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The Magnitude of the Stomatal Response to Blue Light[1]

Modulation by Atmospheric Humidity

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ABSTRACT

The effect of leaf-air vapor pressure difference (VPD) on the magnitude of the stomatal response to blue light was investigated in soybean (*Glycine max*) by administering blue light pulses (22 seconds by 120 micromoles per square meter per second) at different levels of VPD and temperature. At 20 °C and 25 °C, the magnitude of the integrated conductance response decreased with increasing VPD (0.4 to 2.6 kiloPascals), due to an earlier onset of stomatal closure that terminated the pulse response. In contrast, at 30 °C this magnitude increased with rising VPD (0.9 to 3.5 kiloPascals), due to an increasing maximum excursion of the conductance response despite the accelerated onset of stomatal closure. When the feedforward response of stomata to humidity caused steady state transpiration to decrease with increasing VPD, the magnitude of the pulse-induced conductance response correlated with VPD rather than with transpiration. This suggests that water relations or metabolite movements within epidermal rather than bulk leaf tissue interacted with guard cell photobiological properties in regulating the magnitude of the blue light response. VPD modulation of pulse magnitude could reduce water loss during stomatal responses to transient illumination in natural light environments.

Atmospheric humidity and light are principal factors determining levels of gas exchange in leaves (20, 26) yet little is known of their mechanistic interaction. Humidity is manifest at the leaf surface as relative humidity and as evaporative demand (VPD[2]). Stomatal processes are often well described as functions of VPD (2, 8, 12). The stomatal response to light consists of separate metabolic responses to photosynthetically active (PAR, 400–700 nm) and to blue (350–500 nm) radiation (26). The PAR response is mediated by guard cell chloroplasts, while the blue light response is mediated by an unidentified photoreceptor (26). A nonadditive interaction of white light and VPD in determining steady state levels of stomatal conductance has been described in a variety of species (8, 12, 16). However, specific interactions between VPD and the guard cell metabolic responses associated with light stimuli remain to be demonstrated.

Stomatal responses to VPD are mediated, at least in part, through guard cell metabolic responses (9, 14, 15), that could interact directly with the light responses. In species exhibiting a feedforward response (5), transpiration declines with increasing VPD, eliminating bulk leaf transpiration and water status as factors in the stomatal response to VPD and implicating water relations, metabolite transport or other processes within the epidermis (6, 24, 25). These epidermal water relations and transport processes could interact with guard cell photobiology, or alternatively with the hydraulic and osmotic aspects of stomatal movements.

The magnitude of the stomatal response to a pulse of blue light in *Commelina communis* was suppressed by high VPD (1). Such pulse-induced transient stomatal responses are readily quantitated and represent a useful probe of guard cell and stomatal function (1, 2, 11, 13, 27). A photobiological model of the blue light response has been derived from such a protocol (11, 27) that relates response magnitude, with other environmental parameters constant, to the status of a photochemical signal transduction system. A semimechanistic model of stomatal responses to flecks of white light (13) successfully utilized rates of change of (a) a photobiochemical signal resulting from the light stimulus, (b) guard cell uptake of osmoticum, and (c) guard cell uptake of water, to describe transient changes in stomatal conductance following simulated sunflecks. This model did not distinguish between the specific light responses of stomata, nor did it explicitly consider effects of ambient humidity or temperature on transient stomatal responses to light.

These recent experimental and conceptual advances suggested that analysis of the effect of VPD on the magnitude of blue light-induced stomatal responses could probe the mechanism of stomatal responses to each stimulus, nature of their interaction. The present study investigated the regulation of the blue light response over a range of VPD, obtained at several temperatures, and under conditions in which soybean stomata exhibited a feedforward response of reduced steady state stomatal conductance and transpiration with increasing VPD. We hypothesized that as evaporative demand changed

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[2] Abbreviations: VPD, leaf-to-air water vapor pressure difference; M_{rel}, relative integrated response magnitude; T_{rev}, elapsed time from pulse stimulation to reversal from stomatal opening to stomatal closing.

