985), Losch & Tenhunen (1981), Schulze & Hall (1982), Schulze (1986) and Sheriff (1979). Earlier treatments by Cowan (1977) and Raschke (1975a) remain sources of considerable insight.

Abbreviations: V, surface to air vapour pressure difference; D, air saturation deficit; g, g_c, total, cuticular conductance; E, E_c, total, cuticular transpiration; RH, relative humidity; I, photosynthetic photon flux density; e_a, e_s, ambient, saturated water vapour pressure; gc, guard cell.

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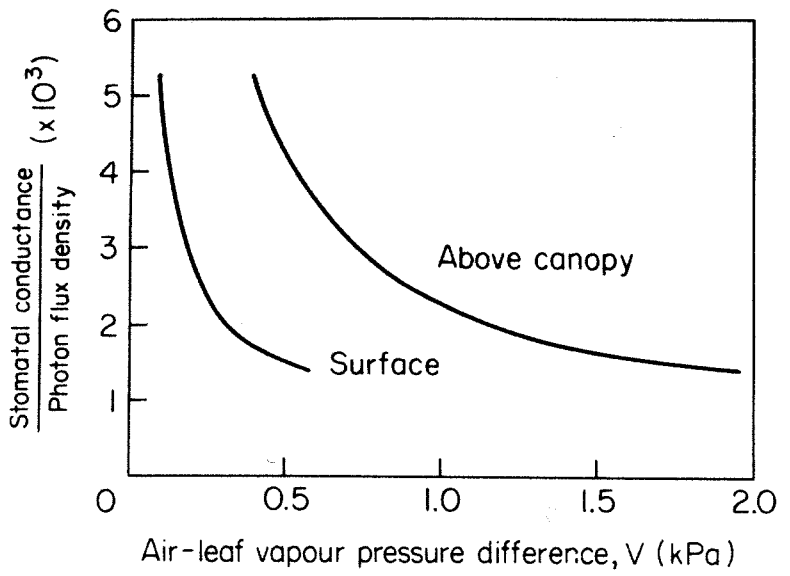

Figure 1. Relationship between stomatal conductance, normalized by prevailing photon flux density, and leaf-to-air vapour pressure difference. V was calculated at the leaf surface or using humidity measured just above the canopy. Data are for field-grown sugarcane in which radiation and evaporative demand were strongly correlated. After Grantz & Meinzer (1990a).

Where might a plant sense humidity?

To the extent that plants sense humidity, they must sense the humidity at their surfaces. This surface humidity may be quite different from that in the bulk atmosphere, even just a few centimetres away, particularly in dense canopies (McNaughton & Jarvis, 1983). In sugarcane, for example, the canopy is humidified by transpiration and low boundary layer conductance so that changes in atmospheric humidity are nearly obscured at the leaf surface (Grantz & Meinzer, 1990a). It is futile under such conditions to seek a physiological response to the humidity prevailing at the level of micrometeorological instruments placed well above the canopy.

The potential differences in interpretation are clear (Fig. 1) when stomatal conductance, normalized for incident light, is plotted against two measures of leaf-air vapour pressure difference (V). The same stomatal movements describe a very sensitive response to humidity (V) at the leaf surface, but a rather less sensitive response to humidity measured in the atmosphere above the canopy. Stomatal responses to humidity have been documented using an array of independent techniques. The recent suggestion (Idso, 1987) that these humidity responses may be artefacts associated with porometer measurements is resolved (Grantz & Meinzer, 1990a,b; Monteith, 1990) by selection of appropriate and compatible reference points for determination of stomatal conductance and humidity. The leaf surface is the only reference point that is independent of differences in natural or experimental boundary layer properties, as has been recognized in the context of single-leaf gas exchange (Ball, 1987; Bunce, 1985).

How might a plant sense humidity?

Interactions between plants and humidity are likely to involve one or both of two distinct processes. Thermodynamic equilibrium may be attained between atmospheric water vapour and the water bound to a structural material or to a humidity sensor. Alternatively, steady state conditions of parameters such as temperature or turgor may be related to steady state fluxes of water vapour. Steady state fluxes can be maintained because most evaporating surfaces in higher plants have continuing access to liquid water.

Poikilohydric plants do not necessarily maintain this access to liquid water, equilibrating with the atmosphere and sensing humidity through the hydration state of the entire organism. In such plants, including many algae and a few angiosperms among other taxa, achievement of the air dry state in low humidity suspends metabolism, which resumes with rehydration. In the more familiar homiohydric plants, equilibration of most plant structures with the atmosphere is largely prevented. Evolution of roots and vascular systems that enhance water supply, cuticles that retard water loss, and vacuoles that buffer water status, all tend to maintain access of evaporating surfaces to liquid water. Under these conditions steady-state models of humidity sensing may be more generally applicable than equilibrium models.

Humidity parameters

Humidity is a general term without specific units. It expresses information regarding the water vapour and energy content of air, and the potential for evaporative cooling. As an equilibrium signal, humidity represents a chemical quantity, rather than a physical quantity such as temperature or pressure. In contrast to other chemical species of interest to plants, water vapour is not a limiting substrate for metabolism, and cannot be sensed via reaction rates or metabolite pools. Unlike physical parameters, with different reporting forms related by linear interconversion of units, specific measures of humidity determine the information content of the measurement. Additional information (generally a temperature) is required to interconvert between alternative reporting forms. Thus the specific measure of humidity that controls a physiological process determines the requirements of any putative biological sensor.

Absolute measures of humidity

Definitions of humidity based on the masses of water vapour (m_w) and dry air (m_a), i.e. specific humidity, q ($q = m_w/[m_w + m_a]$) or mixing ratio, r_a ($r_a = m_w/m_a$), are entirely conservative with respect to temperature and pressure. For meteorological applications, the absolute humidity or vapour concentration (m_w/unit volume) is often used. The partial pressure of water (vapour pressure, e_a) is proportional to the concentration of water and equal to mole fraction times total pressure, ignoring small deviations from idea-

lity. The saturation vapour pressure (e_s) in equilibrium with liquid water is an exponential function of temperature, and provides the reference for relative measures of humidity. It is unlikely that plants respond to absolute or specific humidity.

