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Phytochrome-controlled Nyctinasty in *Albizzia julibrissin*

III. INTERACTIONS BETWEEN AN ENDOGENOUS RHYTHM AND PHYTOCHROME IN CONTROL OF POTASSIUM FLUX AND LEAFLET MOVEMENT[1]

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ABSTRACT

Prolonged irradiation during appropriate parts of the diurnal cycle promotes the opening of *Albizzia julibrissin* leaflets. Leaflets also open without illumination, but such opening starts later and is slower and less complete. Opening in the dark is accompanied by lower potassium efflux from dorsal pulvinule motor cells but equal or greater potassium movement into ventral motor cells than occurs during opening in the light. Far red-absorbing phytochrome inhibits opening in the dark, indicating that its action is similar during endogenously controlled opening and nyctinastic closure; *i.e.*, a high far red-absorbing phytochrome level is associated with low potassium content in ventral motor cells, high potassium content in dorsal motor cells, and a small angle between leaflets.

When open leaflets are darkened, there is an immediate and large potassium flux into dorsal motor cells. This is initially independent of red and far red preirradiation, but prior red light appears to promote continued potassium movement into dorsal cells during the latter part of a 90-minute dark period. The situation in ventral motor cells is different; here the effect of prior red or far red irradiation on potassium efflux is evident after 10 minutes of darkness. Phytochrome controls the direction of potassium movement in ventral motor cells during the early part of the dark period (to 25 minutes); potassium moves out of ventral motor cells if leaflets are preirradiated with red light and into these same cells if leaflets are preirradiated with far red light. Kinetic data are consistent with the suggestion that potassium leaving ventral cells enters dorsal cells. However, there must be an additional source of potassium entering dorsal cells since this potassium movement precedes potassium efflux from ventral cells.

Pulvinules excised from the lamina or rachilla open and close in response to light and darkness and also move during extended periods of constant intensity light or uninterrupted darkness. This shows that the photoreceptor controlling opening and the oscillator controlling endogenously rhythmic leaflet movement are localized in the pulvinule. In addition, all the potassium that enters expanding cells during leaflet movement and the energy for potassium transport must be available from within the pulvinule.

If leaflets are darkened late in the photoperiod, they close more rapidly and show lower inhibition by far red preirradiation or anaerobic conditions. A rhythmic increase in potassium efflux from ventral motor cells appears to be the basis for rhythmic promotion of nyctinastic closure. We suggest this is due to a rhythmic increase in the leakiness of ventral motor cells.

In many species of plants, leaflet movement is regulated by phytochrome and by endogenous rhythms (7, 9, 10, 18). We have studied both phytochrome-controlled nyctinasty (16) and endogenously rhythmic leaflet opening and closure (15) in *Albizzia julibrissin* and have reported that K flux in key pulvinule cells is the crucial event controlling leaflet movement, no matter which regulatory system predominates. In our previous investigations, we attempted to separate the effects of these regulatory systems, *e.g.*, by conducting experiments on phytochrome-controlled nyctinasty during the portion of the diurnal cycle when rhythmic interference is minimal. However, the interaction of these two regulatory systems is also of great interest, particularly since they jointly control other developmental processes such as floral initiation (4, 5) in addition to leaflet movement. The present study was undertaken to explore mechanistic similarities and differences between the two regulatory systems and hopefully to obtain some additional understanding of the mode of action of phytochrome and of the biological clock controlling endogenous circadian rhythms.

MATERIALS AND METHODS

A. julibrissin plants were grown from seed in the greenhouse and were transferred to controlled growth chambers at least 3 days before experiments. Growth conditions, experimental procedures, and light sources for phytochrome experiments were the same as previously described (16).

Irradiation to convert phytochrome to the Pr or Pfr form consisted of R[2] (4 min) followed by FR (1.5 min) or FR (1.5 min) followed by R (4 min). This assured that leaflets in both groups received equal radiant energy. Leaflets in white light prior to darkness were irradiated with R or FR alone.

The leaflet movement studies described in Figures 1 to 4 and Table I utilized pinnule pairs that were excised from an intact plant and floated on water in Petri dishes. For the experiments described in Table II, on the other hand, leaflets remained on the intact plant during the opening process. This explains the more complete opening in the dark in Table II compared to Figure 1, since excision retards the opening process.

Potassium in frozen, cryostated, lyophilized pulvinule sec-

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[2] Abbreviations: DNP: 2,4-dinitrophenol; FR: far red light; R: red light.

