Control of transpiration by radiation

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The terrestrial hydrological cycle is strongly influenced by transpiration—water loss through the stomatal pores of leaves. In this report we present studies showing that the energy content of radiation absorbed by the leaf influences stomatal control of transpiration. This observation is at odds with current concepts of how stomata sense and control transpiration, and we suggest an alternative model. Specifically, we argue that the steady-state water potential of the epidermis in the intact leaf is controlled by the difference between the radiation-controlled rate of water vapor production in the leaf interior and the rate of transpiration. Any difference between these two potentially large fluxes is made up by evaporation from (or condensation on) the epidermis, causing its water potential to pivot around this balance point. Previous work established that stomata in isolated epidermal strips respond by opening with increasing (and closing with decreasing) water potential. Thus, stomatal conductance and transpiration rate should increase when there is condensation on (and decrease when there is evaporation from) the epidermis, thus tending to maintain homeostasis of epidermal water potential. We use a model to show that such a mechanism would have control properties similar to those observed with leaves. This hypothesis provides a plausible explanation for the regulation of leaf and canopy transpiration by the radiation load and provides a unique framework for studies of the regulation of stomatal conductance by CO_2 and other factors.

plant physiology | stomata | micrometeorology

Transpiration, evaporation from plant leaves, plays a key role in the energy and water balance of the land surface; it is a key process in the hydrologic cycle, and because photosynthetic uptake of CO_2 and transpiration are both controlled by stomata, it is strongly linked to plant productivity (1). Models that predict transpiration have important applications in many areas including weather forecasting, understanding climate change, hydrology, ecosystem function, and agricultural production.

Although there is a rich history of studies of transpiration, a full description of the mechanisms that control this process still eludes us. This lack is related to the fact that the control of this process is distributed over a large range of scales from atmospheric turbulence to the regulation of ion transporters in the membranes of cells forming the stomatal pore (2) and it falls into the purview of two separate disciplines. Meteorologists have approached the problem from the top down, emphasizing the energy required to support evaporation of water and the physics of water vapor and heat transport between leaf surfaces and the bulk atmosphere. Stomatal conductance is often used as a fixed boundary condition in such models (3), yet we know that plants are constantly adjusting their conductance according to an internal program as the environmental conditions change. Plant physiologists have focused on sensory systems and turgor-dependent movements that control the aperture of stomata and influence the conductance to diffusion of water vapor across the epidermis (4). They are generally more interested in quantifying the impact of conductance on photosynthesis and water use efficiency at the leaf scale than in understanding how conductance relates to the physics of the environment. This gap in our knowledge limits our ability to model the interplay of physics and physiology in the control of transpiration and the surface energy budget (5). Whereas some models exist that can predict conductance, these models are largely empirical

or theoretical (6–8). There is currently no quantitative, mechanistic basis for predicting this key parameter. We report here on some observations that have stimulated a reexamination of some ideas that have held sway in this field for years. The key difference is that the exchange of energy and water vapor by a leaf with its environment has been considered as a bulk leaf property. We now consider how these exchanges at the outer surface of the epidermis are linked to processes inside the leaf, and we propose a role for the energy and water balance of the epidermis in controlling the rate of transpiration.

In this paper we distinguish between transpiration, E, which is diffusion of water vapor through stomatal pores to the atmosphere, and evaporation, ξ, which we use here to represent the phase change from liquid water to vapor within a leaf. During steady-state transpiration water that evaporates at one place in the leaf may condense at another (a negative ξ); thus gross evaporation can exceed transpiration. Furthermore, the physical controls on transpiration and evaporation are quite different. Transpiration at the leaf scale into a well stirred gas exchange cuvettete can be viewed as "imposed" (3) by the vapor pressure difference (VPD), which is the difference in mole fraction water vapor between that of the air in the cuvettete and that inside of the pore—assumed to be saturated at the leaf temperature. On the other hand, evaporation inside the leaf occurs into air that is essentially saturated with water vapor. There can be no imposed evaporation in such a system. Nevertheless, evaporation can occur if there is an input of energy increasing the temperature and thereby the latent and sensible heat content of the air (9). This so-called "equilibrium" evaporation is one of the limits of the Penman–Monteith evaporation theory (10). Unlike imposed evaporation (3) where water vapor is *pulled* from a wet surface by a diffusion gradient, in equilibrium evaporation, water vapor is *pushed* into the surrounding air by the input of heat. No system undergoing steady-state transpiration can be fully at equilibrium, but it is an adequate approximation for evaporation inside a leaf. The rate of equilibrium evaporation is given by