Relative measures of humidity

Humidity may be expressed relative to isothermal saturation (e_s), as a difference (gradient) or as a proportion. The difference between actual vapour pressure in the atmosphere and the saturation vapour pressure at air temperature gives air saturation deficit, D ($D=[e_s-e_a]$). D adequately predicts canopy transpiration in the absence of measured canopy temperature, but misrepresents the driving force for diffusion when canopy and air are at different temperatures. This uncertainty is largely resolved by the parameter, leaf to air vapour pressure difference, V ($V=[e_{s'}-e_a]$), where $e_{s'}$ is saturated vapour pressure at leaf (not air) temperature. V more accurately represents the diffusional driving force for vapour fluxes under all conditions and can therefore be related to steady state physiological parameters, as noted above. At the leaf surface, i.e. within the unstirred boundary layer, leaf and air temperature are the same, thus ($D=V$).

Relative humidity (RH) represents fractional (per cent) saturation ($RH=100\ [e_a/e_s]$) at a single temperature. RH of the vapour phase is related to the water potential (Ψ_w, volume basis) of liquid water with which it is in equilibrium ($\Psi_w=RT/V_m \ln [RH/100]$), where R is the gas constant, T is Kelvin temperature and V_m is the partial molal volume of water. A linear plot of a physiological parameter against RH is thus a curvilinear plot against Ψ_w. Because of this relationship with Ψ_w, RH is often considered the parameter best suited to serve as an equilibrium signal for plant sensing of humidity.

The moisture content of biological materials is closely related to RH by a number of similar empirical relationships (Skaar, 1988). The sorption isotherm for wood, for example, is well represented as ($M=a+b \ln [100-RH]$) where M is moisture content and a and b are empirically determined (Smith, 1947). As noted above, however, few plant structures equilibrate with the gas phase, a serious limitation of equilibrium hypotheses.

Biological systems are likely to respond to a relative measure of humidity, though determining which one has remained challenging. Normalization of D by e_s yields ($[D/e_s]=1-[e_a/e_s]=1-[RH/100]$). D and RH are thus linearly related, but the slope of the relationship, determined by e_s, depends on temperature (Fig. 2). Measurements at a single temperature cannot distinguish responses to D from responses to RH, though divergence in measurements over a range of temperatures may be diagnostic. The exponential effect of temperature on e_s provides RH with somewhat more temperature compensation than V or D (Ball, Woodrow & Berry, 1987).

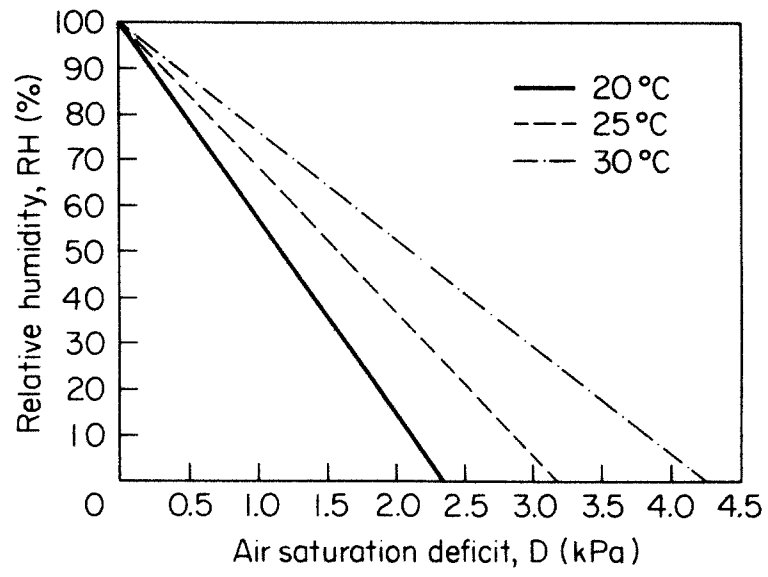

Figure 2. Relationships between relative humidity ($RH=100\ [e_a/e_s]$) and air saturation deficit ($[D=e_s-e_a]$ at three temperatures.

Plant responses to humidity

Ambiguous humidity signals

There are few plant responses that can be uniquely related to direct effects of humidity. Natural changes and experimental manipulations of humidity are frequently associated with changes in other environmental parameters. These often cause independent effects on plants. In the natural or field environment, V is often closely correlated with radiation and thus whith photon flux density (I). In the sugarcane field data shown in Fig. 1, g required normalization by I, a stomatal opening stimulus (Zeiger, 1983), to reveal the typical closing response to V (Grantz & Meinzer, 1990a). Without this normalization the covariance of V and I completely obscured any closing response to humidity (Grantz & Meinzer, 1990a,b). Stomatal responses of sugarcane (Grantz, Moore & Zeiger, 1987), apple (Thorpe, Warrit & Landsberg, 1980) and several conifers (Kaufmann, 1982) have been related to similar composite terms incorporating the antagonistic effects of I and V. When changes in V are associated with changes in other parameters, it is difficult to identify the specific effects of humidity. Over longer periods, acclimation to the other parameters, such as prevailing light, may be more significant than acclimation to prevailing humidity.

Changes in humidity alter transpiration, energy balance and tissue temperatures. These, in turn, may affect ion uptake, carbon assimilation, water transport and other processes, each of which has further physiological consequences that obscure direct responses to humidity. Reduced leaf area expansion at low humidity, for example, initiates a positive feedback cycle. Future radiation interception, growth rate and self-humidification of the leaf microenvironment are reduced, further reducing leaf area expansion. In many cases, apparent humidity effects are associated with reduction in bulk leaf water potential (Ong, Simmonds & Matthews, 1987). The indirect relationship with humidity is demonstrated by enhancement of the impact of high V on growth

(Krizek, Bailey and Klueter, 1971) when soil evaporation is enhanced by growth of plants in porous clay pots rather than in impermeable plastic pots.