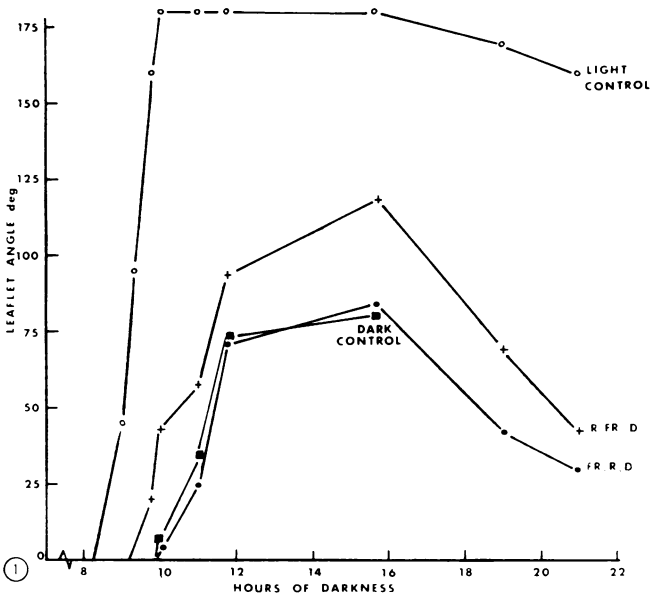


FIG. 1. Phytochrome control of leaflet opening in the dark. Closed leaflet pairs were excised from a plant darkened for 8 hr and given a short FR irradiation followed by R, or R followed by FR, immediately after excision. They were floated on water in Petri dishes and were kept in the dark for 13 additional hr. One group of control leaflets was kept in the dark without R or FR irradiation, while another group was exposed to continuous white light of about 1000 ft-c.

tions was analyzed with an Acton electron microprobe, as previously described (16). Preparative procedures were also the same as before except that pinnule pairs were taken directly from the intact plant rather than from excised pinnae for the experiment described in Table II. In experiments testing phytochrome control of closure (Figs. 5 and 6 and Table III), pinnae were excised and their cut ends were placed in water just prior to R or FR preirradiation and darkening.

All pinnule pairs for a given experiment were taken from the central region of centrally located pinnae. A preliminary experiment revealed that motor cells in pulvinules from these pinnules had about the same K content. Another preliminary experiment indicated that excision of a large number of pulvinules from one leaf had no effect on K movement in other pulvinules excised from the same leaf 24 hr later. These results provide the rationale for the experiments described in Table II.

Changes in K or in the ratio of K to Ca, which was used as a standard to correct for changes in cell volume during leaflet movement, indicate K flux (16).

RESULTS

JOINT RHYTHMIC AND PHYTOCHROME CONTROL OF LEAFLET MOVEMENT

Leaflet Opening. Light promotes the opening of *Albizzia* leaflets, but leaflets will open without irradiation during a long dark period (15). To determine whether the Pfr level influences rhythmic leaflet movement in the dark, closed leaflet pairs, excised from a plant at the end of the usual 8-hr dark period, were floated on water and kept in the dark for 13 additional hr except for brief R-FR or FR-R irradiations immediately after excision. Figure 1 shows that both phytochrome and the endogenous rhythm control opening in the dark. Leaflets started to open earlier and opened more completely if the Pfr level was low. The opening of dark controls was almost the same as that of leaflets given a short exposure to FR followed by R at the 8th hr of the dark period, which suggests that Pfr was stable during 8 or more hr of darkness.

We tested the effects of NaN_3, DNP, and anaerobiosis on opening in the dark to determine whether opening is an energy-requiring process. Leaflet opening was inhibited by 0.5 mM NaN_3 and by 0.5 mM (R-FR) or 1.0 mM (FR-R) DNP (Fig. 2A) and almost completely prevented if leaflets floating on H_2O were in a nitrogen atmosphere (Fig. 2B). These results suggest that opening requires metabolic energy, probably for K transport, since K flux into ventral and out of dorsal motor cells is the basis for leaflet opening (15, 16).

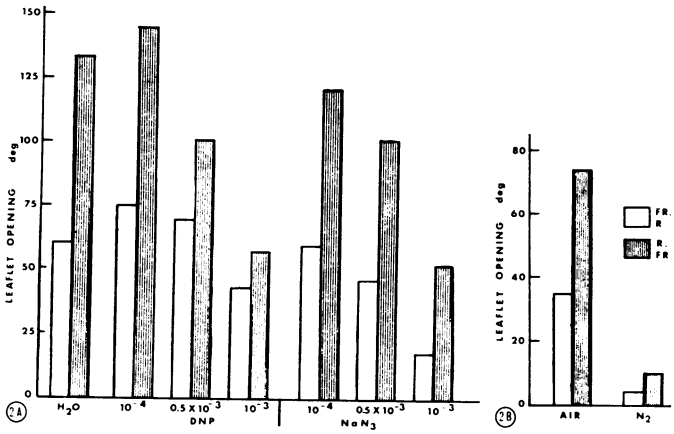


FIG. 2. Opening in the dark of leaflets floating on DNP or NaN_3 in air, or on water in a N_2 atmosphere. Closed leaflet pairs were excised from a plant that had been darkened for 8 hr. A: Leaflets were floated on 0.1, 0.5, and 1.0 mM DNP or NaN_3 in covered Petri dishes in air. B: Leaflets were floated on water in open Petri dishes enclosed in Lucite chambers that were flushed with N_2 and then sealed. All leaflets were exposed to a short FR irradiation followed by R, or R followed by FR, and then kept in the dark for 2.5 additional hr. Leaflet angles were measured at this time.

