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Circadian Rhythms in Stomatal Responsiveness to Red and Blue Light[1]

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Stomata of many plants have circadian rhythms in responsiveness to environmental cues as well as circadian rhythms in aperture. Stomatal responses to red light and blue light are mediated by photosynthetic photoreceptors; responses to blue light are additionally controlled by a specific blue-light photoreceptor. This paper describes circadian rhythmic aspects of stomatal responsiveness to red and blue light in *Vicia faba*. Plants were exposed to a repeated light:dark regime of 1.5:2.5 h for a total of 48 h, and because the plants could not entrain to this short light:dark cycle, circadian rhythms were able to "free run" as if in continuous light. The rhythm in the stomatal conductance established during the 1.5-h light periods was caused both by a rhythm in sensitivity to light and by a rhythm in the stomatal conductance established during the preceding 2.5-h dark periods. Both rhythms peaked during the middle of the subjective day. Although the stomatal response to blue light is greater than the response to red light at all times of day, there was no discernible difference in period, phase, or amplitude of the rhythm in sensitivity to the two light qualities. We observed no circadian rhythmicity in net carbon assimilation with the 1.5:2.5 h light regime for either red or blue light. In continuous white light, small rhythmic changes in photosynthetic assimilation were observed, but at relatively high light levels, and these appeared to be attributable largely to changes in internal CO_2 availability governed by stomatal conductance.

Stomata in plants control the critical tradeoff between beneficial uptake of CO_2 for photosynthesis and normally detrimental water loss through transpiration. Stomatal opening is finely controlled by environmental stimuli such as light (Sharkey and Ogawa, 1987; Zeiger et al., 1987; Zeiger, 1990), humidity (Schulze et al., 1987; Grantz, 1990), and CO_2 concentration (Morison, 1987). Photocontrol of stomata is governed by at least two photoreceptor systems: RL and BL both stimulate opening via photosynthetic photoreceptors, whereas BL also stimulates opening via a specific, as yet unidentified, BL photoreceptor.

There is considerable evidence for the existence of these two photoreceptor systems based on differences in the photobiology of the responses to RL and BL. For example, the action spectrum for light-stimulated stomatal opening does not resemble that of photosynthesis: the action peak in the blue is much larger than that in the red, suggesting that another pigment operates in the blue region of the spectrum and confers additional sensitivity to BL (Sharkey and Raschke, 1981; Karlsson, 1986). Stomata are more sensitive to low levels of BL than RL, the maximum extent of opening is greater under saturating BL than under saturating RL, and the stomata in most species are sensitive to short pulses of BL but not RL (Sharkey and Ogawa, 1987; Zeiger et al., 1987; Zeiger, 1990). Furthermore, BL will cause additional stomatal opening when given over a background of saturating RL irradiation (Ogawa et al., 1978).

The presence of two photoreceptor systems controlling stomatal opening has engendered speculation about the functions of, and interactions between, the two systems. Although both photoreceptor systems could work together much of the time, the BL response might predominate under certain circumstances because of its greater sensitivity to low light levels and to short irradiations. More specifically, the realization that the BL system in stomata is likely to be saturated during most of the day has given rise to the suggestion that the BL system might be important as a signal for the detection of daybreak (Zeiger et al., 1981), and the sensitivity of the BL system to short pulses of light has suggested that perhaps stomatal opening during sunflecks might be predominantly a BL response (Zeiger and Field, 1982; Zeiger, 1990).

In addition to their responsiveness to exogenous, environmental stimuli, stomata also are governed by an endogenous, circadian clock (Meidner and Mansfield, 1968; Gorton, 1990). Stomatal rhythmicity has been observed in whole leaves (see below), in functionally isolated guard cells (Gorton et al., 1989b), and possibly in guard cell protoplasts isolated at different times of day (Dodge et al., 1992). There are two interrelated types of rhythmicity exhibited by stomata. The first type is a simple rhythmicity in the degree of opening (aperture). The opening and closing occurs about every 24 h and persists under constant environmental conditions, causing the stomatal rhythm to "free run." The second type is a rhythmic responsiveness in the rapidity and magnitude of

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Abbreviations: **A**, net carbon assimilation; BL, blue light; *Ci*, intercellular CO_2 concentration; DD, constant darkness; *g*, stomatal conductance; LD, light-dark; LL, constant light; RL, red light; WL, white light.

response to environmental cues such as light, with maximum responsiveness occurring about every 24 h under constant conditions. Rhythmicity in opening is probably linked to rhythmicity in responsiveness because the same cellular mechanisms are likely to be involved. The chain of events involved in stomatal responsiveness to some environmental cue might be summarized as follows: the cue is perceived, a biochemical signal or chain of signals is generated, the water potential of the guard cells is altered, water enters or leaves the guard cells, and finally, the pore opens or closes. A rhythm in opening reflects a rhythm in water potential of the guard cells (Stålfelt, 1967), and, therefore, a response to an environmental cue would presumably be earlier at least by the amount of time required to generate that change in water potential.