In some cases, relatively direct responses to humidity may be identified. Reduction of leaf expansive growth by low humidity (Bunce, 1978) occurs in darkness when stomata are closed (Waldron & Terry, 1987), so that it is not mediated by bulk leaf transpiration or water status. It could reflect reduced epidermal turgor, or humidity-induced stiffening of epidermal walls. Control of tissue growth by turgor and yielding properties of epidermal cells has been hypothesized in a number of systems (e.g. Masuda & Yamamoto, 1972). Direct effects of humidity on epidermal wall properties would represent a humidity sensor, but to date there is little experimental support. Epidermal transpiration is likely to be involved. V is a better predictor of plant growth than RH (Ford & Thorne, 1974) arguing for an effect mediated through vapour fluxes and epidermal water status.

Plantlets cultured *in vitro* often become vitrified (Bornman & Vogelmann, 1984; Ziv, Schwartz & Fleminger, 1987), a state of hyperturgidity and extracellular waterlogging that is associated with the tissue culture environment. Reducing the humidity decreases the proportion of vitrified plantlets (Ziv, Meir & Halevy, 1983), but a causal relationship with humidity is not established. Altering the rigidity or the cytokinin content of the medium has equally profound effects on vitrification (Bornman & Vogelmann, 1984; Ziv *et al.*, 1983, 1987). Disruption of phytohormone distribution by depression of transpirational fluxes may be involved.

Regulation of water loss

Cuticular permeability has been related to the quantity and quality of cuticular waxes rather than to thickness of the cuticle itself (Schonherr, 1982). Removal of these waxes from isolated cuticles and from attached leaves (e.g. douglas fir, Meinzer, 1982) increased cuticular conductance, and enhanced the stomatal response to humidity. The thicker cuticle often observed in species from arid zones is apparently no less permeable to water vapour than the thinner cuticle characteristic of more mesic environments (Schonherr, 1982). Thickness may confer enhanced abrasion resistance to insure cuticle survival in arid environments. Resistance to water vapour of cuticle, once developed, has been shown to respond reversibly to humidity (see below).

Development of epicuticular waxes was retarded in the high humidity of the tissue culture environment in carnation (Sutter & Langhans, 1979) and in plum (Fuchigami, Cheng & Soeldner, 1981). Wax deposition on guard cells and other cells of the stomatal complex was absent at high humidity, and induced at low humidity in plum leaves (Fuchigami *et al.*, 1981), though the impact of these changes on stomatal function and responses to humidity remain untested. Excised leaves from plantlets grown *in vitro* lose considerably more water in dry air than those grown under greenhouse conditions (Brainerd & Fuchigami, 1981).

At lower levels of humidity, in controlled-environment growth chambers, humidity was inversely related to production and morphological complexity of epicuticular waxes (Baker, 1974). Both light and temperature cause additional modifications. Plants grown in humid environments (e.g. maize, Slavik, 1973) exhibit substantially greater cuticular conductance than plants from less humid conditions, and exhibit greater water loss under comparable conditions.

The other component of regulation of water loss that responds to humidity is stomatal control. Growth *in vitro* of apple and carnation resulted in development of stomata that did not close, even when subjected to extreme levels of the closing stimuli, CO_2, darkness, ABA and Ca^{++}. Stomata from greenhouse plants closed rapidly and completely (Brainerd & Fuchigami, 1982; Ziv *et al.*, 1987). Restoration of functional stomata in carnation grown *in vitro* (Ziv *et al.*, 1987) involved changes in guard cell wall properties. Ion transport competence of guard cell protoplasts was maintained throughout the non-functional period. Reduction of humidity caused establishment of normal stomatal regulation of water loss within about 5 d in apple (Brainerd & Fuchigami, 1981), while changes in cuticular waxes may take somewhat longer (carnation, Sutter & Langhans, 1979; plum, Fuchigami *et al.*, 1981).

Stomatal response—toward a humidity sensor

Stomata of many species respond to humidity (for reviews, see Schulze & Hall, 1982; Losch & Tenhunen, 1981). This response is observed in isolated epidermis (Losch, 1977; Lange *et al.*, 1971; Losch & Schenk, 1978), detached leaf sections (Raschke & Kuhl, 1969), individual leaves (Laffray *et al.*, 1984; Schulze *et al.*, 1974; Hall & Hoffman, 1976; Grantz *et al.*, 1987; Kauffmann, 1982) and intact canopies (Grantz & Meinzer, 1990b). In spite of considerable effort, the mechanism remains unknown.

It has been demonstrated in a modest number of species (Farquhar, 1978; Losch & Tenhunen, 1981; Sheriff, 1977c), and generalized to others, that the stomatal closing response to low humidity is independent of feedback control mediated by reductions in leaf water status. Leaf water status may improve in low humidity, if stomatal sensitivity is sufficient (e.g. Fig. 3A) to reduce conductance enough to reduce transpiration, despite the increasing vapour pressure gradient for diffusion (e.g. Fig. 3C; $\Delta E/\Delta V < 0$ as V increases from 2.0 to 3.0 kPa). Feedforward control is indicated since feedback control can maintain, but not reduce, transpiration with increasing V (Cowan, 1977; Farquhar, 1978).

