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Stomatal Responses to Light and Leaf-Air Water Vapor Pressure Difference Show Similar Kinetics in Sugarcane and Soybean[1]

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ABSTRACT

Stomatal responses to light and humidity (vapor pressure difference, VPD) are important determinants of stomatal conductance. Stomatal movements induced by light are the result of a transduction of the light stimulus into modulated ion fluxes in guard cells and concomitant osmotic adjustments and turgor changes. It is generally assumed that this transduction process is a general stomatal property, with different environmental stimuli integrated into guard cell metabolism through their modulation of ion fluxes. In contrast with this notion, the VPD response, which is unique because both its triggering signal and the turgor changes required for aperture modulations involve water molecules, has been considered to be hydropassive and thus independent of guard cell metabolism. We used a kinetic approach to compare the light and VPD responses in order to test the hypothesis that hydropassive changes in guard cell turgor could be faster than the metabolism-dependent light responses. Changes in stomatal conductance in intact leaves of sugarcane and soybean were measured after application of step changes in VPD and in light. In spite of a 5-fold difference in overall rates between the two species, the response rates following light or VPD steps were similar. Although a coincidental kinetic similarity between two mechanistically different responses cannot be ruled out, the data suggest a common mechanism controlling stomatal movements, with the VPD stimulus inducing metabolic modulations of ion fluxes analogous to other stomatal responses.

Stomatal responses to light and humidity are important environmental determinants of stomatal conductance in both intact leaves and isolated stomata (2, 6, 16, 18). The light response, mediated by two different photoreceptor-systems in guard cells (15, 18) requires signal perception, induction of ion transport and subsequent osmotic adjustments and water fluxes leading to turgor-driven changes in stomatal apertures (2, 20, 23). Although sensory transduction in response to signals other than light is less well characterized, it is generally assumed that stomatal conductances in the leaf are the result of an integrated modulation of ion fluxes in guard cells by prevailing environmental stimuli (23).

The stomatal response to humidity (VPD[3]) is unique among stomatal responses because both its triggering signal and the turgor changes resulting in aperture regulation involve water molecules. This unique stimulus-response relationship could underlie a sensory transduction mechanism that bypasses guard cell metabolism. In this hydropassive control of stomatal conductances (7, 11, 19) (also see Ref. 9 and references therein), prevailing levels of air RH would have a direct effect on evaporative demand, water content and turgor relationships of guard cells and surrounding epidermal cells, thus modulating stomatal apertures without the involvement of guard-cell metabolism.

An alternative possibility is that a change in VPD is perceived as a signal, analogous to a light stimulus, which is transduced into a common mechanism controlling ion fluxes and turgor changes in the guard cells. Each alternative has interesting implications for stomatal function. A direct hydropassive response, which does not require an activation of guard cell metabolism, could be faster and rapidly reversible and would primarily interact with other environmental signals in an additive way, thus precluding any metabolic regulation. Metabolic signal transduction would demand the operation of a specific humidity sensor in the epidermis—hitherto unknown in leaves—and, because of its integration into a common metabolic mechanism controlling ion fluxes, could allow a finer regulation of the interaction between the VPD response and stomatal modulation by other environmental stimuli (9).

Available information on the VPD response does not allow a distinction of its underlying mechanism. Stomata in isolated epidermal peels respond to VPD (7, 8) and opposite responses can be induced in adjacent stomata by application of thin air streams differing in humidity (6). Perception of humidity is therefore localized in the epidermis, with no obligate coupling to mesophyll function, but the nature of the sensing remains unknown. VPD-induced stomatal opening in intact leaves of *Vicia faba* (5) and in epidermal peels of *Valerianella locusta* (8) has been shown to involve changes in ionic content of guard cells but there were large temporal discrepancies between the kinetics of aperture and that of ion content changes. These kinetic discrepancies were interpreted (5) as a result of 'follow-up' (9) ion fluxes stabilizing the completed changes in guard cell turgor and volume. On the other hand, the delayed ion fluxes could be related to similar ones recently observed with a metabolically-driven, light stimulation of stomatal opening (4).