$$\xi = \frac{\Delta}{\Delta + \gamma} \times \frac{Q}{\lambda}, \qquad [1]$$

where λ is the latent heat of evaporation of water, Δ is the derivative of the saturation vapor pressure of water with respect to temperature, γ is the psychometric constant, and Q is the available energy. We define a term, $m = \Delta/(\Delta + \gamma)$ to represent the fraction of Q appearing as latent heat; $m = 0.74$ at 25 °C and 0.82 at 35 °C. In meteorological applications, $Q = $ Rn (net radiation) $- G$ (soil heat storage). In the leaf system, heat storage is insignificant, and Q can come from two sources: (*a*) heat

produced by shortwave radiation absorbed by photosynthetic pigments and water (Q_{abs}), and (*b*) heat conducted across the epidermis, (Q_e) which can occur in either direction and can be coupled to exchange of sensible heat and longwave radiation on the outer surface of the epidermis. We use $\xi_Q = mQ_{abs}/\lambda$ and $\xi_e = mQ_e/\lambda$ to denote evaporation driven by absorbed radiation and conduction of heat across the epidermis, respectively. We note that $E = \xi_Q + \xi_e$ and that E is dependent on stomatal conductance whereas ξ_Q is independent of stomatal conductance. We might expect, therefore, that stomatal conductance would have a strong influence on ξ_e—and vice versa. The experiments reported here were designed to investigate this possibility.

Results

Response to Radiation Load. A sunflower leaf was illuminated by an incandescent source with either a "cold mirror" or a fully reflective, front-surface mirror. Changing the mirrors had no effect on the incident photosynthetic photon flux density (PPFD) but the amount of near infrared radiation (NIR) was significantly higher when the front-surface mirror was in place (Fig. 1*A*).

Increasing the radiation load on the leaf resulted in an increase in the dissipation of sensible heat (*H*) and latent heat (*λE*) (Fig. 1*B*). In this experiment air temperature was adjusted to maintain a constant leaf temperature and VPD (Fig. 1 *E* and *F*). Therefore, the increase in *λE* was due to an increase in stomatal conductance (Fig. 1*C*), which also resulted in a corresponding stimulation of photosynthesis (Fig. 1*D*). Fig. 2 shows response curves of photosynthesis to CO_2 concentration with the same leaf measured with alternating the mirrors. These measurements fall on the same curve when plotted on the basis of the calculated intercellular CO_2 concentrations (Fig. 2*A*), but the + and − IR points are on distinctly different curves when plotted on the basis of the ambient CO_2 concentration (Fig. 2*B*). These results support the interpretation that stomatal conductance is influenced by the radiation treatment. This effect was highly reproducible and was not dependent on manipulation of the air temperature to keep leaf temperature constant. Experiments done at the CO_2 compensation point, Γ, gave similar changes in conductance and transpiration (intercellular CO_2 would not vary with light intensity at Γ), indicating that this response is not based on feedback involving intercellular CO_2 concentration. A regression
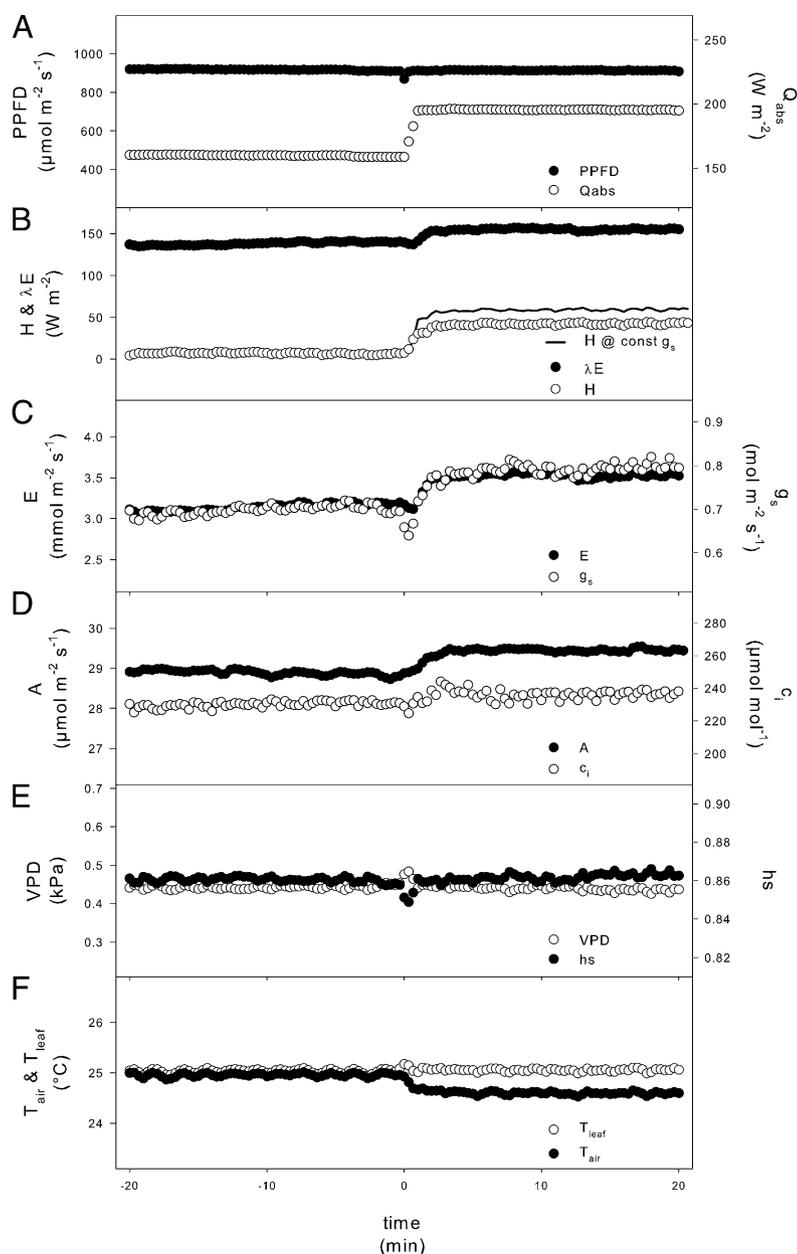