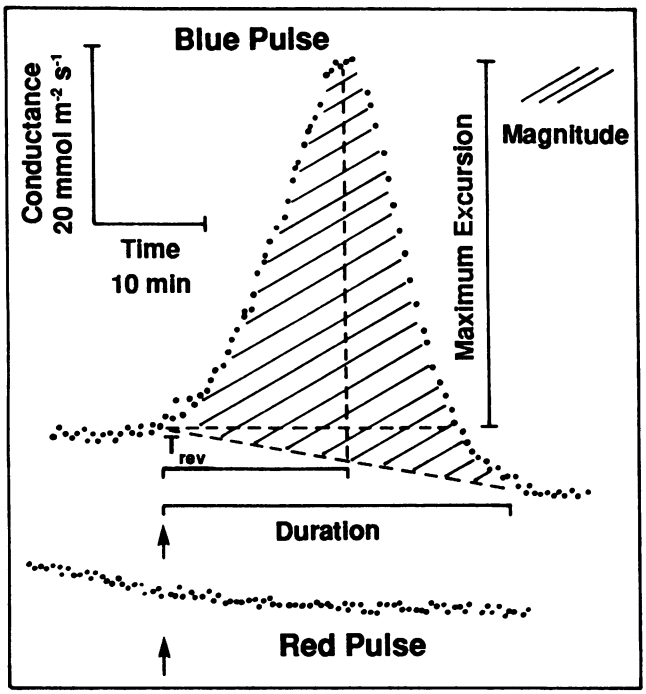

Figure 1. Typical stomatal response to a blue light pulse and absence of any response to an identical red light pulse. VPD was 2.1 kPa and temperature was 20°C in both cases. Parameters used to quantify pulse magnitude and shape are shown as described in "Materials and Methods."

interactive responses to blue light and VPD would act to maintain a constant water cost of the blue light response.

MATERIALS AND METHODS

Plant Growth

Seeds of soybean (*Glycine max*, cv Prize; Honolulu Seed Co.,[3] Honolulu) were planted in a mixture of soil/volcanic ash/peat (1/1/1) in a greenhouse at Aiea, Hawaii under natural illumination with daily irrigation and weekly fertilization (16/16/16). Relative humidity was *ca.* 65/85% and temperature *ca.* 30/20 °C (day/night). One leaflet from healthy, fully expanded leaves of plants three to five weeks old was used for gas exchange experiments.

Gas Exchange

Single, attached leaflets (*ca.* 30 cm^2) were sealed in the cuvette of a null balance gas exchange system (Armstrong Enterprises). The cuvette was modified to enhance leaf boundary layer conductance (*ca.* 4.2 mol m^{-2} s^{-1}). Air flow rate was maintained constant for all experiments at 1.35 1 min^{-1}. Chamber humidity was determined by the dewpoint of the incoming air, regulated with a custom-built humidifier and measured with a dewpoint hygrometer (Dew-10, General Eastern). Incoming air that bypassed the humidifier was passed over magnesium perchlorate to assure dryness. Relative humidity and VPD in the chamber were calculated from chamber temperature, leaf temperature, and the dewpoint temperature of air leaving the chamber, measured with an independent dewpoint hygrometer (Dew-10). The CO_2 content of incoming air was 347 μL L^{-1} for all experiments, and was supplemented with 9934 μL L^{-1} CO_2 in air as necessary to balance CO_2 depletion resulting from photosynthesis. Stomatal conductance, transpiration and assimilation, were calculated from flow rates and differential measurements. Relevant parameters for all calculations were sampled with a micro-computer at 30 s intervals.

[3] Mention of a proprietary product is for the convenience of the reader and does not imply endorsement by the U.S. Department of Agriculture, Agricultural Research Service.

Photobiology

The gas exchange cuvette was shrouded with black cloth to exclude illumination by room light. The protocol was a modification of a previously described dual beam technique (11, 19). Background illumination was provided by a 150 W lamp (ELD; Sylvania) reflected by a 45 ° cold mirror. The light was passed through an interference filter (yellow dichroic) with less than 1% transmission in the blue (< 475 nm) and a layer of Cinemoid (No. 5A), providing high intensity (900 μmol $m^{-2}s^{-1}$) background light that had no measurable blue light as assayed with a quantum sensor (Licor Inc.) through a blue dichroic filter. Pulses of blue light (22 s × 120 μmol $m^{-2}s^{-1}$) were obtained by passing light from a 75 W lamp (EYF;

