

ACTION SPECTRUM FOR THE OPENING OF *ALBIZIA JULIBRISSIN*
PINNULES, AND THE ROLE OF PHYTOCHROME IN THE CLOSING
MOVEMENTS OF PINNULES AND OF STOMATA OF *VICIA FABA*

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Abstract

The opening movements of both *V. faba* stomata and *A. julibrissin* pinnules have been shown previously to depend on a redistribution of potassium ions under the influence of light. Action spectra for both systems show peak effectiveness in blue light, at 440 nm, but for pinnule opening in *A. julibrissin* there is a subsidiary peak in the far red (720 nm). However, opening in far red shows an initial lag period, whereas opening in blue light is rapid. The action spectrum for pinnule opening was not influenced by leaf age, the presence or absence of CO₂, or by simultaneous red light.

Brief exposure to red as against far red irradiation at the beginning of a period in darkness accelerated closure of *A. julibrissin* pinnules, but not of *V. faba* stomata.

It is concluded that whereas potassium ion uptake, driven by blue light, causes opening in both systems, phytochrome is involved in the closing reactions of *A. julibrissin* pinnules but apparently not in those of *V. faba* stomata.

I. INTRODUCTION

The opening and closing movements of stomata of *Vicia faba* and of the pinnules of *Albizia julibrissin* have certain features in common. Both depend on the redistribution of potassium ions, as shown by Fujino (1967) and Fischer and Hsiao (1968) for stomatal guard cells in several species, and by Satter, Marinoff, and Galston (1970) and Satter and Galston (1971) for the ventral and dorsal pulvines of *A. julibrissin*. The low quantum flux action spectrum for the light-promoted influx of potassium ions into the guard cells of *V. faba* stomata shows a pronounced peak in the blue region (Hsiao, Allaway, and Evans 1972), similar to that for the opening of *Mimosa pudica* leaflets (Fondeville *et al.* 1967). Jaffe and Galston (1967) present data showing that blue light is most effective in causing the opening movement of *A. julibrissin* pinnules, and one objective of the present work was to obtain a full action spectrum for this movement under equal quantum flux densities and under the same conditions as those used to determine the action spectrum for stomatal opening and potassium uptake by the guard cells of *V. faba* (Hsiao, Allaway, and Evans 1972).

Phytochrome has been shown to influence the nyctinastic closure of *A. julibrissin* pinnules, brief exposures to red light accelerating closure (Hillman and Koukkari

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1967; Jaffe and Galston 1967) and the redistribution of potassium in the pulvinules (Satter, Marinoff, and Galston 1970). To our knowledge, no influence of phytochrome on the closing reaction of stomata has been reported, although an influence of phytochrome on stomatal movements via effects on the phasing of circadian rhythms has been suggested (Heath, Mansfield, and Meidner 1965). We therefore examined the influence of brief exposures to red and far red light at the beginning of a dark period on the rate of closing of both *A. julibrissin* pinnules and *V. faba* stomata, to extend the comparison of these two systems, and to seek an effect of phytochrome on stomatal movement under conditions where it is clearly expressed in pinnule movement.

II. MATERIALS AND METHODS

(a) *A. julibrissin* Plants

Plants of *A. julibrissin* Durazz. were grown in a greenhouse until they had at least six fully expanded leaves. They were then transferred to an artificially lit cabinet at 21°C for 14 hr of light of about 20,000 lux intensity from fluorescent and incandescent lamps, and at 16°C in darkness for the remaining 10 hr of each day. After at least 1 week in these conditions the experimental material was obtained as individual pairs of pinnules by excision of pinnule pairs 5–14 from the central 4–6 pinnae of leaves 5–7 from the top of each plant. Hillman and Koukkari (1967) have shown these to provide very uniform experimental material. Randomized samples of three pairs of pinnules were placed within a few minutes of cutting on distilled water in aluminium planchets. Treatment replicates usually consisted of two or three such planchets. The remarkable uniformity of response in pinnule pairs selected in this way may be gauged from the results of Hillman and Koukkari (1967), who used only two pairs in each treatment.

For experiments on the opening movements, pinnule pairs were excised in a dark room, under weak green light, prior to the beginning of the daily light period, and were placed on the spectrograph (see below) within 1 hr of the beginning of the expected daily light period. For experiments on closing movements, pinnule pairs were excised under light of high intensity, exposed to red or far red light for 4 min, and then held in darkness at 20°C until measured. The angle between pinnules was measured on a protractor with a moveable arm, to within 1° provided pinnules did not curve.