Fig. 1. Dependence of the rate of transpiration and stomatal conductance on radiation load. Time course of (*A*) absorbed photosynthetic photon flux density (PPFD$_{abs}$, solid symbols) and the corresponding absorbed energy content of the radiation (Q_{abs}, open symbols) when a "cold mirror" was replaced with a full spectrum mirror; (*B*) latent (*λE*, solid symbols) and sensible (*H*, open symbols) heat flux, and the sensible heat flux that would be expected if stomatal conductance did not change (solid line); (*C*) the rate of transpiration (*E*, solid symbols) and stomatal conductance (g_s, open symbols); (*D*) assimilation rate (*A*, solid symbol) and leaf internal CO_2 concentration (c_i, open symbol); (*E*) water vapor pressure difference between the leaf and the air (VPD, open symbols) and the humidity on the leaf surface (h_s, solid symbols); and (*F*) leaf temperature (T_{leaf}, open symbols) and air temperature (T_{air}, solid symbols).

analysis of similar measurements conducted over a range of CO_2 concentrations, temperatures, levels of PPFD, and with additional species, *Xanthium strumarium*, *Zea mays*, *Hordeum vulgare*, *Phaseolus vulgaris*, and *Nerium oleander*, indicated that the rate of transpiration in the presence of NIR was 1.09 times that in its absence, with $R^2 = 0.996$ and $n = 128$, and stomatal conductance was 1.17 times larger with $R^2 = 0.993$ (Figs. S1 and S2). We determined from the absorption spectrum of the leaf and the emission spectrum of the light (Fig. S3) that the flux of radiant energy absorbed by leaves increased by 18% with the full spectrum mirror. Most of this difference is associated with broad absorption features of water at 1,450 and 1,950 nm (Fig. S3) so that the additional radiation should be deposited in the tissues of the leaf roughly in proportion to their water content.

We extended these studies to examine how transpiration was affected by the quantity and quality of photosynthetically active light provided to sunflower leaves by light sources of different colors under uniform conditions (VPD = 0.8 kPa, CO_2 = Γ, and PPFD = 250–700 $\mu mol \cdot m^{-2} \cdot s^{-1}$), selected to avoid CO_2-dependent feedbacks (Fig. S2). In all cases the leaf was held under constant conditions until stomatal conductance reached a stable value. The stable stomatal conductance was proportional to PPFD, but different slopes were obtained with each light source (Fig. 3*A*). All of these lines converged on the same slope (with a positive intercept) when transpiration or conductance was plotted against the absorbed energy content (Q_{abs}, W·m^{-2}) (Fig. 3 *B* and *C*). Similar results were obtained with cocklebur and oleander (Fig. S4). The slope and intercept of a regressions analysis of responses of leaves to radiation load (Table 1) differ among species and decrease with increasing CO_2 for each species. The influence of radiation load on transpiration indicates a possible role for ξ_Q in these experiments, but, as noted above, these measurements were conducted under conditions where transpiration was imposed by a large VPD and vigorous stirring.