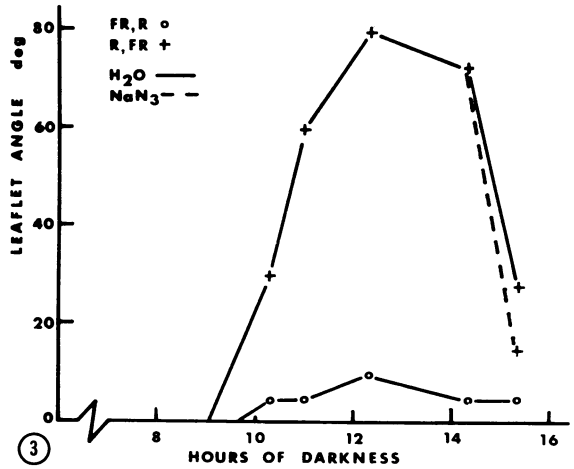


FIG. 3. Effect of NaN_3 on endogenous rhythmic closure of leaflets that opened in the dark. Closed leaflet pairs were excised from a plant that had been darkened for 8 hr and were floated on water in Petri dishes. They were irradiated briefly with R, FR or FR, R immediately after excision and were kept in the dark for 7 additional hr. R, FR irradiated leaflets were transferred to 0.5 and 1.0 mM NaN_3 at the indicated time. Both concentrations of NaN_3 had the same effect on leaflet movement.

Table I. *Effect of Length of Dark Period on Subsequent Rate of Opening in White Light*

Pinnule pairs, excised from a darkened plant and floated on water, were irradiated with white light (10 kiloergs cm^{-2} sec^{-1}) for 1 hr.

Duration of Dark Period	Rate of Opening in Subsequent White Light
hr	*degrees/hr*
2.5	0
4	1
5.5	46 ± 15
7	96 ± 11
8	144 ± 5

Table II. *Electron Microprobe Analysis of K Flux in Dorsal and Ventral Motor Cells of Leaflets That Opened in the Light (Part A) and in the Dark (Part B)*

All leaflets were taken from the central region of central pinnae of the same leaf, on a plant grown under its usual light conditions and 16-hr photoperiod except that the final dark period was extended 1.5 hr. Time was measured from the beginning of the most recent dark period; leaflets for part B were excised about 24 hr after those for part A. At stated intervals, the average angle between the leaflets was measured and six pairs of pulvinules were excised, trimmed, and frozen in modified Tissue Tek at −30 C in a cryostat. Longitudinal dorsi-ventral sections were cut at 24 μ, freeze-dried, and mounted on graphite rods for Acton electron microprobe analysis of K and Ca in dorsal and ventral motor cells. Measured values are scintillations during 25 sec. Changes in K or in K/Ca indicate K flux. Each figure is an average of 36 measurements (6 sections, 3 areas per section, two measurements per area). Standard deviations are included. See Ref. 16 for further details.

Part	Time	Angle between Leaflets	Light (L) or Dark (D)	Ventral		Dorsal	
				K	K/Ca	K	K/Ca
	hr	*degrees*					
A	5	0	D	197 ± 35[1]	0.84 ± 0.17	233 ± 34	1.26 ± 0.19
	7.5	0	D	184 ± 54	0.86 ± 0.20	257 ± 67	1.14 ± 0.26
	8	0	L	...	...	...	...
	9	165	L	181 ± 35	0.87 ± 0.13	185 ± 25	0.97 ± 0.14
	10.3	180	L	202 ± 26	0.92 ± 0.15	167 ± 41	0.66 ± 0.09
B	2	0	D	137 ± 29	0.61 ± 0.13	232 ± 46	1.20 ± 0.24
	5.3	0	D	171 ± 43	0.64 ± 0.14	238 ± 30	1.03 ± 0.23
	7.5	90	D	199 ± 32	0.92 ± 0.21	225 ± 51	0.97 ± 0.13
	9.5	150	D	219 ± 45	1.32 ± 0.13	198 ± 36	0.85 ± 0.08

[1] Values are scintillations/25 sec ± SD.

Leaflets that open in the dark remain opened for about 5 to 6 hr and then begin to close (Fig. 1). Such closure was not inhibited and was even facilitated if leaflets were floated on 0.5 mM NaN_3 for 1 hr (Fig. 3). This may indicate that such closure after prolonged darkness is due to a nonenergetic leaking of K from ventral motor cells.

We also studied the interaction of white light and the rhythm on leaflet opening. Closed leaflet pairs excised from a plant that had been in the dark for varying periods of time were irradiated with white light. The duration of the dark period affected the rate of opening during subsequent illumination (Table I). The blue portion of the spectrum promoted opening most effectively, confirming the results of Jaffe and Galston (10), although they irradiated leaflets with 15 kiloergs cm^{-2} sec^{-1}, while in these investigations the rate of opening was maximal if the blue irradiance was as low as 1 kiloerg cm^{-2} sec^{-1}. Blue was also the most effective spectral region in maintaining leaflets in an open state.

Electron microprobe analysis of K flux in motor cells revealed that during leaflet opening in the light K movement is mainly out of dorsal motor cells, with little change in ventral cells. In the dark, however, K entry into ventral cells exceeds K efflux from dorsal motor cells (15 and Table II).