Action spectra for opening were determined on a spectrograph, with the same set up as that used to determine the action spectrum for stomatal opening in *V. faba* (Hsiao, Allaway, and Evans 1972). The light source was a 2500 W air-cooled Xenon arc lamp, housed in a Zeiss Xenosol III projection unit. By means of a front-aluminized concave mirror, the beam emerging from a slit 1.2 cm wide was projected on a Bausch and Lomb diffraction grating with a ruled area of 206 by 206 mm, with 1200 grooves per millimetre blazed at 4100 Å. From the grating the beam was projected downwards to the plane of the stations 2.7 m below. The linear dispersion at this level was 0.383 mm per 1 Å, and the flux density was up to 4.4 mW cm⁻². Black walls, ceiling, and baffles reduced scattered light to below 2 μW cm⁻². With a slit 1.2 cm wide, the half band width was less than 40 nm. An orange filter removed second-order wavelengths from the red end of the spectrum, and neutral density filters were used at each wavelength station, 20 nm apart, to establish the same quantum flux density for all stations in each action spectrum run (within ±3%), usually 0.78 × 10¹⁵ quanta cm⁻² s⁻¹. Dark controls were of two kinds, one exposed to the scattered light inside the spectrum room, the other in complete darkness. As a white light control, the beam from a high pressure mercury vapour lamp, filtered through 2 cm water and through a layer of Mylar to remove ultraviolet, was used, at a flux density of 14.9 mW cm⁻². For any one run the temperature at all stations was within 1.5°C, the temperature of most runs being between 21 and 25°C.

The exposures to red and far red light were made by placing planchets, in Petri dishes, on a rotating turntable in a light-tight box. The turntable could be illuminated by two Leitz Prado

projectors, one with the light beam filtered through a red (λ_{\max} 660 nm) interference filter, the other through a far red (> 700 nm) filter of black plexiglass, the flux density at 660 or 720 nm being about $0.4 \mu\text{W cm}^{-2} \text{nm}^{-1}$. Earlier experiments with this equipment had shown that exposures at this intensity for 4 min were sufficient to reach photostationary equilibrium of phytochrome in cotyledons of *Pharbitis nil* (Evans and King 1969), and the same was found for acceleration of the closing of *A. julibrissin* pinnules.

(b) *V. faba* Plants

Plants of *V. faba* L. cv. Early Long Pod were grown in well-watered pots in an artificially lit cabinet as used for the *A. julibrissin* experiments, except that the day and night temperatures were 24 and 19°C respectively.

Preliminary experiments showed that stomatal closure on darkening tended to be sluggish in leaf disks or epidermal strips floated on solutions, compared with that in attached leaves. Intact plants were therefore used for the observations on stomatal closure. The light pretreatments were given with the same equipment as that used in the *A. julibrissin* experiments, except that reflecting foil was added to cover the surfaces of the pots, to ensure that the lower leaf surfaces also received the red or far red light. Preliminary experiments with exposures 8 min long gave results similar to those with 4-min exposures. Immediately after the red or far red treatments the plants were placed in darkness at 20°C, and porometer readings taken at 15-min intervals. Hsiao, Allaway, and Evans (1972) have shown that green light is inactive at low intensities for stomatal opening, and dim green light was therefore used during the attachment of the porometer cup on the leaves, which otherwise remained in darkness throughout.

Stomatal conductance was measured with a mass flow porometer similar to that of Spanner and Heath (1951). Results with control plants left in the light for the whole experimental period showed that repeated attachment of the porometer cup to the same leaf, but to different places on it, had little effect on stomata for the first 30 min or so, but thereafter caused some closure.

III. RESULTS

(a) Opening Reaction of *A. julibrissin* Pinnules

(i) Dependence on Quantum Flux Density

Two experiments examined the dependence of pinnule opening on quantum flux density, for exposures of 1, 2, 3, and 4 hr at four wavelength stations on the

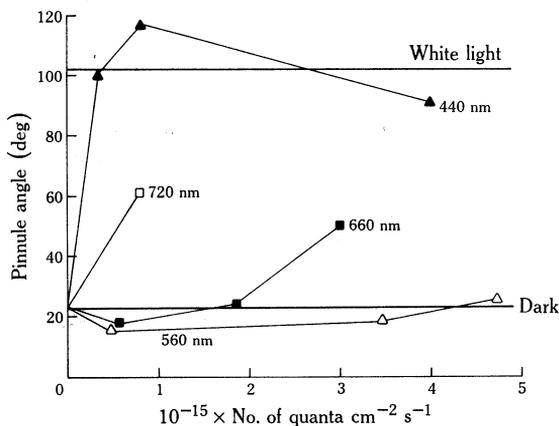


Fig. 1.—Relation between quantum flux density and angle of opening of pinnules of *A. julibrissin* after exposure on the spectrograph for 3 hr.

spectrograph. The results of the two experiments were similar, and those for 3-hr exposures in one experiment are given in Figure 1.