Response to Helox. Proportionality of transpiration to the radiation load was also observed in experiments with helox (also know as "heliox," air with nitrogen replaced by helium). The rate of transpiration in helox (triangles) was similar to that in air (circles) at each radiation level (Fig. 3*C*). This result confirms previous studies (11) showing that stomata regulate to obtain the same rate of transpiration under otherwise constant conditions in air or helox. Diffusion in helox is 2.3 times that in air so a compensating decrease in stomatal aperture is required to obtain the same rate of transpiration in helox and in air (11). Switching from air to helox has a similar effect to increasing the VPD by
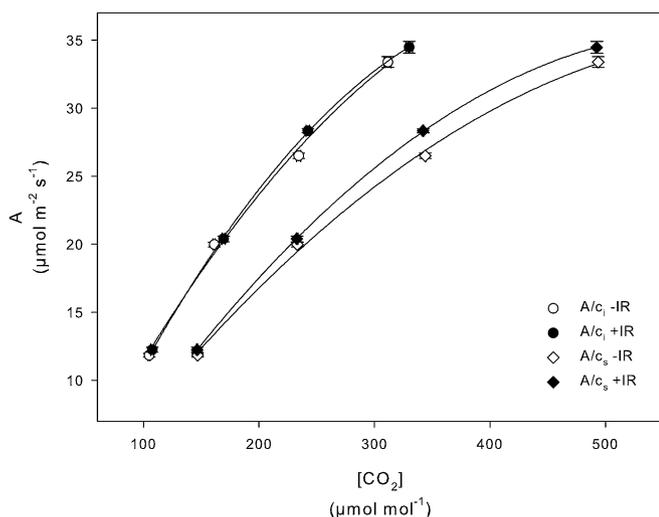

Fig. 2. Dependence of assimilation rate on leaf internal and atmospheric CO_2 concentration. The dependence of assimilation (*A*) is shown on CO_2 concentration ([CO_2]) inside the leaf (circles) and outside the leaf (diamonds), which was measured with the cold mirror (−IR, open symbols) or full surface mirror (+IR, solid symbols).
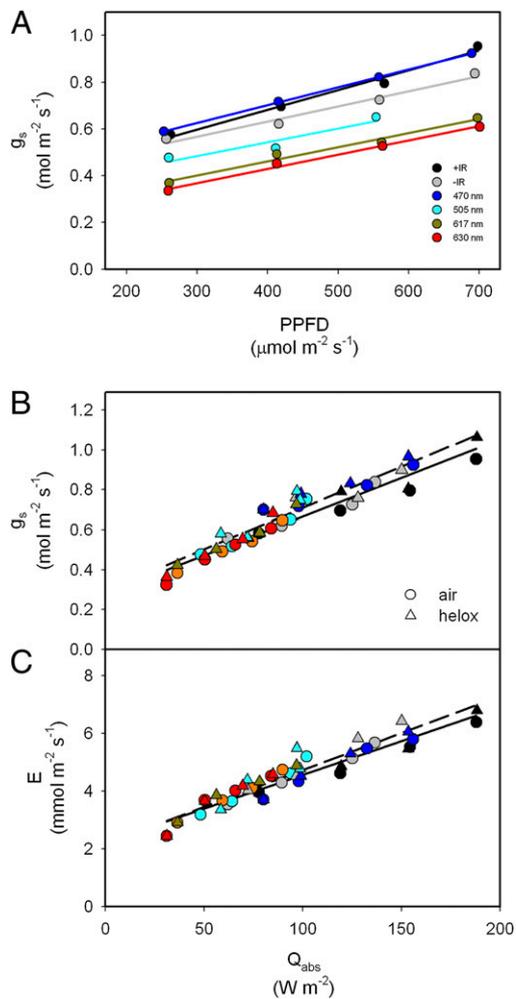

Fig. 3. Dependence of transpiration and stomatal conductance on the absorbed photosynthetic photon flux density (PPFD$_{abs}$) and the absorbed energy content of the radiation (Q_{abs}). (*A*) Stomatal conductance (g_s) as a function of PPFD obtained from different light sources: incandescent light with +IR or −IR treatment, blue, green, orange, and red LEDs. (*B*) g_s obtained in *A* plotted vs. absorbed energy load (Q_{abs}). (*C*) Corresponding transpiration rates (*E*) plotted vs. Q_{abs}.

2.3-fold. Stomatal regulation also tends to keep *E* constant (often with complex kinetics) when the VPD is changed at moderate to high VPD (12) (Fig. S5).

In summary, we observed three phenomena: (*a*) Stomata responded to changes in the radiation load such that transpiration, *E*, changed in proportion to radiation load (W·m^{-2}); (*b*) stomata responded to perturbations in the evaporative demand at constant radiation load to maintain a similar rate of transpiration; and (*c*) changing the CO_2 concentration did not suppress responses to radiation and evaporative demand as in *a* and *b* above but the slope (Table 1) changed.