Nyctinastic Closure. Leaflets grown under a 16-hr photoperiod close if they are darkened during the usual photoperiod. Red irradiation before darkness promotes such closure, but this promotive effect diminishes with increasing length of the light period preceding darkening. Also, leaflets close more rapidly if they are darkened late rather than early in the photoperiod (9, 10, 16 and Fig. 4). These data have led to the conclusion that leaflets show a rhythmic response to the closure stimulus provided by darkness.

Previous investigators studying phytochrome-controlled nyctinasty conducted most of their experiments early in the photoperiod (9–11, 16). Nyctinastic closure at this time requires metabolic energy; inhibition by anaerobiosis was close to 100% in some experiments (16). To ascertain whether the aerobic requirement persists if leaflets are darkened late in the photoperiod, when phytochrome exerts less control over closure, we tested the effect of anaerobiosis on the closure of leaflets exposed to varying periods in the light, irradiated with R or FR and then darkened (Fig. 4). It is interesting to note that: (*a*) there is a parallel decrease in the requirements for oxygen and R preirradiation with increase in the length of the predarkness light period, (*b*) all leaflets closed more rapidly if darkened late rather than early in the photoperiod, and (*c*) R or FR preirradiation had no effect on the closure of leaflets in a N_2 atmosphere. These data suggest that nyctinastic closure is promoted by a rhythmic process as well as by phytochrome, and that the rhythmic process can proceed in air or in nitro-

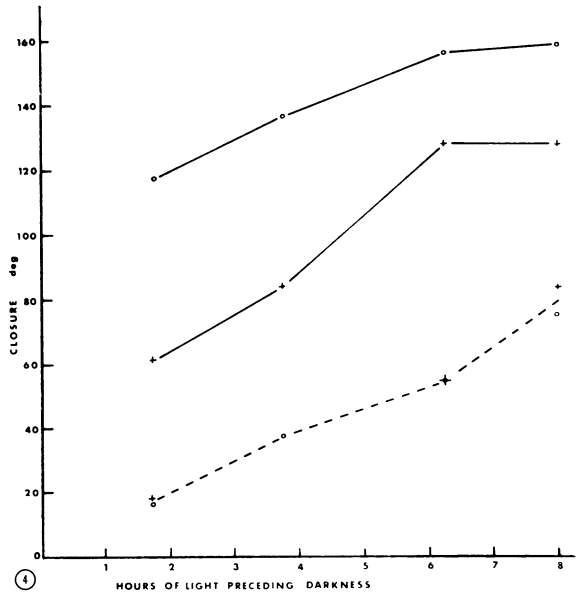


FIG. 4. Closure in air or in nitrogen of leaflets preirradiated with R or FR and darkened after varying periods in the light. Open leaflet pairs excised from a mature leaf of a plant in the light were floated on water in covered Petri dishes in air, or in open Petri dishes enclosed in Lucite chambers that were flushed with N_2 and then sealed. Leaflets were irradiated with R or FR followed by darkness. Closure angles were measured 1 hr later. (R ○, FR +, Air —, N_2 – – –).

gen, although the phytochrome-controlled process is strictly aerobic.

KINETIC ANALYSIS OF K FLUX DURING NYCTINASTIC CLOSURE

Nyctinastic closure is the consequence of K flux into dorsal and out of ventral motor cells; R preirradiation is required for K efflux from ventral motor cells and for optimal K movement into dorsal motor cells (16). In our previous experiments, K measurements were made after leaflets had been darkened for about 90 min and leaflet movement had virtually ceased. In the present investigation, we made comparative kinetic analyses of K flux and leaflet movement. However, there is considerable variation in the physiological behavior of leaflets taken from different plants (9, 10, 16); *e.g.*, the nyctinastic closure of leaflets darkened after 1 hr of light is under stringent phytochrome control in leaflets from some plants but is less rigorously controlled by phytochrome if leaflets are taken from other plants. We studied the behavior of representative leaflets from both populations. Leaflets in the former group hardly moved in an anaerobic atmosphere, but leaflets in the latter group closed 90° under the same conditions. Closure in N_2 was independent of R or FR preirradiation. If leaflet closure is stringently controlled by phytochrome, FR-preirradiated leaflets close slightly during the first 20 to 30 min of the dark period and then reopen (Fig. 5), but if phytochrome control is less rigid, similarly irradiated leaflets continue their closure during the entire dark period (Fig. 6). Opening in the dark and nyctinastic closure also differ in these two populations. If leaflets are in the former group, opening in the dark is under strict phytochrome control and leaflets darkened for 10.5 hr except for a short R-FR light break open almost completely (*e.g.*, R-FR 133° and FR-R 60°), but if leaflets are in the latter group their opening is less rigorously controlled by phytochrome and they open only slightly under these same conditions (*e.g.*, R-FR 56° and FR-R 14°).