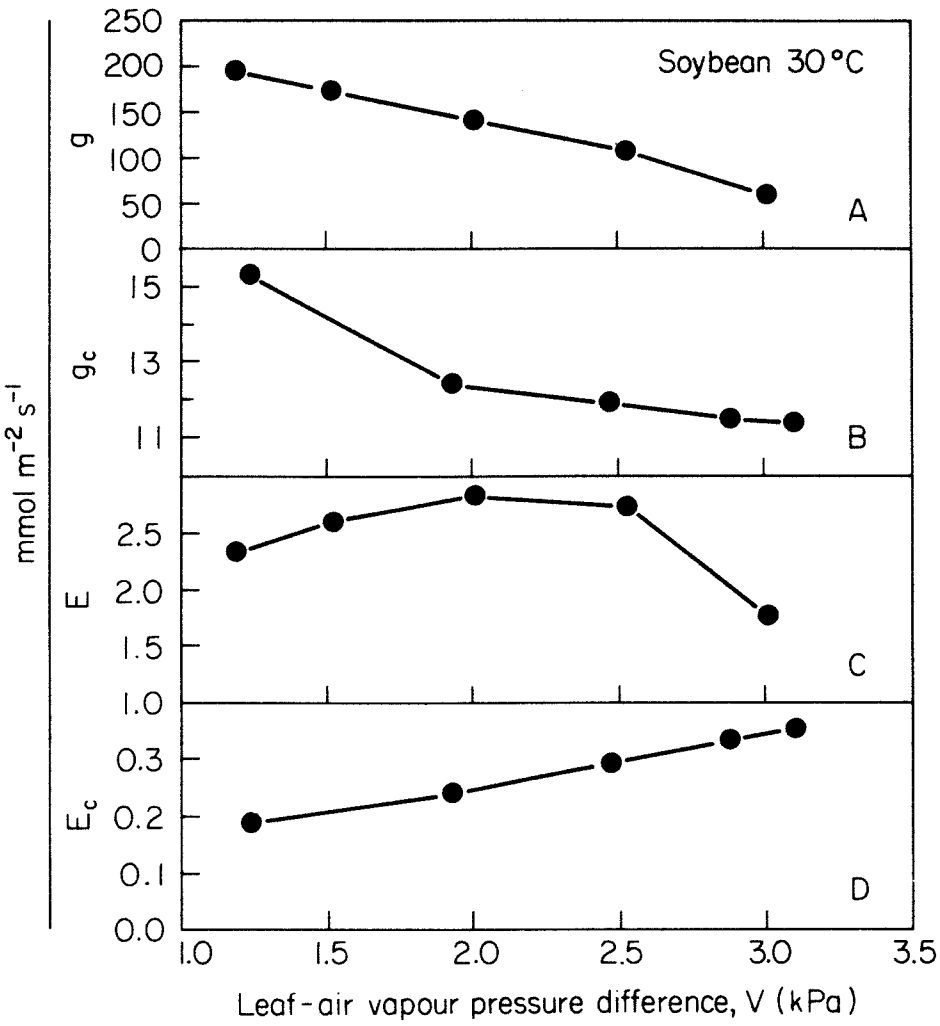

Figure 3. Relationship between leaf-air vapour pressure difference (V) and stomatal conductance (g; A), cuticular conductance (g_c; B), bulk leaf transpiration (E; C), and cuticular transpiration (E_c; D) in soybean. Cuticular conductance was measured in darkness after feeding 5 mol m^{-3} $CaCl_2$ and 10 mmol m^{-3} ABA to excised leaves to insure stomatal closure, and calculated as $[g_c = E_c P/V]$.

At present, steady state gas exchange data indicating $\Delta E/\Delta V < 0$ are diagnostic for feedforward control, and thus for direct stomatal response to humidity (Schulze, 1986). On the other hand, absence of an observed reduction in transpiration with increasing V does not imply that direct stomatal response to humidity is absent (Cowan, 1977). Feedforward behaviour reflects a specific level of stomatal sensitivity, operating over a suitable range of V. In species exhibiting $\Delta E/\Delta V < 0$ over some range of V, the same response mechanism is undoubtedly operational over other ranges of V in which $\Delta E/\Delta V \geq 0$. Similarly, species in which $\Delta E/\Delta V < 0$ has not been observed or does not occur may nevertheless share the same response mechanism. It is noteworthy in this regard that species exhibiting feedforward responses have not been found to share any major morphological, ecological or phylogenetic characteristics that distinguish them from other species (Sheriff, 1977c).

In the effort to distinguish direct from indirect (feedback) responses of stomata to humidity, innovative experimental approaches are required. Steady-state stomatal conductance represents only one parameter, and the diagnostic criterion, $\Delta E/\Delta V < 0$, is unavailable in many species. In contrast, specific components of the stomatal response, when these can be isolated, may contain a great deal of mechanistic information. Effects of V, for example, on these response components, may be contrasted with effects of the associated E even in species that do not exhibit feedforward responses. Kinetic characteristics of the transient stomatal response to blue light were analysed in this way (Assmann & Grantz, 1990a,b). In both sugarcane and soybean, with morphologically dissimilar leaves and five-fold differences in rates of stomatal response, V was a much stronger predictor of opening kinetics than was E (Table 1) in experiments over a range of temperatures. This was also true of closing kinetics in

Table 1. Coefficients of determination between different humidity parameters or bulk transpiration and the kinetics of stomatal response to pulses of blue light[a]. Data obtained at 20, 25 and 30 °C were pooled ($n = 40$ and 52 for soybean and sugarcane). After Assmann & Grantz (1990a)

	r^2		
	VPD	RH	E
Rate of opening:			
Soybean	+0.64**	−0.31**	+0.13*
Sugarcane	+0.27**	−0.18**	+0.10*
Rate of recovery:			
Soybean	+0.34**	−0.19**	+0.11*
Sugarcane	+0.38**	−0.14**	+0.40**

*$P < 0.05$, **$P < 0.01$.

[a]Blue light pulses (22 s × 120 μmol m^{-2} s^{-1}) were administered after steady state gas exchange was established under 900 μmol m^{-2} s^{-1} red light in normal air.

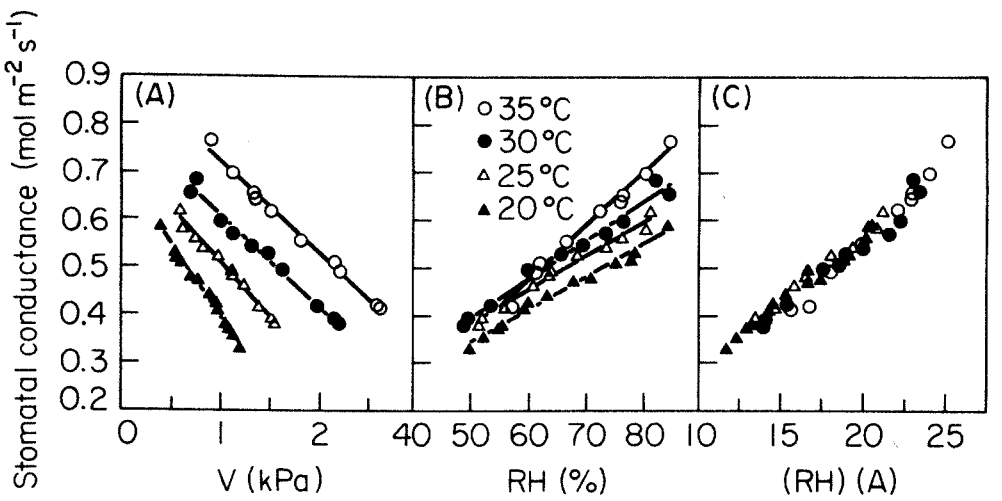

Figure 4. Relationships at four temperatures between stomatal conductance and leaf-air vapour pressure difference (A), relative humidity (B), and the product of relative humidity and net carbon assimilation rate (C). Adapted from Ball *et al.* (1987) and unpublished.