Discussion

This pattern of regulation by radiation loading is not predicted by current concepts of stomatal control (4). The prevailing view among physiologists has been that stomatal control of evaporation is based on sensory systems in guard cells (13–15). Evaporation is thought to occur mostly from the substomatal cavity near the pore, and regulation of the rate of transpiration is generally thought to be by a hydro-active local feedback (4) that senses gradients in water potential associated with liquid water flow to sites of evaporation near the guard cells. The observation that the regulated rate of transpiration in air or helox is similar

Table 1. Linear regression parameters of the equation, $\lambda E = a \times Q_{abs} + b$, relating the energy content of transpiration, λE (W·m^{-2}) to the absorbed radiation load, Q_{abs} (W·m^{-2}) for three different species under different CO_2 concentrations (Fig. 3C)

c_s (µmol·mol^{-1})	H. annuus	X. strumarium (W·m^{-2})	N. oleander
0	$1.07 \times Q_{abs} + 100$ ($R^2 = 0.91$)	$1.22 \times Q_{abs} + 40$ ($R^2 = 0.91$)	$0.43 \times Q_{abs} + 19$ ($R^2 = 0.94$)
150	$0.93 \times Q_{abs} + 87$ ($R^2 = 0.77$)		
350	$0.81 \times Q_{abs} + 51$ ($R^2 = 0.69$)	$0.63 \times Q_{abs} + 63$ ($R^2 = 0.72$)	$0.24 \times Q_{abs} + 91$ ($R^2 = 0.80$)
500	$0.61 \times Q_{abs} + 46$ ($R^2 = 0.63$)		

Measurements of λE were conducted under varying radiation loads with white light and different CO_2 concentrations (c_s), given at the leaf surface. R^2, regression coefficients.

despite a 2.3-fold higher vapor diffusivity in helox (11) has been considered to be the strongest evidence for this hydro-active mechanism. However, the experiments reported here indicate that the rate of evaporation in helox like that in air seems to be coupled to the radiative load on the leaf. There is no convenient way to incorporate control of transpiration by radiation into this framework, leading us to suggest that the conventional view of evaporation and its control in leaves may need to be modified.

It has long been accepted that the thermal conductivity of the cellular matrix is large and that heat from solar radiation absorbed in the mesophyll by pigments (and to a lesser extent water) is conducted via cell-to-cell contacts to the substomatal cavities where evaporation is thought to occur (15). This must take place to some extent, but consider that latent heat flux from a typical leaf in full sun may exceed 100–200 W·m^{-2}. If this evaporation were localized to the substomatal cavity with perhaps 10% of the leaf area, then the heat flux density in these evaporation zones would be very high. Given the open internal structure of a typical leaf (20–70% of the volume is air), it seems more likely that evaporation occurs near the sites where radiation is absorbed (at chloroplasts) and where sensible heat can be transferred from the air to the leaf (at the inner surface of the epidermis). Photoacoustic studies support the idea that evaporation occurs near the sites where light is absorbed. Pressure waves can be detected by a sensitive microphone when heat generated by light pulses absorbed by chloroplasts is transferred to the air (16)—presumably by equilibrium evaporation. The kinetics of this heat transfer indicate a very short diffusion path from the absorption sites to the air (17). The presence of equilibrium conditions inside the leaf would also imply a substantial capacity for heat transport by coupled evaporation/condensation in the interior of the leaf. The possibility that water vapor plays an important role in heat transport in leaves was suggested by Sheriff (18), who observed condensation of droplets on the inner surface of the epidermis when the outer surface was transiently cooled with an air stream; he calculated that a significant fraction of energy deposited inside the leaf is transported to the epidermis by evaporation/condensation.
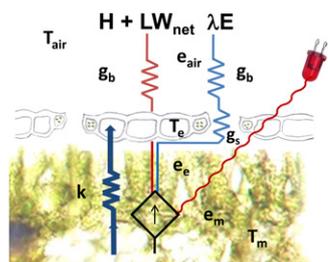
In nature, the leaf temperature reaches a value that satisfies the energy balance relationship that determines how the total energy load on the leaf is partitioned to latent or sensible heat and longwave radiation (LW$_{net}$). These fluxes of energy are transferred to the environment at the outer surface of the epi-
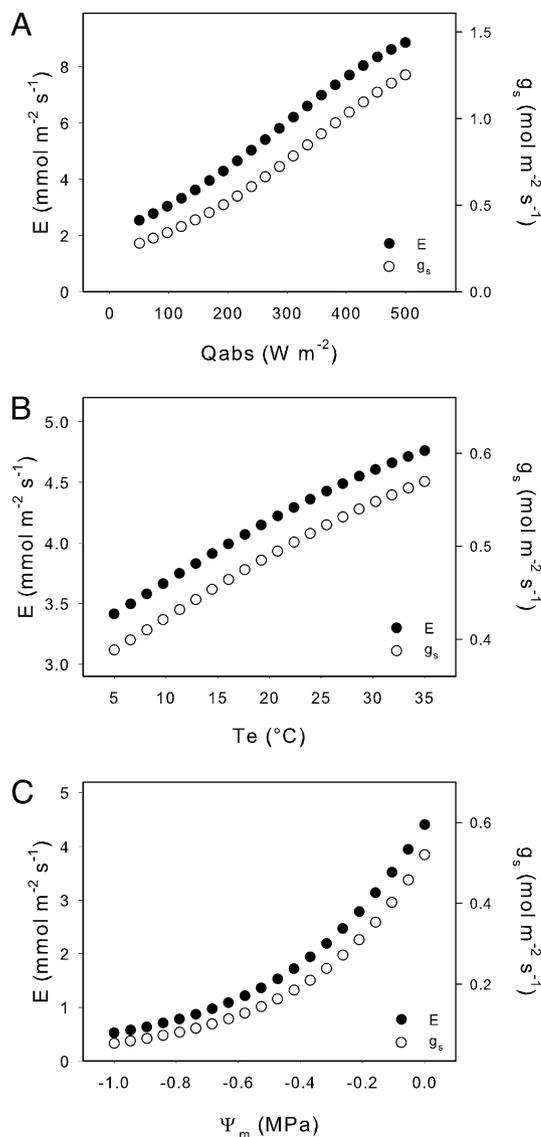
Fig. 4. Schematic representation of the flows of heat and water vapor inside and outside of a leaf. The blue lines represent latent heat fluxes (λE) and the red line sensible heat fluxes (H). The vertical arrow within the diamond is the circuit symbol for a controlled current source (i.e., 73% of absorbed radiation appears as $\lambda \xi_Q$ at 25 °C), LW$_{net}$ is the net thermal radiation, E is transpiration, λ is the latent heat of vaporization of water, T is temperature, e is the partial pressures of water vapor, g is conductance, k is the hydraulic conductance, and the subscripts are as follows: air, air; e, epidermis; m, mesophyll; b, boundary layer; s, stomata. The dark blue line represents liquid flow.