Rhythmicity in Aperture

Many species show free-running rhythmicity in stomatal aperture either in LL or DD, although the rhythms generally damp more quickly under DD than under LL. Such rhythms have been studied microscopically (Stålfelt, 1963; Gorton et al., 1989a, 1989b) or as changes in transpiration rate or stomatal conductance (g) (Heath and Mansfield, 1962; Pallas et al., 1974; Holmes and Klein, 1986; Nixon et al., 1987; Deitzer and Frosch, 1990). The period of the rhythm varies with species from about 22 h (*Vicia* [Stålfelt, 1963]) to about 26 h (*Arachis* [Pallas et al., 1974]; *Avena* [Brogårdh and Johnsson, 1975]). Like all true circadian rhythms, the phase of observed rhythms in stomatal aperture can be reset by light (Mansfield and Heath, 1961, 1963, 1964; Holmes and Klein, 1986; Deitzer and Frosch, 1990) and it is temperature compensated, i.e. the period does not change much with changes in temperature (Gorton et al., 1989b).

A rhythm in **A** frequently occurs in phase with a rhythm in stomatal aperture. The periodicity in **A** may be attributed to periodicity in g, which controls availability of CO_2, but rhythmicity in nonstomatal factors may also contribute (Pallas et al., 1974; Lonergan, 1981; Deitzer and Frosch, 1990; Fredeen et al., 1991; Hennessey and Field, 1991). It is often difficult to distinguish between these two possibilities.

Rhythmicity in Responsiveness to Light

Stomata typically open most quickly in response to light early in the day phase of the rhythm and close more quickly in response to darkness during the night phase of the rhythm (Darwin, 1898). Since Darwin's report, rhythms in stomatal sensitivity to light have been found in many genera including *Musa* (Brun, 1962), *Vicia* (Kana and Miller, 1977), *Xanthium* (Mansfield and Heath, 1963, 1964), *Glycine* (Mansfield, 1963), *Arachis* (Pallas et al., 1974), *Pelargonium* (Scarth and Shaw, 1951), and *Tradescantia* (Martin and Meidner, 1971). All these reports concern stomatal responsiveness to WL. In some, the rhythm was demonstrated by using repeated, short LD cycles (Martin and Meidner, 1971; Pallas et al., 1974); when the length of the circadian day is much different from the length of the LD cycle, the circadian clock cannot entrain and circadian rhythms free-run (Tribukait, 1956; Aschoff, 1978). In others, rhythmicity was suggested by experiments in which the length of the dark period was varied before the lights-on signal. Stomata opened slowly in response to light given after a short, dark period, when the plants are still in early- or mid-subjective night, but opened more rapidly in response to light given after a longer, dark period, when the plants neared or entered the subjective day of their circadian cycle (Brun, 1962; Mansfield, 1963; Mansfield and Heath, 1963; Kana and Miller, 1977).

If, as the evidence suggests, stomata are sensitive to light perceived by two different photoreceptor systems, and if there is a rhythm in sensitivity of stomata to light, several questions arise. Is there a rhythm in sensitivity both to RL perceived by photosynthetic pigments and to BL perceived by a specific BL photoreceptor? If so, are the rhythms of similar magnitude? Are they normally in phase? In this study we address these questions using *Vicia faba*, in which rhythms in aperture (Stålfelt, 1963; Gorton et al., 1989b) have been established, and rhythms in sensitivity to WL (Kana and Miller, 1977) have been suggested. We use gas-exchange methods to monitor **A** and g simultaneously.

MATERIALS AND METHODS

All experiments were performed at Harvard University.

Plant Material

Three-week-old seedlings of *Vicia faba* were grown in a soilless potting mix (Metromix 500; W.R. Grace, Inc., Cambridge, MA) in a plant-growth chamber on a 10-h light:14-h dark regime. The lights were mixed fluorescent (Sylvania F72T12/CW/VHO) and incandescent (General Electric 67W M/CL) and provided 140 to 150 μmol m^{-2} s^{-1} PAR at the growing surface. Temperature was maintained at 21°C during the light period and 20°C at night.

Gas-Exchange Measurements

Plants were removed from the growth chamber 30 min to 1 h before the lights came on in the chamber, and the youngest fully expanded leaf was allowed to equilibrate in the dark in the gas-exchange cuvette for the remainder of the plant's normal dark period. We used an open gas-exchange system operating in differential mode (Field et al., 1991) for measurements of **A**, g, and related parameters. The system (Armstrong Enterprises, Inc., Sunnyvale, CA) employed a dewpoint condenser to control humidity of incoming air, two dewpoint hygrometers (Dew-10; General Eastern, Woburn, MA) to measure humidity before and after the chamber, and a differential IRGA (Li-Cor 6252, Li-Cor, Inc., Lincoln, NE) to determine the difference in CO_2 concentration before and after the chamber. Leaf temperature was 21°C and leaf area was about 15 cm^2. CO_2 concentration in the incoming dry air was 350 μL/L, giving ambient CO_2 concentrations between 335 and 350 μL/L during experiments. Vapor pressure difference was set initially at 1.2 kPa. Boundary layer conductance was about 2.5 mol m^{-2} s^{-1}.

Light Measurements

All measurements of photon fluence rate were made with a quantum sensor from Li-Cor.