Opening was most rapid in blue light (440 nm), but after 3 hr, opening in white light was about the same as that at the lowest flux density at 440 nm. The reduced opening at the highest flux density at 440 nm, found in both experiments, was due to opening ceasing after 1 hr, whereas it continued to greater angles in the lower flux densities. Opening in far red (720 nm) was slow but progressive up to 3 hr. In green (560 nm) and red (660 nm) light there was some initial closing, but at the highest flux density in red light some opening eventually took place. Action spectrum experiments were usually run for 3 hr therefore, to allow for the slower opening at the longer wavelengths, and at the highest quantum flux density that could be obtained across the whole spectrum, namely 0.78×10^{15} quanta $\text{cm}^{-2} \text{s}^{-1}$ as limited by the 720 nm station.

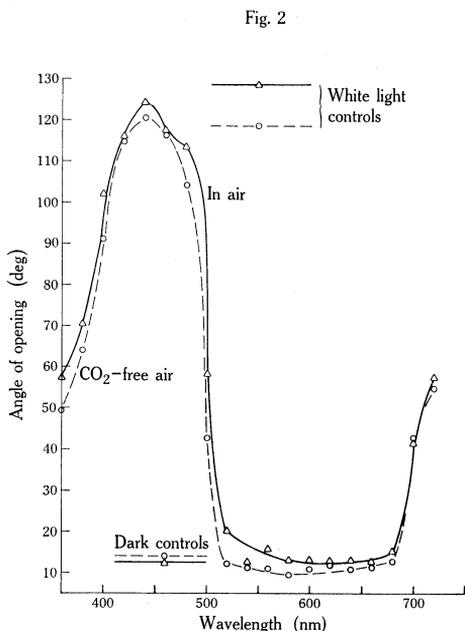


Fig. 2.—Action spectra for pinnule opening after 3 hr under a quantum flux density of 0.78×10^{15} quanta $\text{cm}^{-2} \text{s}^{-1}$. While on the spectrograph the pinnule pairs were held under either circulating air (Δ) or air free of CO_2 (\circ). Each curve combines the results of two experiments, and each point represents 12 pairs of pinnules, on distilled water.

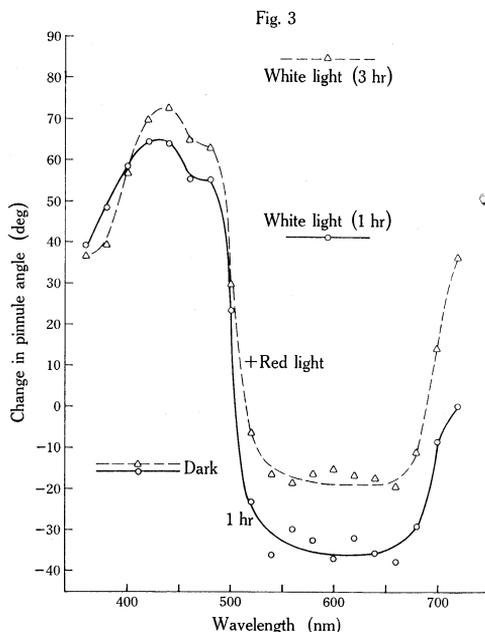


Fig. 3.—Change in pinnule angle during the period on the spectrograph in two experiments with a flux density of 0.78×10^{15} quanta $\text{cm}^{-2} \text{s}^{-1}$, using pinnule pairs which had opened to $35\text{--}40^\circ$ by the end of the dark period. \circ Action spectrum after exposures for 1 hr. Δ Action spectrum after exposures for 3 hr, with red light at a flux density of $135 \mu\text{w cm}^{-2}$ applied across the whole spectrum. Each point represents six pairs of pinnules.

(ii) Action Spectra

Twelve action spectrum runs were made: the results of four of them are presented in Figure 2. A clear action maximum at 440 nm is evident, with no action throughout the green and red regions at this low flux density, but with a secondary peak near 720 nm. Although the presence of CO_2 at atmospheric concentration tends

to suppress stomatal opening, it clearly has no similar effect on the opening of *A. julibrissin* pinnules in light. Other runs with pinnules excised from plants growing under natural light, in a glasshouse, or with pinnules on a solution of 2mM KCl + 0.1 mM CaCl₂ instead of water gave similar results. Unlike the stomata of *V. faba* when isolated epidermal strips are used, pinnule opening is not dependent on exogenously supplied potassium.