soybean (Table 1). Soybean exhibited $\Delta E/\Delta V < 0$ in these experiments (Fig. 3C; Assmann & Grantz, 1990a,b) while sugarcane did not. This is consistent with some similarity of response mechanisms whether or not feedforward responses are observed.

Steady state or equilibrium perception of humidity?

The case for relative humidity. The stomatal response to V is less sensitive at elevated temperature (Hall & Kaufmann, 1975; Ball *et al.*, 1987). This has been interpreted (Ball *et al.*, 1987) as reflecting the smaller fractional change in vapour pressure, relative to saturation (i.e. RH), for a given change in vapour pressure at higher temperature.

One of the more successful empirical models of steady state conductance (Ball *et al.*, 1987) relates g to RH, in combination with assimilation rate (A) and ambient CO_2 concentration (C_a), when this is varied experimentally. The stomatal responses to V (Fig. 4A) or to RH (Fig. 4B), observed in soybean by Ball *et al.* (1987) at several temperatures with constant C_a, suggest that the temperature compensation inherent in RH contributes a great deal towards consolidation of these data. Incorporation of A (Fig. 4C) and C_a when this varies (Ball *et al.*, 1987) also contribute a great deal in this regard. Similarly effective data consolidation using the RH parameter has been attained for soybean and sugarcane (Grantz & Assmann, unpublished) and for a range of prairie grasses (J. Norman, personal communication).

The apparent power of this simple model (Ball *et al.*, 1987) should not be underestimated. It will undoubtedly inspire further direct tests of the RH hypothesis. However, linearization of conductance data at several temperatures may not indicate selection of an appropriate humidity parameter. Unrecognized temperature coefficients could be involved and linearization of eqn 5, below, could be somewhat fortuitous.

A possible response scenario was proposed by Schulze (1986) in which the reported control of cuticular conductance (g_c) by RH or Ψ_w (Schonherr, 1982) was invoked. Both apparent feedback and feedforward responses could be simulated by assuming that stomatal conductance (g) is proportional to cuticular transpiration. If the relationship between g and E_c is not constrained to be linear, and g may respond directly to temperature, then (adapted from Schulze, 1986)

$$g = f_1(E_c, T) \quad (1)$$

$$E_c = (\Psi_w)(g_c) \quad (2)$$

$$g_c = f_2(\Psi_w, T) \quad (3)$$

$$\Psi_w = RT/V_m \ln (RH/100) \quad (4)$$

and therefore

$$g = f_1([RT/V_m \ln \{RH/100\}] \; [f_2\{RT/V_m \ln \{RH/100\}, T\}], T) \quad (5)$$

where f_1 and f_2 are specific but unknown functions, T is Kelvin temperature, Ψ_w is water potential of the vapour phase, and leaf water potential is assumed to be 0. The strongly non-linear relationships between stomatal resistance (r) and conductance (g), i.e. [r = 1/g], and between Ψ_w and RH (eqn 4), make it reasonable to assume that some combination of variables relating a measure of stomatal response to a measure of atmospheric humidity will appear linear. Such a relationship remains empirical rather than mechanistic.

There seems little reason to question the heterogeneous model of some cuticular membranes and the effects of RH or Ψ_w on cuticular pore hydration that underly this proposal (Schonherr, 1982). The role of such cuticular pores in stomatal regulation remains to be established. Cells of the stomatal complex may be protected by highly structured hydrophobic materials, including interlocking filamentous mats (e.g. Sutter & Langhans, 1979) in which resistance to gaseous diffusion may limit cuticular transpiration. Abrasion and solvent removal of embedded and epicuticular waxes has profound effects on epidermal conductance (Pitcairn, Jeffree & Grace, 1986; Meinzer, 1982). Demonstration of hydratable pores

in the cuticle is not inconsistent with a dominant role of gaseous diffusion through epicuticular networks in leaves. In the latter case, vapour pressure gradient (V) would be the more appropriate driving force for E_c (eqn 2) and the appropriate parameter for calculating g_c (eqn 3). Reports (Meidner, 1986) of diurnal trends in g_c associated with leaf (not air) water status also complicate the putative role of RH.

Stomatal response to RH is consistent with operation of a specific sensor, perhaps associated with electron-dense fibrils and other unique properties of the guard cell cuticle (Palevitz, 1981; Sacks & Paolillo, 1983). Equilibration of hypothetical filamentous sensory structures, that protrude outside of the cuticle, could mediate stomatal response to RH. Alternatively, direct RH effects on guard cell wall mechanical properties could modify the relationship between guard cell turgor and stomatal aperture. However, there is little experimental evidence for either scheme to date.

The case for vapour pressure difference. The driving force for diffusion, the principal mechanism of transpiration, is V. Thus, a feedforward system would be expected to sense V rather than RH. The response mechanisms most frequently advanced, involving epidermal turgor, peristomatal transpiration and transient stomatal movements (Maier-Maercker, 1983; Losch & Tenhunen, 1981; Sheriff, 1984) are implicitly responses to V rather than to RH.