Experimental Conditions and Lighting

Experiments investigating rhythmicity in sensitivity to BL or RL were done using a short LD cycle of 1.5-h light:2.5-h dark. This LD cycle of 4 h total duration was repeated continuously for the duration of each experiment, normally 47 h. The plant (except for the leaf in the cuvette) was kept in normal laboratory conditions, about 23°C with 10 μmol m^{-2} s^{-1} fluorescent room light, and these conditions remained reasonably constant (within 5%) for the duration of the experiments. During the first 1.5 h of each 4-h cycle, the leaf in the cuvette received light (140–145 μmol m^{-2} s^{-1}) from above supplied by two incandescent bulbs (General Electric 75W EYF) and passed through a hot mirror and appropriate color filters: red, dichroic filter with less than 1% transmittance below 475 nm plus one layer of orange plastic (Cinemoid 5A); blue, dichroic filter with maximum transmittance between 390 and 480 nm and less than 1% transmittance between 540 and 750 nm. The hot mirror and dichroic filters were from Optical Coating Laboratory (Santa Rosa, CA). Layers of window screen or Kimwipe were employed as neutral-density filters when necessary.

For experiments with continuous WL, the hot mirror was used with neutral-density filters to give the desired photon fluence rate.

RESULTS

Stomatal Responsiveness to BL and RL

Stomatal conductance showed circadian rhythmicity in responsiveness to BL (Fig. 1a) and to RL (Fig. 2a); *g* established during the 1.5-h light periods given during the subjective day was greater than *g* established during the 1.5-h light periods given during the subjective night. A similar effect is observed for *g* established during the 2.5-h dark periods. For neither BL nor RL was there rhythmicity in **A** (Figs. 1b and 2b), although for both *Ci* decreased during each 1.5-h light period as CO_2 was assimilated, and it decreased less during the subjective day than during the subjective night (Figs. 1c and 2c). There was some variability between replicates in the phase of the rhythm, but in all cases, for both BL and RL, the first peak in *g* occurred during either the second light period (lights on h 4 to h 5.5) or third light period (lights on h 8 to h 9.5).

In the experiments represented in Figure 1, stomatal responsiveness to BL is a composite of the responses mediated by photosynthetic pigments and by the BL photoreceptor. One way to separate these two responses to BL is to saturate the photosynthetic response with RL, then give additional BL to promote stomatal opening that cannot be attributed to the photosynthetic pigments (Ogawa et al., 1978). This approach did not work with our system; even subsaturating RL (1000 μmol m^{-2} s^{-1}) given for 48 h caused some necrosis of the leaf, presumably because plants were acclimated to the light levels of their growth conditions. To accomplish the same goal, we calculated the difference between the average response to BL and that to RL; because the response to BL represents responses mediated by both the BL photoreceptor and the photosynthetic pigments, whereas the response to RL is mediated only by photosynthetic pigments, the difference between these two responses should give an estimate of that portion of the BL response attributable specifically to the BL photoreceptor. In confirmation of this approach, O_2 evolution by isolated guard cell chloroplasts did not differ significantly under BL versus RL (W.-h. Wu and S.M. Assmann, unpublished observation), indicating that the increase in *g* in BL versus RL shown here results from the additional contribution of the specific BL photoreceptor.

Average stomatal responses to BL and to RL and the difference between these are shown in Figure 3. Peaks in *g* during the first d are obvious for the average responses to both BL and RL, and one can discern a small peak in *g* in the second d for both (Fig. 3, a and b), but because of variability in rhythmic phase between experiments, rhythmicity is less distinct in the average curves than for any of the individual experiments. The difference between the BL and RL responses, which represents the response attributable to the BL photoreceptor, shows a peak during the 1st subjective day, but not during the 2nd subjective day (Fig. 3c). It is possible that the rhythm in responsiveness to BL damps more rapidly than the rhythm in responsiveness to RL, but it is more likely that variability between individual experiments may have obscured the second peak in Figure 3c. The *g* established during the 2.5-h dark periods should theoretically be the same for both BL and RL experiments, so the closed symbols

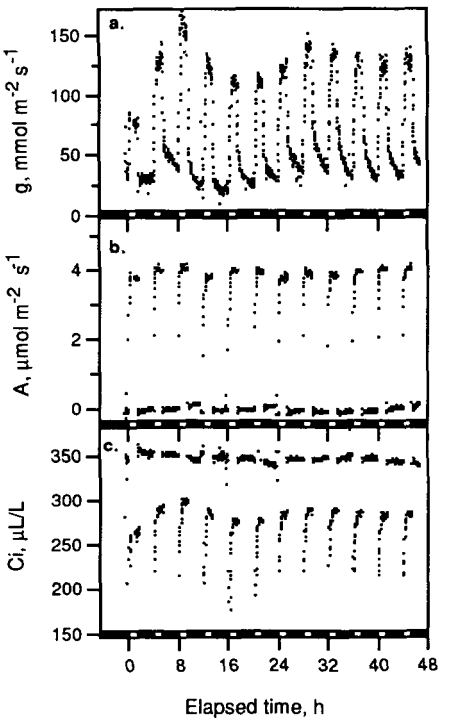

Figure 1. Changes in *g* (a), **A** (b), and *Ci* (c) under cycling BL (1.5 h at 140 μmol m^{-2} s^{-1}) and dark (2.5 h). Leaf temperature was 21°C. The beginning of the first 1.5-h light period coincided with the beginning of the photoperiod in the growth chamber from which the plant was taken; this is h 0 on the abscissa. The alternate light and dark areas on the abscissa represent the repeated 1.5-h light:2.5-h dark cycle. Data are from a single leaf, representative of three replicate experiments.