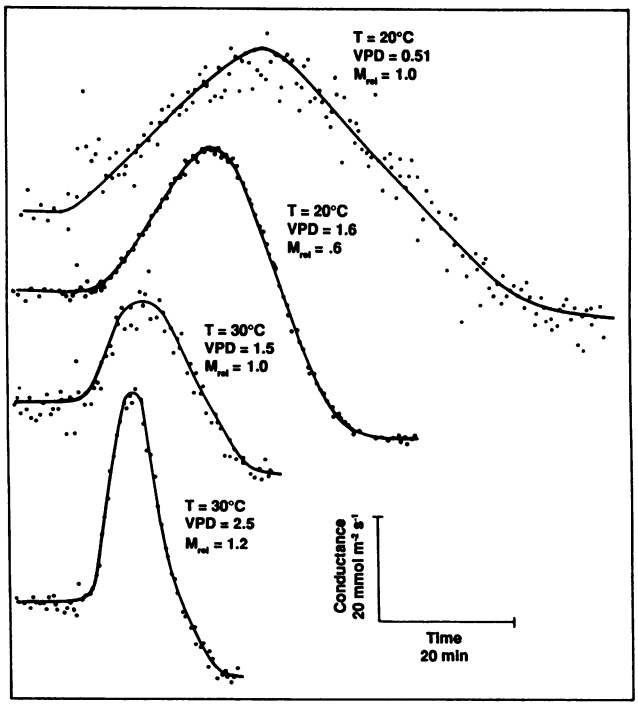

Figure 2. Conductance responses to blue light pulses at two different VPD's and temperatures. Leaf temperature (*T*), VPD, and magnitude of the conductance response relative to the magnitude of the response at the same temperature at low VPD (M_{rel}) are indicated.

Table I. *Correlation Coefficients for Effect of VPD on Magnitude and Associated Components of Conductance Transients Induced by the Blue Light Response*

Temperature	Correlation with VPD (*r*)			
	Response magnitude	Time to reversal	Maximum excursion	Response duration
20°C (*n* = 11)	−0.52	−0.76**	+0.19	−0.60*
25°C (*n* = 15)	−0.10	−0.42	+0.45	−0.52*
30°C (*n* = 14)	+0.65**	−0.40	+0.90***	−0.59*
All (*n* = 40)	−0.30	−0.62***	+0.61***	−0.59***

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.005$.

Sylvania) through a 45 ° hot mirror, a blue interference filter (blue dichroic), and a neutral diffuser. All mirrors and filters except Cinemoid were obtained from OCLI, Santa Rosa, CA. Fluence rates were measured with a quantum sensor (Licor) placed on top of the cuvette and were corrected for transmission through the glass cuvette lid.

Experimental Protocol

The leaf was placed in the cuvette and allowed to reach steady-state levels of assimilation and stomatal conductance at a low VPD (*ca.* 0.4 kPa at 20 °C, 0.9 kPA at 30 °C). A preliminary blue pulse was administered so that all test pulses, including the first, would be preceded by blue light stimulation. Steady-state was defined as near stability over ten minutes and was quantified as the average over that time of conductance, assimilation, transpiration, VPD, and RH. Blue light pulses were then administered and gas exchange parameters monitored without adjusting any system controls until conductance regained a steady state value. The magnitude of the blue light response (Fig. 1) was quantified as the integrated conductance above the baseline following stimulation by blue light (11, 27). Under conditions of slowly changing baseline conductance (increasing or decreasing) the drifting baseline was incorporated into estimates of pulse magnitude as shown diagramatically in Figure 1. The components that determined this magnitude, including duration of response, maximum excursion of stomatal conductance above baseline, and time to reversal from stomatal opening to stomatal closure (T_{rev}) were also determined (Fig. 1). Kinetic parameters associated with rates of conductance increase and recovery were determined from the slopes of tangents to the ascending and descending portions of the pulse response curves as described previously (2). The water cost of a response to blue light was defined as the integrated transpiration above the baseline after a blue light pulse. Several replicate experiments were performed at each of 20 °C, 25 °C, and 30 °C. Figures depict data from representative leaves, while tables contain statistical evaluations of the pooled data.