In the present study, we used a kinetic approach to compare the light and VPD responses, in order to test the hypothesis that hydropassive changes in epidermal and cell turgor, resulting from localized water losses (11) could be faster than the turgor changes induced by light, requiring the additional steps of signal perception and the build-up of ion gradients. Using gas exchange methodology, the kinetics of stomatal responses to step changes in light or VPD were investigated in the C_4 sugarcane and the C_3

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[3] Abbreviations: A, carbon assimilation rate; g, stomatal conductance, C_i, intercellular carbon dioxide concentration; VPD, leaf-air water vapor pressure difference; $t_{1/2}$, half-response time.

soybean, species with drastically different, overall rates of stomatal adjustment. The results show that, in both cases, the half response times for the light and VPD responses were remarkably similar.

MATERIALS AND METHODS

Plant Material. Cuttings of sugarcane (*Saccharum* spp., cv H-65-7052) were grown in a greenhouse in commercial potting mix for at least 1 year. The tallest shoots were periodically removed so that experiments were performed on leaves of rooted tillers, corresponding to the rattoon crop in some commercial management. Seeds of soybean (*Glycine max* L., cv Clark) were grown in potting mix in the same greenhouse. Both species were irrigated daily and fertilized weekly. Plants were grown under natural sunlight (photosynthetic photon fluence up to 0.750 mmol m^{-2} s^{-1}) extended to 12 h with fluorescent bulbs (Sylvania Gro-Lux). Temperature was maintained at 20 ±5°C and RH at about 50%. Experiments were conducted with the youngest fully expanded terminal leaflet of soybean and leaf 3 or 4 (first visible dewlap) of sugarcane.

Experimental. Gas exchange measurements were made by enclosing an attached soybean leaflet, or a portion of an attached sugarcane leaf (30 cm from the tip; about 25 cm^2 for both species), into a gas exchange cuvette. Leaf temperature was maintained at 25.0 ± 0.1°C, measured with a thermocouple appressed to the underside of the leaf. Transpiration and assimilation were measured in a differential system (21) using an IR gas analyzer and two Vaisala sensors (Weather Measure Corp., Sacramento, CA). Light was provided by a Metalarc lamp (M 1000, Sylvania, GTE, Danvers, MA) filtered through Plexiglas cooled with flowing water, and attenuated as required with metal screens. Photosynthetic photon fluence (400–700 nm) was determined with a silicon photocell mounted in the cuvette and calibrated with the same light source and a Licor quantum probe (Lambda Instrument Co., Lincoln, NE). Leaf VPD was adjusted by varying the ratio of humid to dry air entering the chamber, rather than by adjusting the temperature of the dewpoint condensor or of the leaf. This allowed step changes in the RH of the air entering the cuvette and very rapid changes of leaf VPD.

The kinetics of stomatal responses were analyzed by imposing rapid increases or decreases in light or VPD. Sensors in the gas exchange system were sampled 2 to 3 times per min by microprocessor and both primary and calculated parameters were recorded. Time zero was 0.2 min prior to the first data point acquired following perturbation of light or VPD. Half-response time ($t_{1/2}$) was measured from time zero to the time at which one half of the maximal response was observed. The half-response times determined in this manner were consistent with half-times calculated from log-linear plots of conductance *versus* time, and were more appropriate since the transients were sigmoidal rather than exponential in time.

The response time of the gas exchange system was determined by replacing the leaf in the chamber with an open pan of distilled H_2O. Conductance was measured as with a leaf, with temperature of the evaporating surface determined with a submerged thermocouple. The $t_{1/2}$ for equilibration of chamber humidity, including mixing and sensor response was less than 1 min, with full equilibration in less than 3 min (Fig. 2B; trace g_{pan}). The system response kinetics were the same for step changes of increasing or decreasing humidity. The data have not been deconvoluted or otherwise adjusted mathematically to correct for system response kinetics. Measurements of responses to each class of stimulus (increase or decrease in light or VPD) were repeated several times (Table 1) using different plants. Representative traces are presented.