Use of the inverse of the parameter, V, multiplied by photon flux density (I/V), effectively consolidated conductance data for field-grown sugarcane, whether obtained with a porometer ($r^2=0.78$; $n=32$; Grantz & Meinzer, 1990a; $r^2=0.68$, $n=23$, Grantz *et al.*, 1987) or with micrometeorological techniques ($r^2=0.72$, $n=277$; Grantz & Meinzer, 1990b), over a substantial diurnal range of temperatures. Use of I in this context may be functionally equivalent to use of assimilation rate (A) in the model of Ball *et al.* (1987), though application of A to sugarcane might further consolidate the data. The kinetics of stomatal opening responses to blue light pulses (Assmann & Grantz, 1990a) were accelerated in both sugarcane and soybean by increasing V. The rates of stomatal opening and closing were consistently more strongly related to V than to RH (Table 1) though the regressions were highly significant in all cases. In stomatal physiology, as in other areas of biology, the appropriate humidity parameter is not yet established.

Humidity signal perception

Stomatal responses to stimuli such as light or CO_2 involve a sequence of discrete events. Signal perception leads to transduction involving metabolically-mediated ion transport, osmotic adjustment, liquid water fluxes, and turgor-driven changes in stomatal aperture. The stomatal response to humidity is unique in that the initial triggering stimulus and the final turgor response are both mediated by water. Thus, it is not immediately clear that the intervening transduction events must be metabolic.

Stomatal aperture reflects the differential turgor between guard cells and neighbouring cells (Sharpe, Wu & Spence, 1987). Epidermal turgor is affected by humidity (Shackell & Brinckmann, 1985; Frensch & Schulze, 1988) so that appropriate stomatal responses could occur passively (i.e. hydraulically) without any active (i.e. metabolic) involvement. On the other hand, a metabolic response would better integrate responses to humidity with the metabolic responses to other environmental stimuli. Each would act through regulation of guard cell ion transport (Zeiger, 1983; Hsiao, 1976). In fact, both passive and metabolic phases can be distinguished during the stomatal response to humidity.

The first stomatal response to a change in humidity is rapid, passive, and in the opposite direction (whether opening or closing) from the subsequent stomatal movement toward the new steady state. With moderate decreases in humidity these passive responses are in the opening direction and are transient (e.g. Hall & Hoffmann, 1976), giving way to metabolic responses in the closing direction. These passive responses are caused by reduction in back pressure exerted by surrounding cells on the cells of the stomatal complex. It is not known whether guard cell water content and turgor also decline under such conditions, though this seems likely. If these surrounding epidermal cells lose turgor, the situation reverses. Stomata close passively with further desiccation as guard cell water content and turgor decline in the absence of substantial back pressure (Fig. 5, solid circles). This level of epidermal water deficit is not generally associated with reversible stomatal responses to humidity, but may explain reductions in aperture in isolated epidermis subjected to dry air (e.g. Lange *et al.*, 1971).

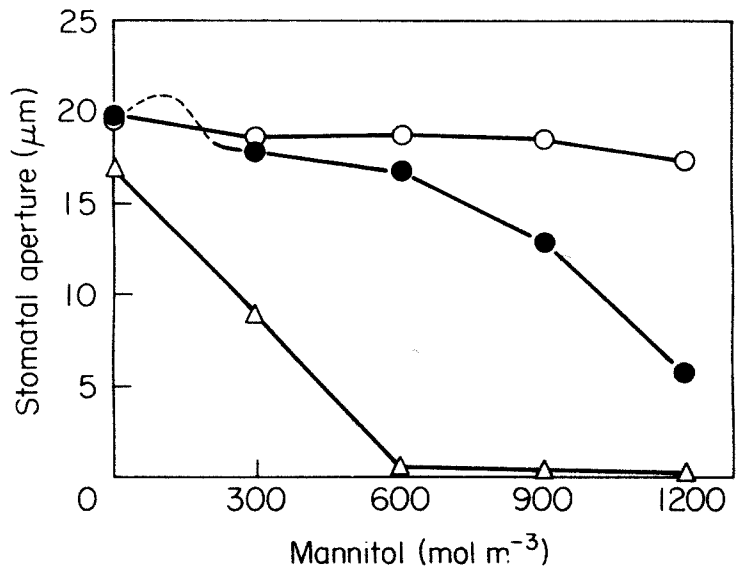

Figure 5. Apertures of initially open stomata in isolated epidermis (○) and in leaf disks (△) of *Commelina* following 3 h incubation in a range of mannitol concentrations. Apertures were measured in the mannitol concentrations in which peels were incubated (●) or following transfer of all peels to the same osmotic environment (△ and ○). After Grantz & Schwartz (1988).

Passive, transient stomatal responses are associated with a variety of manipulations of epidermal water status. They are observed following changes in humidity surrounding leaves (Grantz & Zeiger, 1986; Hall & Hoffmann, 1976; Raschke & Kuhl, 1969; Sheriff, 1979; Laffray *et al.*, 1984), changes in water potential of the medium surrounding roots (Hoglund & Klockare, 1987), leaf excision in air (Raschke, 1970) or under water (Myers, Kuppers & Neales, 1987), and following changes in the medium bathing isolated epidermis (Grantz & Schwartz, unpublished). Changes in steady state epidermal turgor may cause persistent, passive changes in steady state stomatal apertures in leaves (Jarvis, 1980; Koch & Maier-Maercker, 1986) and in isolated epidermis (Fig. 5, solid circles). The passive opening responses at moderate levels of epidermal water deficit (unpublished observations indicated by broken line in Fig. 5) give way to passive closing responses as epidermal cells lose turgor.

These passive responses have no counterpart in metabolic responses to stimuli such as light (Grantz & Zeiger, 1986; Assmann & Grantz, 1990a,b). They are completely hydraulic. They indicate that either epidermal or subsidiary cells, that exert back pressure on guard cells, exchange water more rapidly than guard cells themselves. More rapid vapour loss from guard cells than from surrounding cells would accelerate stomatal movement toward steady state conductance, rather than causing transient changes in the opposite direction, as observed. Loss of turgor from subsidiary cells, but not from guard cells, has been observed visually during transient opening responses (Raschke, 1970; Maier-Maercker, 1979a,b). This is in apparent contrast with morphological evidence of thinly cuticularized areas of guard cell wall in several species (Appleby & Davies, 1983a,b; Jarvis, 1980).