Thus we conducted two similar kinetic analyses of nyctinastic closure, each with leaflets from a different population. We followed the same procedure in both experiments: several pinnae were excised from the same leaf of a plant in the light, and their cut ends were placed in water. One pinna was kept in the light as a control, while the others were irradiated with R or FR light and then darkened. After about 10, 25, or 90 min of darkness, pairs of pulvinules were excised and frozen in preparation for microprobe analysis.

In both of these experiments (Figs. 5 and 6 and Table III), the direction of K movement in ventral motor tissue during the early part of the dark period was controlled by phytochrome. The effect of prior R or FR irradiation was most evident between the 10th and 25th min of the dark period; K moved out of ventral motor cells if leaflets were exposed to R light and into these same cells if leaflets were exposed to FR light. Thus phytochrome controls the direction of K movement in the ventral motor cells during part of the dark period.

The flux direction during the latter part of the dark period was independent of prior exposure to R or FR light but differed for the two populations of leaflets. K moved into ventral motor cells in Figure 5 and out of these same cells in Figure 6. Thus differences in the physiological behavior of leaflets from the two populations can be attributed to differences in the K permeability of their ventral cell membranes. It is likely that some of the K leaving ventral motor cells in Figure 6 diffused out passively, since these leaflets closed partially under anaerobic conditions. Thus we infer that leaflets with dark closure not strictly controlled by phytochrome have leaky ventral cell membranes.

In these as in all previous experiments, darkening caused a large increase in the K content of dorsal motor cells whether leaflets were preirradiated with R or FR (16). K flux into dorsal cells was very pronounced during the first 15 min of the dark period; the K content of these cells increased about 30 to 65% during this interval. Red preirradiation was promotive in Figure 5 and in most similar experiments we conducted.

THE MOVEMENT OF PULVINULES WITH LAMINAE REMOVED

We excised open leaflet pairs from a plant in the light, carefully removed the laminar tissue and all the rachilla except the small piece connecting the two pulvinules, and floated these delaminated pulvinules on water. They closed when darkened, even though controls in the light remained wide open. We also conducted an experiment with the reverse conditions; *i.e.*, the lamina was removed from closed pinnule pairs in the dark prior to irradiation with white light. These pulvinule pairs opened in the light, while dark controls remained closed. In addition, delaminated pulvinule pairs excised from a closed, darkened plant opened during a long dark period, while those excised from an illuminated, open plant closed during a long photoperiod, just as did controls with laminae. However, pulvinule pairs without laminae gradually lose their mobility. Their movement is similar to that of complete leaflet pairs during the 3- to 4-hr period following laminar removal.

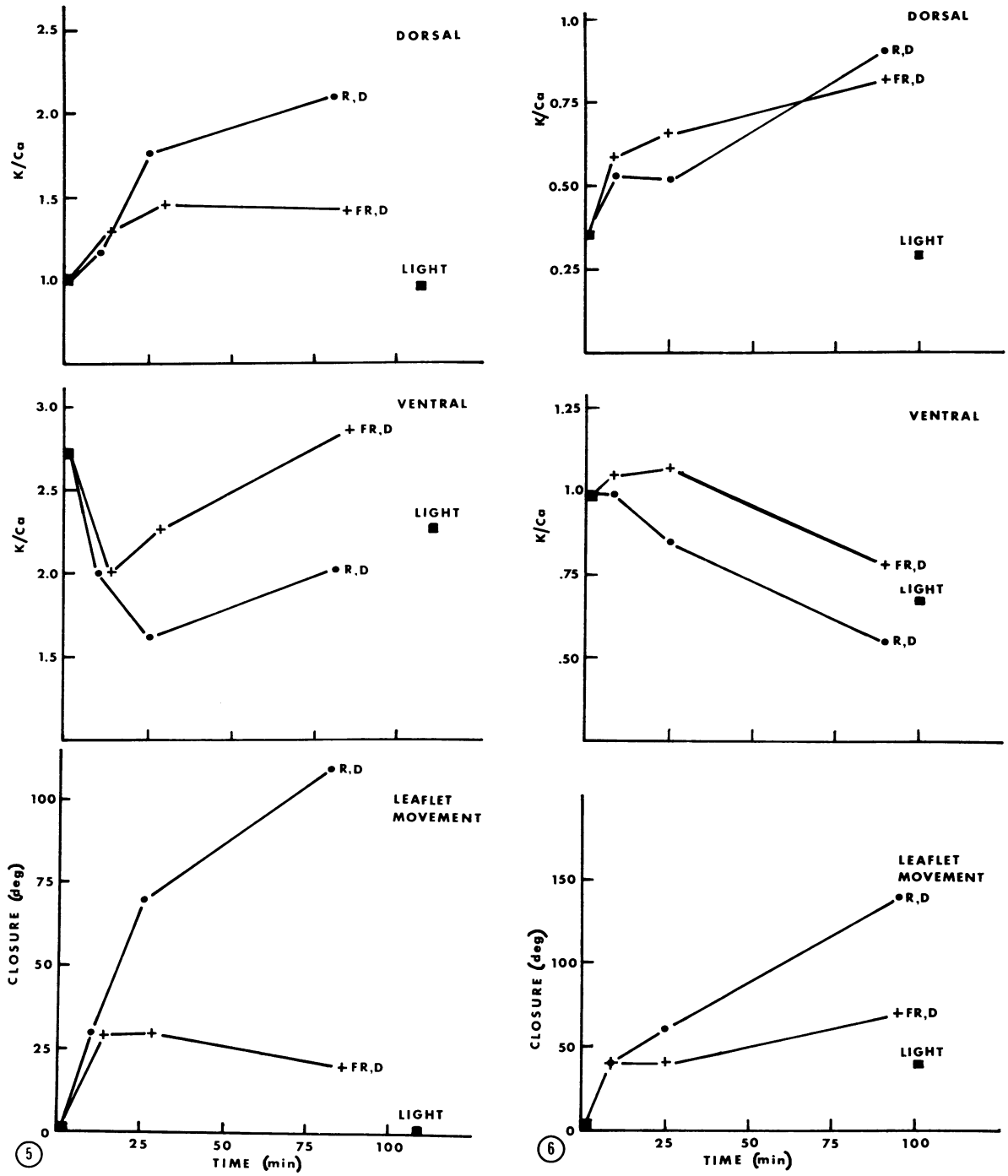
In another experiment, we detached the pinnule, including the pulvinule, from the rachilla, and found that this did not alter pulvinule movement in either the light or the dark. Thus, turgor changes in pulvinar motor cells are controlled by light, darkness, and the endogenous rhythm, even when the pulvinule is detached from the lamina or rachilla. Koukkari and Hillman (11) reported that the phytochrome controlling *Albizzia* leaflet movement is located within the pulvinule. Our experiments indicate that the blue photoreceptor is similarly localized, as is the oscillator that controls endogenous leaflet movement. Also, all or most of the K that enters expanding motor cells during leaflet movement must be stored within the pulvinule, and pulvinar cells must supply the energy for K transport.