RESULTS

Sugarcane. Stomatal responses to step changes in light were very rapid in sugarcane (Fig. 1) with half-response times of 1.9 min for opening in response to increased light (Fig. 1A, Table I) and 2.3 min for closing in response to decreasing light (Fig. 1B). No latency period in the conductance response following step changes in light was observed, although the time course began slowly and accelerated in a sigmoidal fashion. Assimilation began to increase immediately after the step change, with the combined responses of assimilation and conductance resulting in only slight changes in intercellular CO_2 (C_i). VPD in the chamber was initially constant, indicating that the stomata were responding only to light. Eventually, the change in conductance was sufficient to cause a small change in chamber humidity and thus in VPD. Half-response times were measured from the time of the step change to the time of the maximum response, even though in both opening and closing directions this represented an overshoot which was followed by partial recovery, before attainment of a new steady-state conductance (Fig. 1, A and B).

Stomatal responses to step changes in VPD were also rapid in sugarcane (Fig. 2, A and B). The step changes in humidity imposed on the gas stream entering the chamber were convoluted by mixing with the gas already present in the chamber. For this reason, the changes in VPD actually imposed on the leaf had some delay. The time constant for mixing within the chamber was shorter than those of the stomatal responses (Fig. 2B, traces g and g_{pan}) but the stomatal responses were sufficiently rapid to impose some uncertainty to the measurements. With soybean,

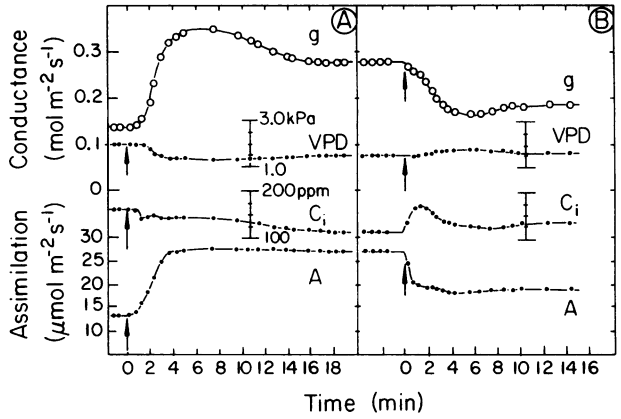

FIG. 1. The response of conductance (g), assimilation (A), VPD, and C_i in sugarcane, to a step change in photon fluence (arrows) from 0.275 to 0.620 mmol m^{-2} s^{-1} (A) or from 0.620 to 0.390 mmol m^{-2} s^{-1} (B). Half-response times ($t_{1/2}$) of conductance were (A) 2.2 min and (B) 2.1 min.

Table I. *Kinetics of Stomatal Responses to Step Changes in Light and VPD in Sugarcane and Soybean*

	Half-Response Time	
	Opening	Closing
	min	
Sugarcane VPD	3.7 ± 2.2[a]	2.5 ± 1.2
	$n = 11$	$n = 13$
Sugarcane light	1.9 ± 0.5	2.3 ± 0.8
	$n = 5$	$n = 6$
Soybean VPD	18.4 ± 4.2	9.3 ± 1.6
	$n = 3$	$n = 3$
Soybean light	13.2 ± 2.1	9.3 ± 3.3
	$n = 4$	$n = 4$

[a] Mean ± SE.

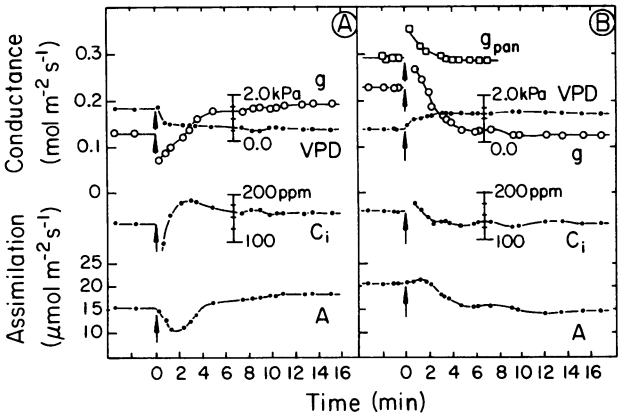

FIG. 2. The response of conductance (g), assimilation (A), and C_i in sugarcane, to a step decrease (A) or increase (B) in VPD (arrows). The kinetics and magnitude of the VPD step are indicated (VPD). Half-response times ($t_{1/2}$) of conductance were (A) 2.4 and (B) 1.9 min. Photon fluence was 0.600 mmol m^{-2} s^{-1}. The response kinetics of the gas exchange system (Fig. 2B, trace g_{pan}; relative units) to a step change in VPD were obtained by replacing the leaf with an open pan of water.