Fig. 5. Dependence of simulated response of transpiration (E) and stomatal conductance (g_s) on driving forces. Dependence is shown on (A) absorbed radiation load (Q_{abs}), (B) leaf temperature (T_e), and (C) water potential of the mesophyll (Ψ_m) under otherwise constant conditions with $\Psi_e = -0.1$ MPa, $Q_{abs} = 200$ W·m^{-2}, $T_e = 25$ °C, vapor pressure difference between the air at the leaf surface and that of intercellular air space (VPDs) 1 kPa, and maximum stomatal conductance of 2.5 mol·m^{-2}·s^{-1}.

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dermis, and, to conserve energy, an equivalent flux must be provided on the inside [i.e., $(\lambda E + H)_{in} = (\lambda E + H + LW_{net})_{out}$] (Fig. 4). This two-sidedness of the epidermis has important implications. On the inside, heat transfer occurs under conditions of equilibrium evaporation/condensation, so the latent heat flux arriving from the mesophyll is fixed (a function of the leaf temperature and the absorbed solar radiation). This may, or may not, balance the flux of water vapor escaping through the stomata. Any imbalance would need to be made up by evaporation from, or condensation on, the inner surface of the epidermis, since $E = \xi_Q + \xi_e$ (Fig. 4).

The water balance of epidermal tissues could provide needed sensory information for stomatal regulation. If $\xi_e \neq 0$, the epidermal water potential should increase or decrease relative to that of the bulk leaf until a flow of water to, or from, the bulk leaf (k, Fig. 4) balances the vapor exchange. Therefore the water potential of the epidermis should hinge, or pivot, on the balance between the flux of water vapor produced by the absorbed radiation and the flux of water vapor that exits the stomata. We suggest that regulation of stomatal conductance could be based on a mechanism that senses and maintains homeostasis of epidermal water potential.

There is already strong evidence for humidity responses associated with the inner surface of the epidermis. Lange and coworkers (19) showed that stomata respond to manipulations of air humidity in contact with the inner surface of a detached epidermis—opening in moist air and closing in dry air. A recent study (20) confirmed Lange's observations and showed that the water potential in the vapor phase is the operative variable. We propose that water potential changes associated with the water balance of the epidermis provide the sensory information needed for feedback regulation of stomatal conductance. It is important to note that we are not suggesting a simple control by vapor phase water potential. Rather the water potential of the cells and the gas phase determines whether they gain or lose water. If there is a significant resistance to liquid flow to or from the rest of the leaf (k, Fig. 4), then evaporation or condensation at the epidermis would cause a steady-state displacement of the water potential.

We do not address here the mechanism of these stomatal responses to epidermal water status. What seems clear from the published work is that when the epidermis gains water, the stomatal pores open, and when it loses water, they close (19, 20). The kinetics associated with stomatal adjustments suggest that these responses are more complex than simple uptake or release of water from guard cells (15). Furthermore, the presence of a cuticle lining the inner surface of the epidermis in the substomatal cavity and stomatal pore in some species (21) makes it difficult to identify what part of the epidermis is in contact with water vapor of the intercellular air spaces. For the present, we assume that it is the water relations of the whole epidermis that elicit feedback on stomatal conductance. A heuristic model based on analogy to a control system in a greenhouse is provided in *SI Text*.