DISCUSSION

To facilitate the ensuing discussion we have summarized the kinds of leaflet movements observed and our understanding of the physiological and chemical basis for such movement (Table IV).

Leaflet Opening. Leaflets illuminated with white light opened at a rate that increased with the length of the preceding dark period but did not open at all if the dark period was less than 4 hr (Table I). Thus blue or white light can advance the opening phase of the leaflet movement rhythm, but only if light is given at an appropriate time, presumably after metabolic changes prerequisite to opening have been completed. When the dark period was 10 hr long, leaflets opened without illumination (Fig. 1). Thus the pervasive influence of the endogenous rhythm controls leaflet opening both in the light and in the dark.

Leaflets open when K moves into ventral and out of dorsal motor cells (Table II). Absorption of blue light controls both the opening response and the inhibition of closure by continuous irradiation. There is considerable evidence suggesting that the blue-absorbing photoreceptor might be a photosynthetic pigment. Photosynthesis would provide energy for K transport; also, photosynthetic pigments appear to control the effect of light on K flux in stomatal guard cells (12, 19, 22) and in giant algal cells (20). In addition, light-dark and the



FIGS. 5 and 6. Kinetic analysis of phytochrome controlled leaflet movement and K flux. These experiments were conducted on different days with different plants, but with the same procedures. Several pinnae with open leaflets were excised from a plant in the light at photoperiod hr 1.5. They were irradiated with R or FR light followed by darkness, or they were kept in the light (control). Pinnae treated with FR light were irradiated 6 min after those irradiated with R light, so that pulvinules receiving both treatments could be kept in the dark for about the same period of time before they were excised, trimmed, and frozen in preparation for microprobe analysis. K and Ca scintillations/25 sec were measured in the electron microprobe, as described in Table II. Changes in K/Ca indicate K flux. Leaflet closure in Figure 5 was under stringent phytochrome control, but closure in Figure 6 was less rigorously controlled by phytochrome. Additional data from these same experiments are presented in tabular form in Table III.

reverse transition causes H^+-K^+ exchange between isolated chloroplasts and their bathing solution (6, 13, 14).

Our observation that closed *Albizzia* pulvinules excised from the rachilla or lamina in the dark open in response to light suggests that the photoreceptor controlling opening is localized within the pulvinule. If a photosynthetic pigment, it could be in the motor cells, which contain a normal but not unusually large number of chloroplasts, or in the inner cortical cells surrounding the vascular core. Most of the photosynthetic tissue in the pulvinule is localized in these latter cells (17).

Microprobe analysis revealed that K efflux from dorsal cells was primarily responsible for leaflet opening in the light

but K flux into ventral cells was the most significant K movement during leaflet opening in the dark (Table II). These results are consistent with other data showing that (*a*) the K content of dorsal motor cells is higher when leaflets are in the dark rather than in the light (Figs. 5 and 6, Table III and [15, 16]), and (*b*) there is substantial K movement into the ventral motor cells when leaflets are darkened (Fig. 5).

The phytochrome system has the same effect on opening in the dark as it has on nyctinastic closure; an increase in the Pfr level causes a reduction in the angle between leaflets (Figs. 1, 5, and 6). Thus Pfr inhibits K movement either into ventral motor cells or out of dorsal motor cells or both, during leaflet opening as well as during nyctinastic closure. Opening in the dark appears to require metabolic energy (Fig. 2).