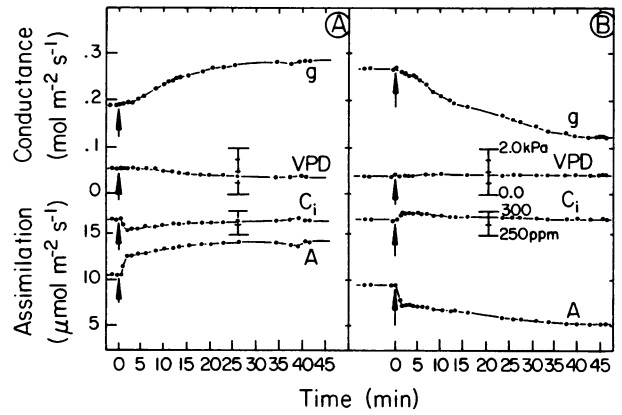

FIG. 3. The response of conductance (g), assimilation (A), VPD, and C_i in soybean to a step change in photon fluence (arrows) from 0.350 to 0.500 mmol m^{-2} s^{-1} (A) or from 0.500 to 0.350 mmol m^{-2} s^{-1} (B). Half-response times ($t_{1/2}$) of conductance were (A) 11.0 min and (B) 12.0 min.

on the other hand, mixing was many times faster than the actual stomatal responses (Figs. 3 and 4), eliminating any ambiguity in the conductance measurements.

Contrary to the prediction ensuing from a hydropassive mechanism, VPD-induced closure in sugarcane was not faster than light-induced closure, with the $t_{1/2}$ for the two processes being very similar (Table I; *cf.* Figs. 1B and 2B). Furthermore, VPD-induced opening was somewhat slower than the homologous light response (Table I; Figs. 1A and 2A). Thus, within the precision of these kinetic data, sugarcane responses to light and VPD seemed to be characterized by similar time constraints.

Soybean. The kinetics of stomatal responses in soybean were about five-fold slower than those in sugarcane (Table I), with half-response times of 13.2 min for opening and 9.3 min for closing in response to light steps (compare Fig. 3 with Fig. 1, note the compressed time scale in Fig. 3). No latency period was observed with either g or A, although initially A responded more rapidly than g, causing transient changes in C_i (Fig. 3). Unlike sugarcane, there was little overshoot in the stomatal response to light in soybean (*cf.* Figs. 1 and 3) so that the measured $t_{1/2}$ represented the half-response time of the final, attained steady state conductance.

Stomatal responses to step changes in VPD (Fig. 4, A and B,

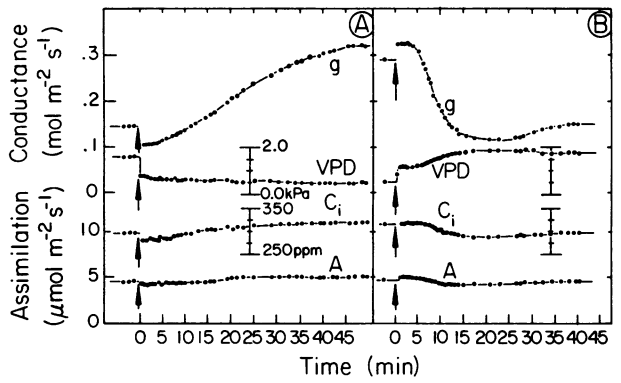

FIG. 4. The response of conductance (g), assimilation (A), and C_i in soybean to a step decrease (A) or increase (B) in VPD (arrows). The kinetics and magnitude of the VPD step are indicated (VPD). Half-response times ($t_{1/2}$) of conductance were (A) 19.0 min and (B) 7.5 min. Photon fluence was 0.140 mmol m^{-2} s^{-1}.

note compressed time axis) were also slower in soybean than in sugarcane (Table 1; Figs. 2 and 4). As in sugarcane, VPD-induced closure in soybean was not faster than light-induced closure (Table 1; Figs. 3B and 4B) while the VPD-induced opening was noticeably slower than the homologous light response. Thus, despite the vastly different overall rates of conductance in the two species and their contrasting carbon metabolism, both sets of data pointed to very similar time constants for the stomatal responses to light and VPD.