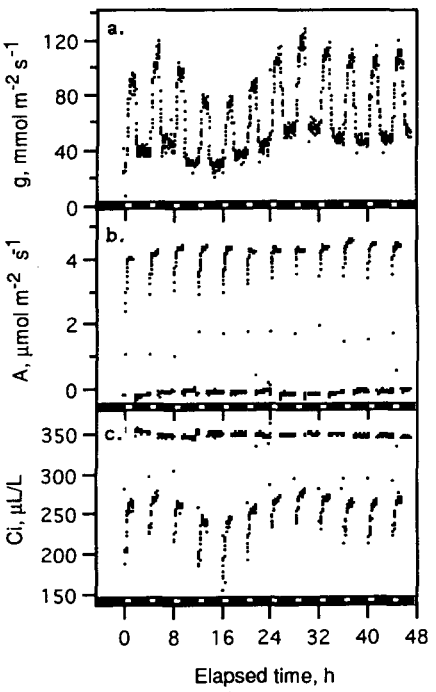

Figure 2. Changes in *g* (a), **A** (b), and *Ci* (c) under cycling RL (1.5 h at 140 μmol m^{-2} s^{-1}) and dark (2.5 h). Other conditions as in Figure 1.

in Figure 3c should be at $g = 0$ mmol m^{-2} s^{-1}. Deviation from this value is an indication of differences between plants used in the two sets of experiments.

The rhythmicity observed in the *g* established by 1.5 h of BL or RL has two additive components: first, during the subjective day, stomata open more in response to light than during the subjective night, and second, the *g* established during the dark periods changes such that stomatal opening in response to light begins with a greater initial *g* during the subjective day (Figs. 1a, 2a, 3, a and b). Thus, in Table I we compare the amplitude both in the overall rhythm with BL and with RL (first two columns of data) and in the responsiveness to BL and to RL (second two columns of data). Data given are for three replicate experiments done with BL (BL#1–3) and with RL (RL#1–3); average values for replicate experiments (mean BL and mean RL) are also shown. The extent of opening under BL is greater than under RL, and the difference between opening established during the subjective day and during the subjective night is also usually greater under BL than under RL (Table I, first column). If this difference is expressed as a percentage of the *g* established during the subjective day, these differences largely disappear (Table I, second column). Similarly, the responsiveness of *g* to BL is greater than for RL (Figs. 1a and 2a), and the difference in this responsiveness between subjective day and subjective night is also correspondingly greater (Table I, third column). Again, when this difference is expressed as a percentage of the responsiveness during the day, differences between BL and RL largely disappear (Table I, fourth column).

The BL response analyzed in Table I should be a composite of responses mediated by photosynthetic pigments and by a specific BL photoreceptor. An analysis of rhythmicity expressed in Figure 3c should give an estimate of the rhythmicity in the response mediated specifically by the BL photoreceptor, but it may be an underestimate of rhythmic amplitude because of the rhythm-flattening effects of phase variability between experiments. For comparison with Table I, the rhythmicity in responsiveness shown in Figure 3c shows about a 39% difference between subjective day and subjective night.

Rhythms in Continuous WL

The experiments described above demonstrated a clear rhythm in stomatal responsiveness to BL and RL, but no corresponding rhythm in **A**. Even though stomates were more open during the subjective day than during the subjective night and there was a corresponding change in *Ci*, **A** was essentially unchanged. Rhythms in **A** under constant WL have frequently been observed in other species, and one possible explanation for our finding was that it might somehow be an artifact of the unusual nature of the light regime used, cycling at 1.5-h light:2.5-h dark. Therefore, we con-

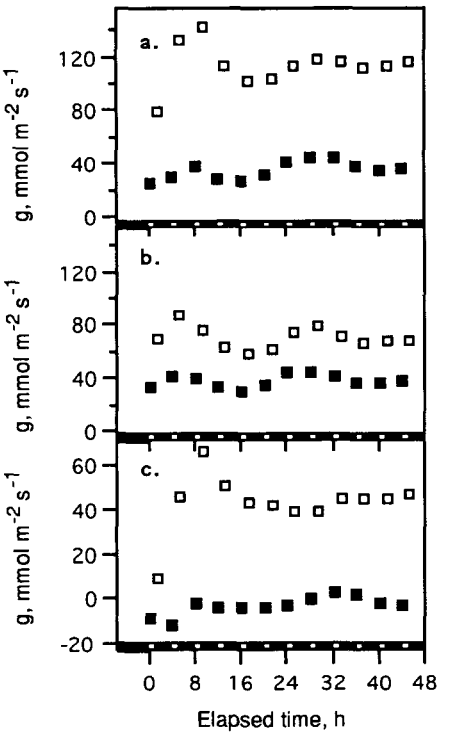

Figure 3. Average changes in stomatal conductance for three experiments conducted as in Figure 1 with BL (a), and for three experiments conducted as in Figure 2 with RL (b). For each experiment, five consecutive values at the peak of conductance for each 4-h LD cycle were averaged together to give a single value, and these values from three experiments were averaged together and represented by the open symbols in a and b. Similarly, points at the trough of conductance in each 4-h LD cycle are averaged and represented by the closed symbols in a and b. c, Difference between the BL response in a and the RL response in b.