RESULTS

The Blue Light Response

A transient increase in stomatal conductance was observed following a brief pulse (22 s) of blue light whereas no comparable response was seen following a pulse of red light of the same intensity and duration (Fig. 1). Pulses were administered on a background of high intensity red light (900 μmol m^{-2} s^{-1}) so that direct irradiance effects on guard cell and mesophyll photosynthesis were not observed (not shown). Thus the response of conductance was a direct and specific response to blue light. Slowly increasing, or more commonly decreasing, baseline levels of conductance were observed from time to time (*e.g.* Fig. 1). The pulse responses in Figure 1 illustrate the method of calculating response magnitude under conditions of slowly changing baseline conductance.

Interaction between Blue Light and Humidity

The level of ambient humidity strongly influenced the stomatal response to a pulse of blue light. Identical pulses administered to the same leaf at different levels of leaf-air VPD (Fig. 2) elicited conductance responses differing in magnitude and shape. At the lowest temperature investigated (20 °C) the integrated magnitude of the response decreased by *ca.* 40% (M_{rel} = 0.6; Fig. 2) with increasing VPD, whereas at the highest temperature (30 °C) the magnitude increased by *ca.* 20% (M_{rel} = 1.2; Fig. 2) with VPD. While exact values of M_{rel} varied between experiments, these trends were consistently observed (Table I).

The contrasting results at 20 °C and 30 °C originated from differential effects of VPD on the components of the conductance response (Table I). At all three temperatures (20 °C, 25 °C, 30 °C), response duration and the time to onset of stomatal recovery, *i.e.* reversal from opening to closing, declined with increasing VPD. As described previously (2), the

Table II. *Correlation Coefficients for Relationship between Integrated Magnitude of the Blue Light-Induced Conductance Response and Components of That Response*

Temperature	Correlation with Conductance Magnitude (*r*)				
	Time to reversal	Maximum excursion	Response duration	Rate of opening	Rate of closing[a]
20°C (*n* = 11)	+0.77**	+0.51	+0.81**	−0.05	−0.03
25°C (*n* = 15)	+0.21	+0.74***	+0.36	+0.45	+0.38
30°C (*n* = 14)	+0.062	+0.71***	+0.031	+0.50	+0.16
All (*n* = 40)	+0.60***	+0.41**	+0.68***	−0.10	+0.07

[a] Absolute value. ** $P \leq 0.01$; *** $P \leq 0.005$.

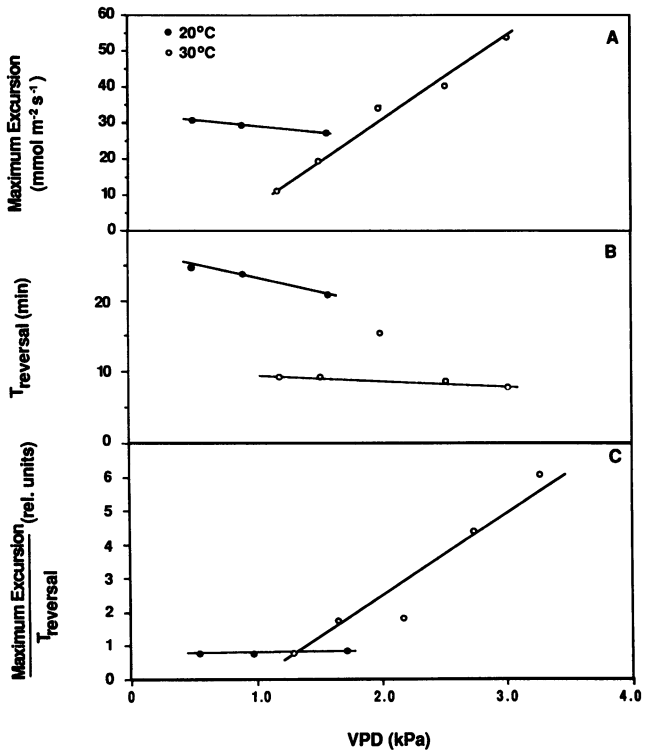

Figure 3. Effect of VPD on (A) the maximum excursion of the conductance response, (B) the time to initiation of stomatal closure (T_{rev}), and (C) the ratio of these two parameters, at 20 °C and 30 °C. Data are from the same leaves as those of Figure 2.

rate of stomatal opening increased with increasing VPD at each of these temperatures. In contrast, the maximum excursion of the conductance response was unaffected (at the P = 0.05 level) by VPD at 20 °C, but was significantly ($P \leq 0.005$) correlated with VPD at 30 °C (Table I). Despite their significant correlations with VPD (2) and the obvious geometric connection between opening or closing kinetics and total response magnitude (*e.g.* Fig. 1), stomatal kinetics alone were not good indicators of integrated response magnitude (Table II).