In the experiments reported in Figure 2 all the pinnule pairs used were almost completely closed at the end of the dark period when they were placed on the spectrograph. Older leaves often show some anticipatory opening of the pinnules before the end of the dark period, and several such leaves were used to see if the action spectrum for their opening was different. Also, being 35–40° open when placed on the spectrograph, the possibility that light of some wavelengths might cause closure could be examined. The action spectrum given in Figure 3 for exposures of 1 hr indicates that green and red light did cause initial closure. Far red light (720 nm) had little effect during the first hour, whereas blue light gave greater opening than the white light control treatment. In another experiment red light was added across the spectrum to see if the red light-induced closure would override the opening reaction induced by blue or far red light, but the results (in Fig. 3) indicate that this did not happen, and that the action spectrum was unchanged, except for a possible enhancement of the response to far red light (720 nm).

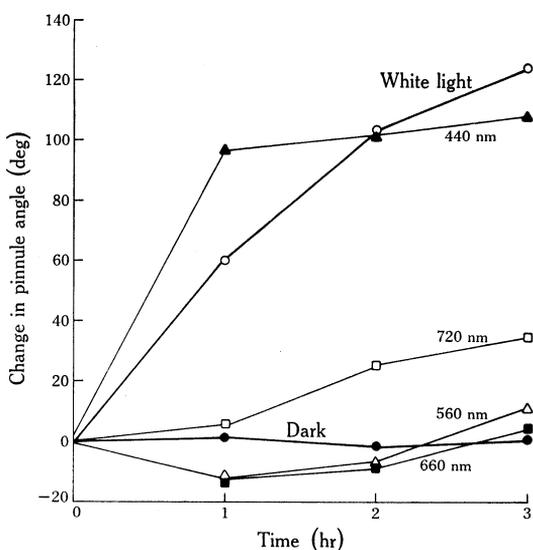


Fig. 4.—Change in pinnule angle with time at four stations on the spectrograph—440, 560, 660, and 720 nm, and also under intense white light and in darkness. Flux density at the spectrograph stations was 0.78×10^{15} quanta $\text{cm}^{-2} \text{s}^{-1}$.

Pinnule pairs which opened 35–40° by the end of the diurnal dark period were also used in two experiments to follow the time course of opening at several wavelengths. The results of one experiment are given in Figure 4, for flux densities of 0.78×10^{15} quanta $\text{cm}^{-2} \text{s}^{-1}$. As noted above, opening in blue light was more rapid than in the far more intense white light source. Opening in white light continued over a longer time, as it did also in lower intensity blue light. Opening under far red light was initially slow, while in red and green light there was an initial closing of

pinnules which had opened somewhat in darkness. Longer exposures at those wavelengths led to some opening, which was more pronounced at higher flux densities.

(b) *Closing of A. julibrissin Pinnules*

As found by Hillman and Koukkari (1967), closing of pinnules after a period in high intensity light was more rapid when they were exposed briefly to red light rather than to far red light prior to darkness, and was more rapid after a longer period in high intensity light (Fig. 5). Nevertheless, the closure following brief exposure to far red light was quite marked, compared with control pinnules kept under white light.