Table I. *Summary of conductance results for three BL (BL #1–3) and three RL (RL #1–3) experiments like those represented by Figures 1 and 2*

g_L, Conductance established during a 1.5-h light period; g_D, conductance established during a 2.5-h dark period. $g_{L\,day} - g_{L\,night}$, *absolute*, The difference between the maximum conductance value in the light during the subjective day and the conductance value in the light during the subjective night. $g_{L\,day} - g_{L\,night}$, % $g_{L,\,day}$ expresses this same difference as a percentage of the maximum conductance value in the light during the subjective day. $(g_L - g_D)$ is the difference between g_L and the average of the g_D values on either side. $((g_L - g_D)_{day} - (g_L - g_D)_{night})$, *absolute*, The difference in light stimulation of conductance during the subjective day and subjective night. $((g_L - g_D)_{day} - (g_L - g_D)_{night})$, % $(g_L - g_D)_{day}$ is this same difference expressed as a percentage of the light-stimulated conductance during the subjective day. Each value of g is the average of the five consecutive values at the peak in the light (for g_L) or trough in the dark (for g_D), normally the five values immediately before a change in light. Hence, g_L is normally determined from the average of five g values just before the light is turned off and g_D from the average of five g values just before the light is turned on.

Expt.	Overall Rhythmicity		Rhythmicity in Responsiveness	
	$g_{L\,day} - g_{L\,night}$ *absolute*	$g_{L\,day} - g_{L\,night}$ % $g_{L\,day}$	$((g_L - g_D)_{day} - (g_L - g_D)_{night})$ *absolute*	$((g_L - g_D)_{day} - (g_L - g_D)_{night})$ % $(g_L - g_D)_{day}$
	mmol m^{-2} s^{-1}		*mmol* m^{-2} s^{-1}	
BL #1	53	33%	50	42%
BL #2	23	22%	34	40%
BL #3	56	34%	42	34%
Mean BL	44	30%	42	39%
SD	18	7%	8	4%
SE	10	4%	5	2%
RL #1	11	22%	13	43%
RL #2	37	33%	31	41%
RL #3	34	37%	15	54%
Mean RL	28	31%	20	46%
SD	14	8%	10	7%
SE	8	4%	6	4%

ducted a series of experiments with constant WL at different photon fluence rates (Fig. 4). At low light levels (e.g. Fig. 4, a–c), there was a rhythm in g without a corresponding rhythm in **A**, just as we observed with the experiments using cycling at 1.5-h light:2.5-h dark regime. A small first peak in **A** was often observed, but no second increase in **A** corresponding to the second increase in g. With increasing light, the extent of stomatal opening was greater, the amplitude of the rhythmicity in g was correspondingly greater, and the rhythmicity in **A** increased, but in no case was this rhythm in **A** very large.

Another possible explanation for the lack of rhythmicity in **A** was that, at low light levels, the levels of *Ci* established might be saturating or nearly so. Therefore, we tested the effect of different levels of *Ci* on photosynthesis during both the subjective day and the subjective night under different light conditions: BL, RL, and WL at the level used for the experiments represented by Figures 1, 2, and 4, a to c (140 μmol m^{-2} s^{-1}), and WL at the higher level used in the experiment represented by Fig. 4, j to l (770 μmol m^{-2} s^{-1}). *Ci* values were chosen to cover the range observed in the previous experiments. Only under the highest light condition was there much change in **A**, with changes in *Ci* between about 200 and 300 μL/L during either the subjective day or subjective night. In the experiment represented in Figure 5, the **A***Ci* curves obtained during the subjective day and subjective night were nearly superimposable. In a replicate experiment, the **A***Ci* curve obtained during the subjective night for 770 μmol m^{-2} s^{-1} WL was slightly lower (less than 1 μmol m^{-2} s^{-1} at all points) than that obtained during the subjective day.

DISCUSSION

In most previous work (but not all; Deitzer and Frosch, 1990), circadian rhythms in **A** accompany rhythmic changes in g and are attributed partly to stomatal factors (differences in *Ci* caused by changes in g) and partly to nonstomatal factors (Pallas et al., 1974; Fredeen et al., 1991; Hennessey and Field, 1991). In the present experiments on *Vicia*, circadian changes in **A** were observed only at high light levels, much higher than the light conditions under which the plants were grown. Under lower light levels, the small changes in *Ci* that resulted from rhythmic changes in g did not affect changes in **A**. It is interesting that under these conditions there was no evidence for any rhythmic change in **A** derived from nonstomatal factors. A rhythmic change in **A** associated with nonstomatal factors would also be evidenced by a change in the **A***Ci* curves between subjective day and subjective night. A change such as that observed in *Phaseolus*, with lower **A** during the subjective night than during the subjective day (Fredeen et al., 1991; Hennessey and Field, 1991), would suggest a lower **A***Ci* curve during the subjective night. We observed a slight change of this sort in one experiment