As temperature and VPD increased, the dominant factor determining the integrated conductance response magnitude (Table II) changed from duration or time to reversal (at 20 °C) to maximum excursion (at 30 °C). As temperature and VPD increased, there was a dramatic increase in the maximum change in stomatal conductance (Fig. 3A), relative to the much smaller change in the time to reversal of the pulse-induced opening process (Fig. 3, B and C).

At 30 °C, soybean exhibited a decline in steady state transpiration (a feedforward response [5] of stomata to humidity) as VPD increased from *ca.* 2.0 to *ca.* 3.0 kPa (Fig. 4). Steady state transpiration would be expected to resume increasing with further increase in VPD, as noted previously in similar leaves (2). The decline in baseline transpiration over the range of VPD utilized allowed an experimental distinction between effects of transpiration and those of VPD. Through the choice of a species exhibiting such a feedforward response, the integrated conductance response could be shown to describe a uniquely-valued function of VPD (Fig. 5A), but not of steady state transpiration prior to the blue light pulse (Fig. 5B). The representative traces of Fig. 5 are confirmed by the significant correlation between magnitude and VPD observed over several experiments at 30 °C ($r = 0.65$, P = 0.01; Table I) and lack of such correlation with transpiration ($r = -0.079$, P = 0.79; Table III). Maximum excursion, the primary determinant of response magnitude at this temperature, also correlated more strongly with VPD than with transpiration (*cf.* Tables I and III).

The water cost of the conductance response under these experimental conditions was evaluated as total blue light-induced transpiration above the baseline level of steady state transpiration. Despite decreases in integrated magnitude of the conductance response with increases in VPD observed at 20 °C and 25 °C, the combination of integrated magnitude and prevailing evaporative demand resulted in an increase in blue light-stimulated water loss with increasing VPD at all three temperatures (Table IV). Integrated blue light-induced transpiration increased sharply with VPD (Fig. 6) with no indication of saturation even at the highest levels of VPD and water loss.

DISCUSSION

Because stomata respond to many environmental variables, definition of a stomatal response to any one parameter is difficult. In studying the stomatal response to blue light, the introduction of a gas exchange protocol involving pulse stimulation superimposed on high intensity background red light (11) allowed induction of a rapid response to blue light (Fig. 1) without direct excitation by PAR of photosynthesis in either mesophyll or guard cells (1, 11, 27). This allowed quantitation of the magnitude of the blue light response, independently of photosynthetic effects. Once the blue light response could be defined in this way, it became feasible to address the physiologically and ecologically relevant questions of how the magnitude of this response was affected by other environmental parameters.

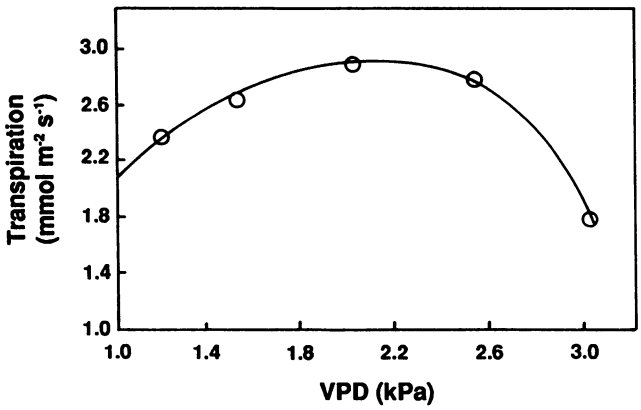

Figure 4. Effect of VPD on steady-state transpiration in soybean at 30 °C. Data are from the 30 °C leaf of Figure 2.