Nyctinastic Closure: Kinetic Analysis. The rapid initial closure of darkened leaflets is due primarily to K movement into dorsal cells. In Figure 6, the K content of dorsal motor cells increased 65% during the first 10 min of the dark period while the K content of ventral motor cells either remained constant (R preirradiated) or increased slightly (FR preirradiated). Thus the K entering dorsal motor cells early in the dark period cannot come from ventral motor cells although it must be stored within the pulvinule, since excised pulvinules close when darkened. Our kinetic curves (Figs. 5 and 6) suggest, however, that K leaving ventral motor cells might enter dorsal motor cells and thus be responsible for the increase in the K content of dorsal motor cells during the latter part of the dark period. Thus the promotive effect of R irradiation on K flux into dorsal cells might be a consequence of its promotion of K efflux from ventral motor cells. If K moves from ventral to dorsal cells, it could move through cell membranes as previously suggested (16) or it might move from cell to cell via plasmodesmata.

Although the K content of the dorsal motor cells of both R- and FR-pretreated leaflets always increases when leaflets are darkened, R preirradiation is required for K efflux from the ventral motor cells when closure is under stringent phytochrome control (Fig. 5 and [16]). The effect of prior R on K flux is evident about 10 min after R irradiation. The state of phytochrome determines the direction of K flux during the 10 to 25 min of the dark period; K moves *out* of ventral motor cells if leaflets are preirradiated with R and *into* these same cells if leaflets are preirradiated with FR (Figs. 5 and 6). These data can be interpreted to indicate activity by both Pr and Pfr; an alternative explanation is that K moves into ventral as well as dorsal motor cells of darkened leaflets, but this influx can be masked by Pfr-promoted active secretion of K ions through ventral cell membranes. We favor the latter view, since there is little evidence from other species suggesting that Pr is physiologically active. The latter view is also supported by the substantial K flux into ventral cells during endogenously controlled opening in the dark (Table II).

Interaction of the Endogenous Rhythm and the Phytochrome System. K movement both into dorsal and out of ventral motor cells is required for leaflet closure (16). K always moves into the dorsal cells of darkened leaflets (Figs. 5 and 6) but FR preirradiation can prevent K efflux from ventral cells if leaflets are darkened early in the photoperiod (Fig. 5). Thus it is reasonable to expect that the rhythm, which reduces the inhibitory effect of FR and promotes the closure of all leaflets darkened late in the photoperiod (Fig. 4), does so by promoting K efflux from ventral motor cells. We previously reported (16) that phytochrome-controlled nyctinasty early in the photoperiod requires metabolic energy and aerobic conditions. We hypothesized that Pfr promotes the active transport of K ions through ventral cell membranes and that mitochondrial oxidative phosphorylation is probably the primary energy source. However, the rhythmic process appears to promote K efflux from ventral cells of darkened leaflets even if leaflets are in a nitrogen atmosphere and thus cannot produce much ATP by oxidative phosphorylation (Fig. 4). There are two mutually antagonistic interpretations. (*a*) The rhythmic process promotes closure by the active secretion of K ions out of ventral cells, utilizing ATP from a pool that increases in size during the photosynthetic period. According to this hypothesis, the ability of leaflets to close under anaerobic conditions is determined by the titer of endogenous ATP. (*b*) The rhythmic process promotes closure by passive diffusion of K ions through pores in the ventral cell membranes of darkened leaflets. According to this hypothesis, the leakiness of the ventral cell membranes increases with the length of the light period preceding darkening. We favor the latter hypothesis, for two reasons. (*a*) The same behavioral characteristics that differentiate the closure of leaflets darkened late in the photoperiod from those darkened earlier (*i.e.*, more rapid closure, less inhibition by FR preirradiation, and partial closure in a nitrogen atmosphere [Fig. 4]) also differentiate

Table III. *Kinetic Analysis of Phytochrome-controlled Leaflet Movement and K Flux*

Tabular presentation of data for the experiments described in Figures 5 and 6. Changes in K or in K/Ca indicate K flux.

Experiment	Treatment	Time	Closure	Ventral		Dorsal	
				K	K/Ca	K	K/Ca
		min	*degrees*				
Figure 5	Light	0	0	351 ± 76[1]	2.60 ± 0.44	172 ± 69	1.04 ± 0.21
		108	0	300 ± 60	2.17 ± 0.56	150 ± 30	0.95 ± 0.25
	R, dark	10	30	287 ± 48	1.92 ± 0.30	194 ± 35	1.12 ± 0.19
		25	70	256 ± 61	1.54 ± 0.30	255 ± 41	1.70 ± 0.20
		81	110	307 ± 91	1.93 ± 0.53	319 ± 45	2.03 ± 0.43
	FR, dark	13	30	280 ± 50	1.92 ± 0.40	223 ± 49	1.23 ± 0.27
		28	30	324 ± 71	2.19 ± 0.48	247 ± 57	1.41 ± 0.30
		86	20	360 ± 85	2.76 ± 0.46	219 ± 59	1.36 ± 0.39
Figure 6	Light	0	0	453 ± 79	0.99 ± 0.29	232 ± 66	0.35 ± 0.11
		100	40	329 ± 101	0.65 ± 0.29	218 ± 63	0.28 ± 0.13
	R, dark	8	40	401 ± 80	0.99 ± 0.29	285 ± 70	0.52 ± 0.14
		25	60	374 ± 79	0.85 ± 0.13	275 ± 47	0.51 ± 0.10
		90	140	257 ± 67	0.55 ± 0.15	422 ± 66	0.91 ± 0.13
	FR, dark	8	40	471 ± 83	1.05 ± 0.18	320 ± 73	0.59 ± 0.08
		25	40	435 ± 129	1.14 ± 0.24	316 ± 68	0.66 ± 0.12
		90	70	318 ± 42	0.77 ± 0.14	381 ± 108	0.82 ± 0.27