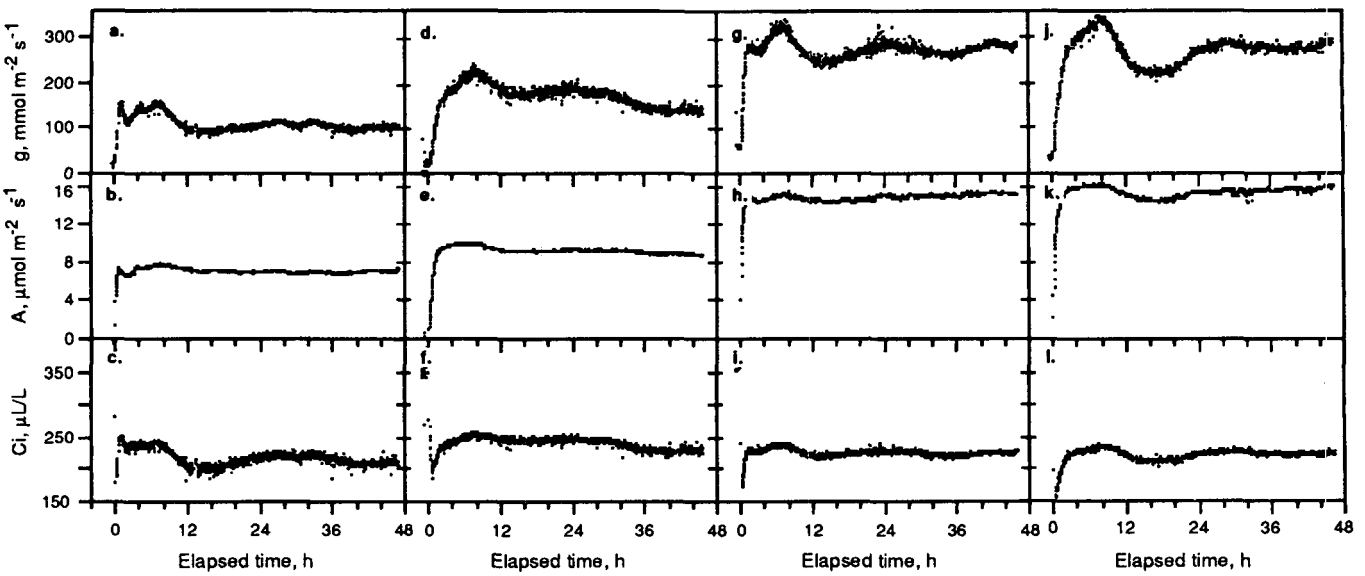

Figure 4. Changes in *g* (a, d, g, j), **A** (b, e, h, k), and *Ci* (c, f, i, l) under continuous WL at 140 μmol m^{-2} s^{-1} (a-c), 260 μmol m^{-2} s^{-1} (d-f), 400 μmol m^{-2} s^{-1} (g-i), or 770 μmol m^{-2} s^{-1} (j-l). Other conditions as in Figure 1.

for 770 μmol m^{-2} s^{-1} WL, but usually **A***Ci* curves were virtually unchanged between subjective day and subjective night (Fig. 5). Existing data suggest, then, that in *Vicia* circadian rhythms in **A** are small and predominantly stomatal in origin, whereas in *Phaseolus* they are much more robust and both stomatal and nonstomatal factors appear to be important. Differences in growth conditions or measurement techniques employed in different laboratories, as well as intrinsic differences between species, may all contribute to this difference in photosynthetic rhythmicity.

Within the resolution of the techniques used here, there were no significant differences in either phase or amplitude between circadian rhythms in overall stomatal opening under cycling at 1.5-h RL:2.5-h dark and those under cycling at 1.5-h BL:2.5-h dark (Figs. 1 and 2; Table I, second column). Nor were there significant differences in either phase or amplitude of circadian rhythms in responsiveness to BL and RL (Figs. 1 and 2; Table I, fourth column). The time of maximum responsiveness to either BL or RL was in the middle of the subjective day, as was the time of maximal *g* under constant WL. This is in contrast to earlier work with *Vicia* by Kana and Miller (1977), who used microscopic techniques to study the effect of the length of the dark period on the rate of stomatal opening in subsequent WL. They found that the maximum rate of opening was at the end of the plants' normal dark period, whether the plants were grown under L:D photoperiods of 8:16, 12:12, or 16:8 h. In addition, they saw no further increase in the rate of opening if the plants were held in the dark for an additional 4 h after the end of the normal dark period. The plants were grown under qualitatively similar conditions in both studies; it is likely that the different measurement and irradiation techniques explain the different results.

One growth parameter that should be considered when interpreting these and related results is light level. The *Vicia* plants used in these experiments were grown in a growth

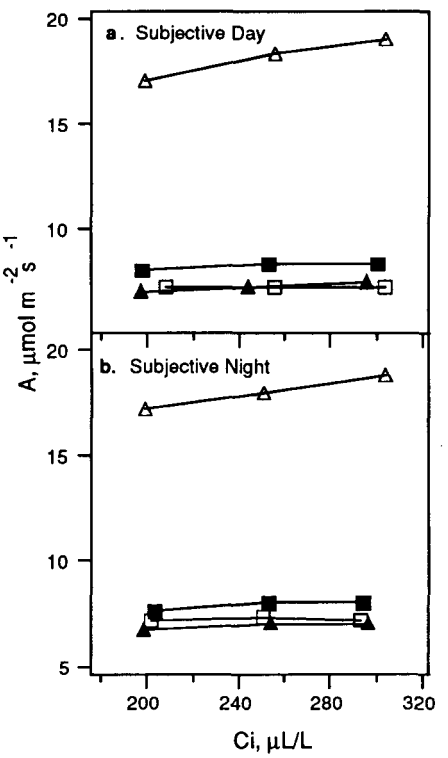

Figure 5. **A***Ci* curves for different light conditions taken during the subjective day (a) and during the subjective night (b). □, BL at 140 μmol m^{-2} s^{-1}; ■, RL at 140 μmol m^{-2} s^{-1}; ▲, WL at 140 μmol m^{-2} s^{-1}; △, WL at 770 μmol m^{-2} s^{-1}. All data were collected during 4.5 h during the middle of the subjective day or subjective night.

chamber at relatively low light, which could lead to changes in the development of stomatal photoreceptor systems (Assmann, 1992; Assmann et al., 1992). Nevertheless, the differences in responses to BL and RL reported here are similar to results from many species grown under a variety of conditions (Sharkey and Raschke, 1981; Zeiger et al., 1981, 1983; Sharkey and Ogawa, 1987): whereas **A** established under RL was similar to that established under BL, *g* was almost twice as high under BL (Figs. 1–3). Although chamber-grown and field-grown plants will have many differences, both appear to have distinct stomatal responses to RL and BL (e.g. Assmann et al., 1992). Plants adapted to high light intensities would be more likely to withstand the continuous, saturating RL necessary for dual-beam experiments (Iino et al., 1985; Zeiger et al., 1985). Such experiments would be a valuable additional test of our conclusions about circadian rhythmic responses to BL.