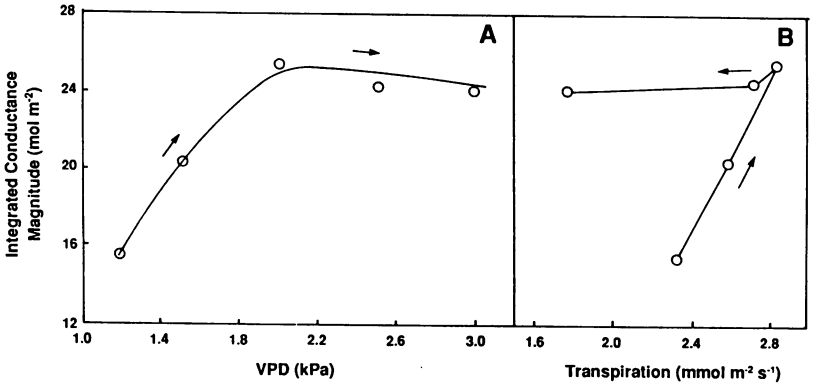

Figure 5. Integrated magnitude of blue light pulse-induced conductance increase as a function of (A) VPD and (B) prevailing transpiration before the pulse. *Arrows* indicate the order of experimentally increased VPD. Data from the 30 °C leaf of Figure 2.

At 20 °C and 25 °C, over a VPD range of 0.4 to 3.5 kPa, the integrated magnitude of the blue light response in soybean was negatively correlated with increasing VPD (Fig. 2; Table I) as had been previously observed at 25 °C with *Commelina communis* (1). At 30 °C, over a VPD range from 0.9 to 3.5 kPa; however, there was a positive correlation between response magnitude and increasing VPD. Thus, the apparently simple interactive model of evaporative demand and blue light-induced pulse response magnitude suggested by earlier studies (1) was found to be considerably more complex.

An analysis of the components of the conductance response showed that integrated response magnitude and stomatal kinetics were not well correlated (Table II). These results do not imply that stomatal kinetics were unrelated to response magnitude. An increase in maximum excursion of the conductance response, despite a decrease in time to onset of stomatal closure, implies accelerated opening kinetics. However, the kinetic parameters alone were not good predictors of the total response magnitude (Table II). For example, the rate of opening increased with VPD at all temperatures tested (2) while the magnitude of the pulse response declined with VPD at all temperatures except 30 °C (Table I). The combined results of the analyses in Tables I and II indicate that at 20 °C and 25 °C accelerated onset of stomatal recovery accounted for the decrease in integrated response magnitude with increasing VPD. At 30 °C, the effect of VPD on maximum excursion dominated the effect on recovery time (Fig. 3), resulting in the observed positive correlation between response magnitude and VPD (Table I). Possible effects of temperature, independent of VPD, remain to be further characterized.

Because stomata of soybean exhibit a feedforward response to humidity (5), steady-state transpiration does not continually increase with increasing VPD (Fig. 4) as would be expected from consideration of evaporative demand alone. Over the range of VPD in which bulk leaf transpiration declines with increasing evaporative demand, bulk leaf water status improves (5, 24), although epidermal water status is likely to continue to decline. Soybean exhibited the most pronounced feedforward response at 30 °C (2) (Fig. 4). Experiments at this temperature therefore afford the best opportunity for separation of effects of VPD from those of stomatally controlled bulk leaf transpiration. At 30 °C, and in the combined data set from all temperatures, the time to initiation of stomatal closure and the maximum excursion of the pulse-induced conductance response, were both more highly correlated with VPD than with transpiration. This implicates epidermal water relations, as opposed to bulk leaf water status, in the modulation of the blue light response by ambient humidity. These epidermal processes merit further investigation as potential mechanistic components of the steady-state stomatal response to VPD (17, 25).

The time at which stomatal closure is initiated following blue light stimulated opening may be controlled by a quantitative interaction of the metabolic response of guard cells to VPD (9, 14, 15), with the photobiochemical features of the guard cell response to blue light (11, 19, 27). Available kinetic models of stomatal responses to pulses of blue light (11, 27) suggest several potential loci of interaction at the level of

Table III. *Correlation Coefficients for Effect of Steady State Transpiration on Magnitude and Components of Conductance Transients Induced by the Blue Light Response*

Temperature	Correlation with Transpiration (*r*)		
	Magnitude	Time to reversal	Maximum excursion
20°C (*n* = 11)	+0.49	+0.15	+0.38
25°C (*n* = 15)	−0.027	−0.38	+0.30
30°C (*n* = 14)	−0.079	−0.18	+0.19
All (*n* = 40)	+0.015	−0.36*	+0.38*

* $P \leq 0.05$.