DISCUSSION

Rates of Stomatal Responses in Sugarcane and Soybean. Stomatal responses to step changes in both light and VPD were nearly five-fold faster in sugarcane than in soybean. These contrasting rates probably underlie both mechanical and physiological differences. Grasses have been shown to have a rapid stomatal response to blue light, a feature not found in dicots (3) despite their overall capacity for a blue light response (25). Stomatal characteristics of grasses include a shuttle of K^+ and Cl^- between guard cells and subsidiary cells (14) and a "dumbbell" guard-cell shape (12); both features might accelerate the stomatal responses in these species as compared with non-grasses requiring ion accumulation from the apoplast and a somewhat less efficient volume regulation by kidney-shaped guard cells. In addition, soybean and sugarcane also differ in their C_3 *versus* C_4 metabolism, although the precise relationship between stomatal kinetics and the two pathways of carbon fixation remains to be established.

Comparative Kinetics of the Light and VPD Responses. In both species, the $t_{1/2}$ of opening and closing in response to light were very similar. Previous studies have shown interspecific differences in relative rates of light-dependent opening and closing (1, 13, 22), with a trend among shade-tolerant species to open faster than they close (13). Sugarcane and soybean, both sun species, exhibited symmetric opening and closing. In recent studies of the blue light response of stomata, it has been shown that opening in response to blue light results from the establishment of an electrochemical gradient with its magnitude dependent on incident fluence rates (2, 20, 25). In addition, steady-state apertures and rates of closing also depend on ion permeability and efflux rates (10). Interspecific differences in these processes probably result in distinct kinetics of opening and closing and might also account for the variable occurrence of overshoots in conductance, following light steps, which were observed with sugarcane but not with soybean (*cf.* Figs. 1 and 2). Similar, light-dependent overshoots have been observed with *Xanthium* (24),

but in contrast with their predominant association with the opening response in sugarcane, those in *Xanthium* were characteristic of the closing response.

The kinetics of the VPD response differed from that of the light response because of the slower opening rates. VPD-dependent opening was clearly slower than closing in soybean and marginally but consistently slower in sugarcane (Table I). A larger $t_{1/2}$ for opening than for closing in response to VPD has been reported in intact leaves (17) as well as in epidermal peels (6, 7). The longer time required for opening suggests the involvement of an additional step, or of a step that is rate-limiting for opening but not for closing. Since the kinetics of VPD-induced opening was not altered by leaf excision under water (D Grantz, unpublished data), transport of water into the leaf is unlikely to be the rate-limiting process. A rate-limiting step specific for VPD-induced opening could involve sensory transduction of the VPD signal.

In contrast with the light response, overshoots in conductance following VPD-induced closure were observed with soybean (Fig. 4). The time courses of these overshoots were slow and are thus unlikely to represent hydraulic equilibration within the epidermis. Further studies are needed to establish whether the kinetics of water fluxes have slow time courses or if these overshoots reflect metabolic processes occurring during or following the VPD-induced movements.

In spite of the five-fold difference in response rates, the overall pattern of the two responses were remarkably similar in the two species (Table I). Since comparable amounts of water must be taken up by the guard cells during equivalent responses to light or VPD, the differences in rates at which the driving force for these water fluxes is established could be diagnostic for the operation of either a metabolic or a hydropassive mechanism. The established features of sensory transduction for the light response (2, 20) clearly indicates a requirement for the generation of an electrochemical gradient across the guard cell membrane and the transport of ions leading to the osmotic changes driving the hydraulic adjustment associated with stomatal movements. In contrast, the VPD-induced response could generate a water gradient simultaneously with the application of the stimulus; thus, in theory, a hydropasssive mechanism for the VPD response could result in faster rates of stomatal adjustments than the metabolically dependent light responses. Although the data reported here cannot rule out a coincidental kinetic resemblance of the observed rates, the close similarity between the kinetics of the light and VPD responses are suggestive of a common regulatory mode of adjustment of stomatal apertures, presumably through the control of ion transport at the guard cell membrane (23). In contrast with a hydropassive mechanism which could only allow for additive interactions, a common metabolic link between the VPD response and those induced by other environmental signals affecting stomatal movements, would make possible a tighter regulation of the integrated stomatal response to the environment, which is consistent with the empirically observed, close interactions between the VPD response and other stomatal stimuli (9).

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