To better explore this idea we constructed a model based on energy balance of the leaf system, taking into account the proposed coupling between internal evaporation and the exchanges

of a leaf with its external environment as illustrated in Fig. 4. The model is defined by three conservation equations:

(*i*) The exchange of energy with the leaf's external environment is represented as

$$Q_{abs} = LW_{net} + c_p \times g_b(T_e - T_{air}) + \lambda \times \frac{g_t(e_s - e_{air})}{P_{air}}. \quad [2]$$

This equation states that the sum of longwave (LW_{net}), sensible (second term), and latent heat (third term) exchanged from the leaf equals its energy gain from absorbed shortwave radiation, Q_{abs}. Locations of temperatures, T, water vapor partial pressures, e, and conductances, g, are identified in the legend to Fig. 4 and $g_t = (1/g_b + 1/g_s)^{-1}$. Additional symbols (Table S1) include the heat capacity of air, c_p, the latent heat of vaporization of water, λ, and atmospheric pressure, P_{air}. The exchange of energy by longwave radiation, LW_{net} is treated separately rather than as a component of net radiation because LW_{net} interacts with the epidermis whereas Q_{abs} is mostly deposited at the chloroplasts.

(*ii*) The exchange of latent heat through the stomata is the sum of internal production of latent heat by equilibrium evaporation, $\lambda \xi_Q$, and the loss or gain of latent heat by the epidermis, $\lambda \xi_e$, which is equated here to the flow of water between the epidermis and the mesophyll via the hydraulic conductance (k) in response to a steady-state water potential gradient, ($\Psi_e - \Psi_m$):

$$Q_{abs} \cdot m = \lambda \times \frac{g_t(e_s - e_{air})}{P_{air}} + \lambda \times k(\Psi_e - \Psi_m). \quad [3]$$

(*iii*) The internal production of sensible heat from the absorbed radiation at equilibrium, $Q_{abs}(1 - m)$, plus the heat exchanged at the epidermis by evaporation/ condensation of water vapor must balance the heat transferred across the epidermis to the air and to net longwave exchange:

$$Q_{abs} \cdot (1 - m) + \lambda \times k(\Psi_e - \Psi_m) = LW_{net} + g_b(T_e - T_{air}). \quad [4]$$

At steady state, these three conservation equations must be satisfied. A schematic diagram (Fig. 4) depicts the flows of water vapor and sensible heat in the leaf system. We solved this system of equations with MATLAB (Dataset S1) for Ψ_e for a range of plausible values of stomatal conductance (Fig. S6) as external inputs of light, temperature, humidity, and soil water availability are varied. From this result a conductance is selected that matches an arbitrarily chosen set point value of Ψ_e. To avoid solutions with infinitely high or negative stomatal conductances, we specified a maximum (g_{max}) or minimum (0) value with a
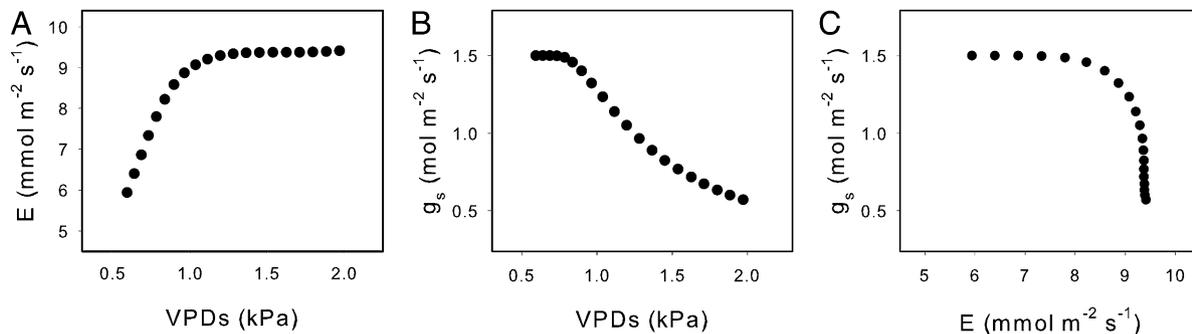
Fig. 6. Simulated response of transpiration (E) and stomatal conductance (g_s) to changes in the surface vapor pressure difference (VPD). (*A*) Dependence of E on VPDs; (*B*) dependence of g_s on VPDs; (*C*) the relation of E vs. g_s under changing VPDs under otherwise constant conditions with $\Psi_e = -0.2$ MPa, $Q_{abs} = 500$ W·m^{-2}, $T_e = 25$ °C, maximum stomatal conductance of 1.5 mol·m^{-2}·s^{-1} (for details on the model see *SI Text*).