Two of the main functions of circadian rhythmicity are to provide temporal coordination of the multitude of biochemical and physiological processes within an organism and to assure that these events are properly timed with respect to the daily environmental cycle. In nyctinastic plants, for example, circadian rhythmicity in membrane channel activity, H^+ pumping, and ion transport must be properly coordinated with each other in two distinct pulvinar regions to bring about leaf opening, and they must occur at the proper time of day to effect opening during the day and closure at night (Lee, 1990; Moran, 1990). In mammals, a host of endocrine, cytological, and physiological changes occur daily, each peaking at a different time (Moore-Ede et al., 1982). Normally, the timing of a rhythm makes functional sense: circadian control of stomatal aperture causes opening during the subjective day, when photosynthesis normally would occur, not during the subjective night. The observation that rhythms in sensitivity to BL and RL both peak at about the middle of the subjective day suggests that these two stomatal light responses might normally have their main function at that time, when natural light level normally peaks and demand for CO_2 would be greatest. This observation would argue, albeit in a speculative way, against a role of the BL response strictly at dawn (Zeiger et al., 1981), but it is consistent with a role of the BL response in controlling stomatal opening during sunflecks (Zeiger and Field, 1982; Zeiger, 1990).

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LITERATURE CITED

Aschoff J (1978) Circadian rhythms within and outside their ranges of entrainment. *In* I Assenmacher, DS Farner, eds, Environmental Endocrinology. Springer-Verlag, New York, pp 172–181

Assmann SM (1992) Effects of light quantity and quality during development on the morphology and stomatal physiology of *Commelina communis*. Oecologia **92:** 188–195

Assmann SM, Lee DM, Malkus P (1992) Rapid stomatal responses to red light in *Zea mays*. Photochem Photobiol **56:** 685–689

Brogårdh T, Johnsson A (1975) Regulation of transpiration in *Avena*: responses to white light steps. Physiol Plant **35:** 115–125

Brun WA (1962) Rhythmic stomatal opening responses in banana leaves. Physiol Plant **15:** 623–630

Darwin F (1898) IX. Observations on stomata. Phil Trans R Soc Lond B **190:** 531–621

Deitzer GF, Frosch S (1990) Multiple action of far-red light in photoperiodic induction and circadian rhythmicity. Photochem Photobiol **52:** 173–180

Dodge SM, Marsh PB, Tallman G (1992) Comparison of physiological responses of guard cell protoplasts of *Nicotiana glauca* isolated from leaves collected before dawn or at midday. Physiol Plant **86:** 221–230

Field CB, Ball JT, Berry JA (1991) Photosynthesis: principles and field techniques. *In* RW Pearcy, J Ehleringer, HA Mooney, PW Rundel, eds, Plant Physiological Ecology. Chapman and Hall, London, pp 209–253

Fredeen AL, Hennessey TL, Field CB (1991) Biochemical correlates of the circadian rhythm in photosynthesis in *Phaseolus vulgaris*. Plant Physiol **97:** 415–419

Gorton HL (1990) Stomates and pulvini: a comparison of two rhythmic, turgor-mediated movement systems. *In* RL Satter, HL Gorton, TC Vogelmann, eds, The Pulvinus: Motor Organ for Leaf Movement. American Society of Plant Physiologists, Rockville, MD, pp 223–237

Gorton HL, Williams WE, Binns ME (1989a) Repeated measurements of aperture for individual stomates. Plant Physiol **89:** 387–390

Gorton HL, Williams WE, Binns ME, Gemmell CN, Leheny EA, Shepherd AC (1989b) Circadian stomatal rhythms in epidermal peels from *Vicia faba*. Plant Physiol **90:** 1329–1334

Grantz DA (1990) Plant response to atmospheric humidity. Plant Cell Environ **13:** 667–679

Heath OVS, Mansfield TA (1962) A recording porometer with detachable cups operating on four separate leaves. Proc R Soc B **156:** 1–13

Hennessey TL, Field CB (1991) Circadian rhythms in photosynthesis. Oscillations in carbon assimilation and stomatal conductance under constant conditions. Plant Physiol **96:** 831–836

Holmes MG, Klein WH (1986) Photocontrol of dark circadian rhythms in stomata of *Phaseolus vulgaris* L. Plant Physiol **82:** 28–33

Iino M, Ogawa T, Zeiger E (1985) Kinetic properties of the blue-light response of stomata. Proc Natl Acad Sci USA **82:** 8019–8023

Kana TM, Miller JH (1977) Effect of photoperiod on stomatal opening in *Vicia faba*. Plant Physiol **60:** 803–804