Transient movements are not necessarily dominated by external cuticular vapour loss. In *Commelina* (Maier-Maercker, 1981), hydropassive responses induced with osmoticum added to roots were only observed when stomata were greater than ca. 20% open. In *Tradescantia,* epidermal turgor was independent of V when stomata were closed in the dark (Frensch & Schulze, 1988). In sugarcane, the effect of V on g was greatly reduced when baseline g was reduced in low light (Grantz *et al.*, 1987). All of these experiments, performed under non-feedforward conditions, suggest that vapour loss from mesophyll or internal epidermal evaporation sites dominated epidermal turgor and thus passive stomatal responses. Nevertheless, an important role for external cuticular transpiration remains.

Transpiration from surfaces inside the stomatal pore (Sheriff & Meidner, 1975) cannot mediate feedforward responses in which $\Delta E/\Delta V < 0$. Potential interior evaporation surfaces are cuticularized in some species (Boyer, 1985; Nonami & Schulze, 1989). In isolated epidermis of *Nicotiana glauca* and *Tradescantia virginiana*, the effect of humidity on stomatal apertures was reduced but not abolished by preventing evaporation from the inner surface (Sheriff, 1977b), suggesting a substantial role for external cuticular transpiration. In high light, condensation rather than evaporation has been observed on the inner epidermal surfaces (Sheriff, 1977a). Whether or not cuticular transpiration controls epidermal turgor, it could play a mechanistic role in the humidity response by delivering and distributing signal metabolites within the epidermis, as discussed below. The mechanistic relationship between the transient stomatal responses and induction of guard cell metabolic responses remains largely untested.

Humidity signal transduction

Humidity-induced changes in steady state stomatal apertures apparently involve guard cell ion fluxes, though measured changes in guard cell K^+ content exhibit a delay relative to changes in stomatal aperture. This lag is longer during humidity-induced responses than during light-induced responses (Laffray *et al.*, 1984; Losch & Schenk, 1978) and is longer in isolated epidermis than in intact leaves. The similarity between the kinetics of stomatal response to light and to humidity, in both sugarcane and soybean despite five-fold faster response times in sugarcane, is consistent with a common metabolic basis involving guard cell ion transport in both cases (Grantz & Zeiger, 1986). Overshoots in soybean conductance following changes in humidity were similar to overshoots observed during light responses (Grantz & Zeiger, 1986). These were much too slow to be hydraulically-mediated. Humidity-induced stomatal closure while epidermal turgor remained positive (Shackell & Brinckmann, 1985) indicates a metabolic response, since passive closure requires more severe epidermal desiccation (Sharpe *et al.*, 1987) as observed in osmotically stressed isolated epidermis (Fig. 5, solid circles).

The metabolic response to humidity could be triggered by a direct guard cell response to epidermal water status (Edwards & Meidner, 1978; Schulze, 1986). Membrane potential in protoplasts of epidermal cells of *Commelina* responded to external osmoticum (Pantoja & Willmer, 1986), and stretch activated ion channels have been tentatively identified in patches of guard cell plasma membrane (Schroeder & Hedrich, 1989). These data suggest a mechanism for transduction of humidity-induced changes in epidermal turgor into guard cell ion fluxes. Humidity-induced stomatal closure in *Tradescantia virginiana* was preceded by just such a decrease in epidermal turgor (Shackell & Brinckmann, 1985). However, direct tests do not support this hypothesis.

Stomata in isolated epidermis of *Commelina communis* opened passively in dilute solutions and

closed passively and progressively in increasingly concentrated solutions of mannitol (Fig. 5, solid circles). Apertures from all mannitol treatments were similar when transferred to a common osmoticum for measurement following incubation (Fig. 5, open circles). There was no indication of either guard cell ion loss, that could mediate humidity-induced stomatal closure, nor ion uptake, that would indicate guard cell turgor maintenance. It is possible that the relevant range for membrane stretch-receptors lay between 0 and 300 mol m^{-3} mannitol and was overlooked in these experiments. Stomata in intact leaves, subjected to the same treatments, closed progressively up to the turgor loss point (600 mol m^{-3} mannitol; Fig. 5, open triangles) at which complete closure was observed.

In a series of elegant gas exchange/pressure probe experiments (Shackell & Brinckmann, 1985) small transient responses were observed beginning immediately with the onset of the humidity change, coincident with changes in epidermal turgor. In contrast, metabolic movements only became apparent when the change in humidity was complete, ca. 18 min later. Epidermal turgor was proportional and in phase with changes in V throughout the experiments, but at no time was stomatal conductance proportional to epidermal turgor, suggesting a less direct coupling between V and g.

Wilted leaves of *Atriplex hastata* exhibited the same stomatal sensitivity to V as turgid leaves (Sheriff & Kaye, 1977). While information on epidermal water status during these experiments is lacking, a direct stomatal response to epidermal turgor is not suggested. Nor do these data support the hydraulic balance mechanism proposed by Maier-Maercker (1983), in which guard cell water status is both sensor and transducer. Stomatal responses to humidity do not seem to be mediated by direct guard cell responses to epidermal water status. Alternative hypotheses are required.

Metabolites in the transpiration stream

Stomatal responses to plant and soil water deficits are mediated indirectly, by signal metabolites such as abscisic acid (ABA) imported from outside of the epidermis (Cowan *et al.*, 1982; Radin & Hendrix, 1988; Zhang & Davies, 1989; for a recent review see Cornish & Radin, 1990). Considerable ABA is present in the transpiration stream of unstressed plants (Loveys, 1984; Cornish & Zeevaart, 1985a,b; Hartung, Radin & Hendrix, 1988; Singh *et al.*, 1979; Loveys, 1977; Zeevaart, 1971; Table 2). Signal metabolites, including ABA, other inhibitors (Ogunkamni, Wellburn & Mansfield, 1974; Munns & King, 1988) or Ca^{++}, or promoters such as cytokinins (Itai & Vaadia, 1965; Blackman & Davies, 1985) or K^{+}, could mediate stomatal responses to humidity.