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gradual transition of g_s imposed when approaching these values. Simulated responses of transpiration and stomatal conductance to radiation (Fig. 5A), leaf temperature (Fig. 5B), and decreasing leaf water potential, Ψ_m (Fig. 5C), mimic observed responses of leaves, except that the temperature response of transpiration is less than observed in some studies (6, 11). This simulation could be improved by small changes in the model structure, but we chose to keep the model simple, leaving this issue for future research. The simulated response to radiation (Fig. 5A) shows an intercept similar to the observations (Fig. 3C). In the model, the source of vapor for transpiration in darkness is evaporation from the inner surface of the epidermis driven by heat from the air, and it occurred because the Ψ_e "set point" was lower than Ψ_m. The linear response up to very high radiation loads is probably not realistic; other factors such as a fall in Ψ_m at high flux (12) and signaling from the mesophyll (8) may limit this response. Simulated responses of stomatal conductance to VPD are of particular interest. Monteith (12) noted that nearly all measurements of stomatal conductance as a function of VPD fall into three regimes defined by maximum stomatal conductance, maximum transpiration, and the transition between these two phases. These regimes are clearly seen in our simulations. Stomatal conductance reached a maximum level under low VPD and decreased with increasing VPD (Fig. 6B) and transpiration was controlled to a constant value at high VPD (Fig. 6A).

The mechanism as we have described it to this point would have a fixed yield of transpiration for all leaves under the same radiation load. However, this prediction is inconsistent with theoretical arguments and a great deal of data showing elegant "fine tuning" of the exchange of water vapor for CO_2 (Table 1) (22). For example, leaves of *N. oleander* that have stomata only on their lower surface had a lower yield of transpiration ($\lambda E = 0.43 \cdot Q_{abs} + 19$) than sunflower ($\lambda E = 1.07 \cdot Q_{abs} + 100$) or cocklebur ($\lambda E = 1.22 \cdot Q_{abs} + 40$), both with amphi-stomatous leaves. Regions of the epidermis without stomata or between stomata might condense vapor ($-\xi_e$), or more compact tissues might conduct more heat from the mesophyll to the epidermis—both decreasing the amount of vapor left to interact with regulatory portions of the epidermis. Developmental controls over the density and size of stomata (23) and thus maximum conductance (g_{max}) by light and CO_2 levels during growth (24) appear to have a similar effect, although the basis for this effect is less clear than for hypostomatous leaves. Physiological responses to CO_2 seem to maintain the same pattern of response to radiation but change the yield of latent heat (Table 1). Simulations indicate that this might be effected by solute levels in epidermal tissues and/or the conductance to liquid flow, k (Fig. 4).

Conclusions

A hypothesis for stomatal regulation is presented. This hypothesis is based on observations of stomatal responses to humidity in epidermal strips first made by Otto Lange and coworkers (19) in 1971. This observation has lain dormant for many years because it could not be integrated with the then current concepts of how evaporation is controlled in leaves. We argue on the basis of experiments reported here that these concepts are probably wrong, and we present an alternative synthesis linking heat and water transport from its source in the leaf mesophyll across the epidermis to the leaf's surroundings. The water balance of the epidermis emerges as being very sensitive to the difference between the rate of transpiration and the rate of production of water vapor inside the leaf by absorbed radiation. Lange's observations fit nicely into this framework—providing precedence to propose that stomatal regulation senses and controls epidermal water balance. A model of this mechanism reproduced the general patterns of stomatal regulation in plants, and we discuss how this mechanism might be integrated with other factors (CO_2, blue light, g_{max}, sun–shade adaptation, phytohormones, etc.) that also regulate transpiration and photosynthesis.

Methods

Plants were grown at the greenhouse facilities at the Carnegie Institution. Gas exchange of leaves was measured with open gas exchange systems LI-6400 (LI-COR) and MPH-1000 (Campbell Scientific). Two-sided boundary layer conductance was determined to be 2.84 and 3.15 $mol \cdot m^{-2} \cdot s^{-1}$ for these chambers, respectively.

Hemispherical reflectance and transmittance from 400 to 2,500 nm were measured on attached leaves ($n = 5$ per species). The leaf spectra were collected with a custom-designed, full-range spectrometer using 1.4-nm sampling (FR-Pro with Select Test detectors and a custom exit slit; Analytical Spectra Devices), an integrating sphere modified for high-resolution spectroscopic assays (Labsphere), and a custom illumination collimator. Measurements were collected with 200-ms integration time per spectrum. The spectra were then calibrated for dark current and stray light and referenced to a calibration block (Spectralon; Labsphere) within the integrating sphere.

Light intensity within the PAR range was measured with a LI-COR quantum sensor (LI-190) and total radiation load was measured with a three-eighths-inch laboratory thermopile (unknown manufacturer) calibrated against a Li-200 pyranometer using the direct solar beam. The incandescent light source was a tungsten halogen lamp (Sylvania ELC, 24 V, 250 W; the spectrum of the light source is shown in Fig. S3). Light-emitting diodes were LUXEON K2 series diodes (Philips Lumileds Lighting Co.).

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