Karlsson PE (1986) Blue light regulation of stomata in wheat seedlings. II. Action spectrum and search for action dichroism. Physiol Plant **66:** 207–210

Lee Y (1990) Ion movements that control pulvinar curvature in nyctinastic legumes. *In* RL Satter, HL Gorton, TC Vogelmann, eds, The Pulvinus: Motor Organ for Leaf Movement. American Society of Plant Physiologists, Rockville, MD, pp 130–141

Lonergan TA (1981) A circadian rhythm in the rate of light-induced electron flow in three leguminous species. Plant Physiol **68:** 1041–1046

Mansfield TA (1963) Length of night as a factor determining stomatal behaviour in soybean. Physiol Plant **16:** 523–529

Mansfield TA, Heath OVS (1961) Photoperiod effects on stomatal behavior in *Xanthium pennsylvanicum*. Nature **191:** 974–975

Mansfield TA, Heath OVS (1963) Studies in stomatal behaviour. IX. Photoperiodic effects on rhythmic phenomena in *Xanthium pennsylvanicum*. J Exp Bot **14:** 334–352

Mansfield TA, Heath OVS (1964) Studies in stomatal behaviour. X. An investigation of responses to low-intensity illumination and temperature in *Xanthium pennsylvanicum*. J Exp Bot **15:** 114–124

Martin ES, Meidner H (1971) Endogenous stomatal movements in *Tradescantia virginiana*. New Phytol **70:** 923–928

Meidner H, Mansfield TA (1968) The role of rhythms in stomatal behaviour. *In* Physiology of Stomata. McGraw-Hill, New York, pp 102–169

Moore-Ede MC, Sulzman FM, Fuller CA (1982) The Clocks that Time Us. Harvard University Press, Cambridge, MA

Moran N (1990) The role of ion channels in osmotic volume changes in *Samanea* motor cells. *In* RL Satter, HL Gorton, TC Vogelmann, eds, The Pulvinus: Motor Organ for Leaf Movement. American Society of Plant Physiologists, Rockville, MD, pp 142–159

Morison JIL (1987) Intercellular CO_2 concentration and stomatal response to CO_2. *In* E Zeiger, GD Farquhar, IR Cowan, eds, Stomatal Function. Stanford University Press, Stanford, CA, pp 229–251

Nixon EH, Markhart AH III, Koukkari WL (1987) Stomatal aperture oscillations of *Abutilon theophrasti* Medic. and *Hordeum vulgare* L. examined by three techniques. *In* JE Pauly, LE Scheving, eds, Advances in Chronobiology Part A. Alan R Liss, New York, pp 67–79

Ogawa T, Ishikawa H, Shimada K, Shibata K (1978) Synergistic action of red and blue light and action spectra for malate formation in guard cells of *Vicia faba* L. Planta **142:** 61–65

Pallas JE Jr, Samish YB, Willmer CM (1974) Endogenous rhythmic activity of photosynthesis, transpiration, dark respiration, and carbon dioxide compensation point of peanut leaves. Plant Physiol **53:** 907–911

Scarth GW, Shaw M (1951) Stomatal movement and photosynthesis in *Pelargonium*. I. Effects of light and carbon dioxide. Plant Physiol **26:** 207–225

Schulze E-D, Turner NC, Gollan T, Shackel KA (1987) Stomatal responses to air humidity and soil drought. *In* E Zeiger, GD Farquhar, IR Cowan, eds, Stomatal Function. Stanford University Press, Stanford, CA, pp 311–321

Sharkey TD, Ogawa T (1987) Stomatal responses to light. *In* E Zeiger, GD Farquhar, IR Cowan, eds, Stomatal Function. Stanford University Press, Stanford, CA, pp 195–208

Sharkey TD, Raschke K (1981) Effect of light quality on stomatal opening in leaves of *Xanthium strumarium* L. Plant Physiol **68:** 1170–1174

Stålfelt MG (1963) Diurnal dark reactions in the stomatal movements. Physiol Plant **16:** 756–766

Stålfelt MG (1967) Endogenous diurnal rhythm in the osmotic surplus of the guard cells. Physiol Plant **20:** 57–64

Tribukait B (1956) Die aktivitatsperiodik der wissen maus im kunnsttag von 16 bis 29 studen lange. Z Vgl Physiol **38:** 479–490

Zeiger E (1990) Light perception in guard cells. Plant Cell Environ **13:** 739–747

Zeiger E, Assmann SM, Meidner H (1983) The photobiology of *Paphiopedilum* stomata: opening under blue but not red light. Photochem Photobiol **38:** 627–630

Zeiger E, Field C (1982) Photocontrol of the functional coupling between photosynthesis and stomatal conductance in the intact leaf. Blue light and PAR-dependent photosystems in guard cells. Plant Physiol **70:** 370–375

Zeiger E, Field C, Mooney HA (1981) Stomatal opening at dawn: possible roles of blue light response in nature. *In* H Smith, ed, Plants and the Daylight Spectrum. Academic Press, New York, pp 391–407

Zeiger E, Iino M, Ogawa T (1985) The blue light response of stomata: pulse kinetics and some mechanistic implications. Photochem Photobiol **42:** 759–763

Zeiger E, Iino M, Shimazaki K-I, Ogawa T (1987) The blue-light response of stomata: mechanism and function. *In* E Zeiger, GD Farquhar, IR Cowan, eds, Stomatal Function. Stanford University Press, Stanford, CA, pp 209–227