[1] Values are scintillations/25 sec ± SD.

Table IV. *Summary of the Movements of Albizzia Pulvinules*

Leaflet Movement	Necessary Conditions	Prevented by Metabolic Inhibitors?	Chemical Basis[1]		References
			K, dorsal	K, ventral	
Nyctinastic closure	Darkness	Yes	++		Table III, Figures 5, 6, and References 15, 16
	Darkness, Pfr	Yes	+++	−−	
Rhythmic closure	Prolonged light	?	++	−−	Reference 15
Rhythmic closure	Prolonged darkness	No	+	−−−	Figures 1, 3
Rhythmic nyctinastic closure	Several hours of light followed by darkness	No	++	−−−	Figure 4
Light-promoted opening	Blue light	?	−−−	+	Table II and Reference 15
Rhythmic opening	Prolonged darkness	Yes	−	+++	Table II and Figures 1, 2

[1] + indicates influx and − indicates effiux.

the "stringent" leaflets (Fig. 5) from those we described as "leaky" (Fig. 6). As the length of the light period preceding darkening increases, the behavior of previously stringent leaflets becomes increasingly similar to that of leaky leaflets. Thus we suggest that the rhythmic change in the rate and extent of phytochrome-controlled nyctinastic closure is due to a rhythmic change in the leakiness of ventral cell membranes. (*b*) This hypothesis is also consistent with our data on endogenous rhythmic leaflet opening and closure during a long dark period. Leaflet opening in the dark is primarily due to a large increase in the K content of ventral motor cells (Table II); thus it is reasonable to expect that K efflux from ventral cells is an essential prerequisite for closure. Addition of NaN_3 to the bathing solution facilitates closure (Fig. 3), suggesting that in this case K ions diffuse passively *out of* ventral motor cell membranes. Comparative analysis of leaflet movement during a long dark period and of nyctinastic closure (Figs. 1, 2B, and 3 compared to 4) suggests that the same regulatory systems function in similar ways under both these conditions.

Our hypothesis is supported by reports of endogenous circadian rhythmic variation in the energy required for leaf movement in *Phaseolus multiflorus* (1, 21) and for floral induction in *Chenopodium rubrum* (3). It is also consistent with data (8) showing rhythmic alteration in flowering of *Lemna perpusilla* upon transfer to water.

Although we have ignored the role of the dorsal cells in our analysis of rhythmic control of leaflet closure, we do not mean to imply that there are not significant rhythmic changes in the K permeability of these cells also. Such changes are certainly indicated by previously published data showing a 150% increase in the K content of dorsal motor cells during endogenously controlled closure in the light (15). However, our data indicate that K efflux from ventral cells most severely limits the nyctinastic closure of leaflets darkened early in the photoperiod.

CONCLUSION

Our previous study showing that endogenous, rhythmic K flux in dorsal and ventral motor cells of *A. julibrissin* is the basis for rhythmic leaflet movement provided evidence for endogenous, rhythmic changes in membrane permeability (15). Bünning and Könitz's report (2) of circadian rhythmic variation in the distance between the two layers of the endoplasmic reticulum in *Helodea canadensis* suggests a possible biophysical basis for this change. Data on the effect of R and FR light on opening in the dark (Fig. 1) show that a low Pfr level permits maximal expression of the endogenous rhythmic variation in leaflet angle. Preirradiation with FR to reduce the Pfr level also permits maximal rhythmic variation in the rate and extent of nyctinastic closure (Fig. 4). It is likely that ventral motor cells show the most pronounced rhythmic permeability change, at least if leaflets are darkened during the portion of the diurnal cycle that we investigated. Thus, damping of the rhythmic variation in leaflet angle by Pfr is probably due to its damping of the rhythmic variation in K efflux from ventral motor cells.

Metabolic energy and aerobic conditions are required for Pfr-controlled K flux in motor cells of darkened leaflets. We suggest that the rhythmic promotion of K efflux is due to a rhythmic increase in the leakiness of ventral cell membranes; if this is true, then the rhythm and Pfr control K flux by controlling different metabolic events. Pfr, by permitting K efflux from ventral cells during a portion of the diurnal cycle when such flux is not promoted by the rhythmic process, establishes a basis for control by phytochrome of a process otherwise solely under rhythmic control.

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