Passive stomatal responses to humidity may be experimentally isolated from ensuing metabolic

Table 2. Analysis of ABA transport to the epidermis in relation to the stomatal response to humidity in *Commelina* and sugarcane

Commelina communis		
Xylem [ABA][a]	9.1 μmol m^{-3}	
$t_{1/2}$ closure[b]	20 min	
	Time to deliver 3 amol gc^{-1}[c]	
	VPD = 1.0 kPa	VPD = 3.2 kPa
Cuticular Pathway[d]	6.5 h	2.6 h
Stomatal Pathway	20.2 min	15.2 min
Saccharum spp.		
Xylem (ABA)[a]	100 μmol m^{-3}	
$t_{1/2}$ closure[b]	2.5 min	
	Time to deliver 3 amol gc^{-1}[c]	
	VPD = 1.0 kPa	VPD = 3.2 kPa
Cuticular Pathway[d]	1.9 h	1.5 h
Stomatal Pathway	4.8 min	3.7 min

[a]Xylem exudate was obtained, using root pressure under well-watered conditions, from single representative plants, and analyzed by ELISA.

[b]Half times of stomatal response were taken from Grantz & Zeiger (1986) and Grantz & Perry (unpublished).

[c]Estimate of 3 amol gc^{-1} to initiate stomatal closure obtained from Weyers & Hillman (1979) and stomatal densities of ca. 120 and 400 gc mm^{-2} for *Commelina* and sugarcane, respectively.

[d]Representative cuticular and stomatal conductances were obtained as in Fig. 3.

responses by treatment of leaves with norflurazone, which inhibits ABA synthesis (Hoglund & Klockare, 1987), or by removal of the epidermis from the mesophyll (Grantz & Schwartz, 1988), which prevents transfer of such metabolites to the epidermis (Fig. 5). In both cases, rapid, passive responses are observed in all treatments, while metabolic responses are observed only when the epidermis remains in contact with ABA producing mesophyll tissue.

In the experiments of Fig. 5, neither synthesis (Cornish & Zeevaart, 1985a, 1986; Dorffling *et al.*, 1980; Loveys, 1977) nor redistribution (Behl & Hartung, 1986; Heilmann, Hartung & Gimmler, 1980) of ABA within the epidermis was sufficient to induce metabolic responses, in spite of severe epidermal water deficit. Nor did stress-induced release of ABA from guard cells or epidermal cells (Cornish & Zeevaart, 1986; Behl & Hartung, 1986; Hartung, Kaiser & Burschka, 1983; Lahr & Raschke, 1988) induce metabolic responses, even though this mechanism would deliver ABA directly to the active site at the guard cell exterior (Hartung, 1983). If signal metabolites such as ABA are involved in the stomatal response to humidity, then they are likely to be imported from outside the epidermis, and their steady state concentration in the guard cell apoplast must depend on humidity.

The quantity of ABA in the guard cell environment depends on the site of cuticular transpiration, the rate of catabolism by guard-cells (Grantz *et al.*, 1985) and other epidermal cells (Singh *et al.*, 1979) and on the ABA concentration in the cuticular portion of the

transpiration stream. It can be shown (Table 2) that cuticular transpiration alone cannot deliver adequate ABA, at stem xylem concentrations, to mediate the humidity response. However, bulk leaf transpiration can do so. ABA is delivered to the mesophyll evaporation sites (Table 2) at just about the rate required to account for observed rates of humidity-induced closure in both sugarcane with rapid responses and *Commelina* with slower responses. This delivery rate is assumed to be ca. 3 amol per guard cell, delivered within the half response time (3 amol $[gc^{-1}]$ $[t_{1/2}]^{-1}$). Evaporation of the apoplastic solution in the mesophyll would enrich the cuticular transpiration stream in ABA and other constituents, allowing delivery of substantially more ABA by cuticular transpiration than would be predicted from xylem concentrations. Very small amounts of ABA carried in the transpiration stream are known to mediate stomatal response, even when much larger amounts are present in the leaf tissue (Raschke, 1975b).

Cuticular transpiration (E_c) of soybean increases with V (Fig. 3D) even when stomatal transpiration declines (Fig. 3C). Changes in E_c alone could achieve humidity-dependent changes in the guard cell apoplastic environment. Changes in humidity could redistribute cuticular transpiration between regions of cuticle that differ in the humidity-sensitivity of g_c. ABA deposition could take place closer to the guard cells in low humidity. Measurements of cellular turgor and cellular and apoplastic levels of ABA in epidermal and mesophyll tissues, under contrasting levels of V chosen so that $\Delta E/\Delta V < 0$, would allow a convincing test of this hypothesis. Recent development of the required microanalytical, pressure probe, and gas exchange techniques has made such measurements feasible.

Concluding observations

Plants respond in a variety of ways to atmospheric humidity, but there is little evidence for a specific humidity sensor. In many cases, water deficits in the air or in the soil have similar effects, apparently mediated through tissue water status. Other responses to both soil and atmospheric water status may represent feedforward responses with respect to tissue water status. Both may be mediated by chemical communication within the plant, involving signal metabolites such as ABA carried in the transpiration stream. This integration of response mechanisms could lead to close coupling of A and g, and still allow considerable scope for direct responses by guard cells to environmental parameters, without invoking a specific humidity sensor.

Few of the studies involving putative stomatal responses to humidity have involved species that exhibit feedforward responses. This is because such species do not have large epidermal cells or fail to yield readily detachable epidermis. In the absence of feedforward responses, in which $\Delta E/\Delta V < 0$, a direct stomatal response to humidity is assumed, not established. Under these conditions it is difficult to distinguish mechanisms associated with stomatal transpiration from those associated with cuticular transpiration. Application of modern pressure probe, gas exchange, and phytohormone microanalytical techniques, in appropriate material over appropriate ranges of humidity in which feedforward responses are observed, seems to offer great potential for characterizing direct and indirect response mechanisms, and for linking specific humidity parameters with specific biological responses. Application of similar reasoning and techniques to putative physiological responses to humidity other than stomatal responses could be equally rewarding.

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