Table IV. *Correlation Coefficients for Effect of VPD on Integrated Magnitude of Transpiration Transients Induced by the Blue Light Response*

Temperature	Correlation Coefficient (*r*)
20°C (*n* = 11)	+0.70*
25°C (*n* = 15)	+0.95***
30°C (*n* = 14)	+0.94***
All (*n* = 40)	+0.86***

* $P \leq 0.05$; *** $P \leq 0.005$.

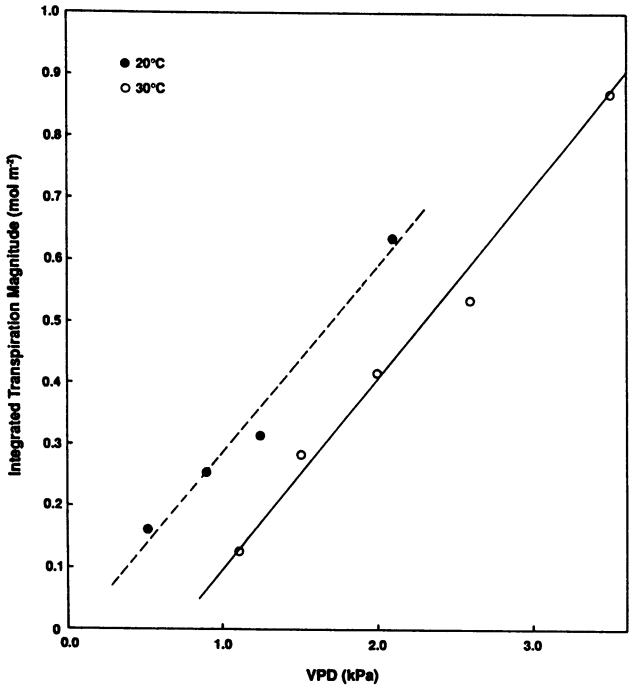

Figure 6. Integrated magnitude of blue light pulse-induced transpiration increase as a function of VPD at 20 °C and 30 °C in two representative leaves.

guard cell ion transport, or rates of light- and dark-photoreceptor interconversions. Interactions could be mediated by VPD-induced redistribution within the epidermis of signal metabolites present in the transpiration stream (*e.g.* see refs. 7 and 28).

The increase in the maximum excursion of stomatal conductance with increasing VPD that was observed at 30 °C (and occasionally at high VPD at lower temperatures) and the acceleration of opening kinetics with increasing VPD (2–4) are more likely to reflect hydropassive effects of temperature and VPD on epidermal water relations (6, 10, 21, 25). Accelerated opening kinetics could result from reduced epidermal backpressure on the stomatal complex. Reduced epidermal turgor could arise from enhanced cuticular conductance at high temperature (22, 23) and from the increased driving force for peristomatal or cuticular transpiration at high VPD (17) as well as from enhanced stomatal transpiration in the absence of feedforward responses (18, 25). Accelerated opening kinetics would increase the maximum excursion of stomatal opening, unless time to initiation of stomatal closure were decreased in a compensatory manner. This accelerated onset of closure could be metabolic, as suggested above, or could also reflect VPD-induced limitations in water availability to the guard cells.

The components of the blue light response were better correlated with VPD than with transpiration (Tables I and III; 30 °C data). This is consistent with the observation, under feedforward conditions (5) (Fig. 4), that the integrated magnitude of the blue light response was a uniquely-valued function of VPD, but not of transpiration (Fig. 5). These data indicate that changes in epidermal water relations or in VPD-induced redistribution of metabolites within the epidermis may affect not only steady state stomatal conductance and stomatal kinetics, but also the magnitude of stomatal responses to independent stimuli such as blue light. The ecological ramifications of these interactions remain to be evaluated.

We had initially hypothesized that a physiologically integrated stomatal response to blue light and VPD could maintain a constant transpirational water cost during a blue light response, even as evaporative demand increased. However, this hypothesis was not supported. Integrated water loss attributed to the blue light response increased with increasing VPD, even as the stomatal response magnitude itself declined (Fig. 6, Table IV). Nevertheless, the acceleration of stomatal recovery at high VPD reduced the rate at which the water cost would otherwise have increased. In natural, dynamic light environments this interactive response may reduce the water loss due to the blue light component of stomatal response under conditions of high evaporative demand and low inherent water use efficiency.

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