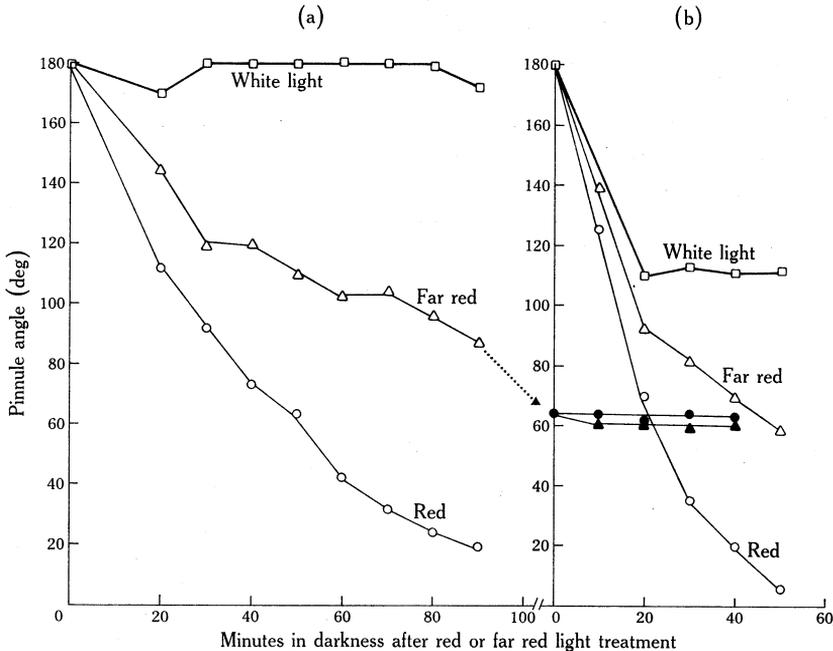


Fig. 5.—Closing of pinnules in darkness at 20°C after opening under white light for either 2 hr (a) or 6 hr (b) as influenced by exposure to either red or far red light for 4 min immediately before the period in darkness. White light controls were excised pairs of pinnules kept in the artificially lit cabinet following cutting. Other groups of pinnules were exposed to far red light for 4 min after 2 hr in white light, then kept in darkness for 4 hr, and then exposed to either red or far red light for 4 min, before return to darkness (solid symbols).

Several experiments of this kind were done, and the results in all instances were similar, except that the acceleration of closure by red light was slight after exposure to white light for 6 or 7 hr in some experiments. The accelerating effect of red light on closure was always marked after exposures to only 1½–2 hr of light, even when the pinnules were opened in blue light. Where pinnules were exposed to far red light after only 2 hr in light, and were then kept in darkness for 4 hr when they were exposed briefly for a second time, red or far red light at that stage had no further effect on their closure [Fig. 5(b)]. Thus, the more rapid closure evident after longer periods in light is unlikely to be a rhythmic phenomenon, but rather a direct response to the length of the light period.

Pinnule pairs were also taken from older leaves in which they had begun to open in darkness just before the beginning of the light period, presumably in response to an endogenous rhythm governing potassium redistribution (Satter and Galston 1971). These were then exposed to red or far red light for 4 min, and returned to darkness. Under these conditions brief exposure to red light caused a slight initial closure, but brief exposure to far red light led, after a lag of about 30 min, to progressively greater opening in darkness.

(c) Closure of *V. faba* Stomata

Preliminary experiments established that the stomata of upper leaves closed slightly more quickly in the dark than those of the lower leaves, as is true for the closing of *A. julibrissin* pinnules (Hillman and Koukkari 1967). Also, stomatal closing on darkening was somewhat slower when leaves had been only a few hours in light than later in the day, as is also the case for the closing of *A. julibrissin* pinnules (Fig. 5). To allow for these effects the data presented in Figure 6 include the same

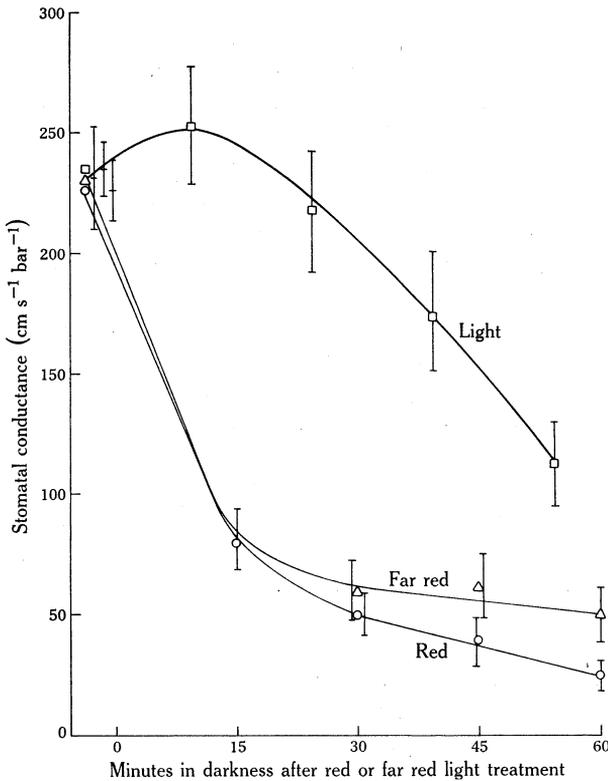


Fig. 6.—Stomatal closure in darkness following exposure for 4 min to red or far red light. White light controls were kept in the artificially lit cabinet during the experiment. Each point is the mean of measurements on 25 leaves. The vertical bars are two standard errors in length.

number of upper and lower leaves, and of observations earlier or later in the light period, in each treatment. Leaves with approximately the same stomatal conductance in the light before the red or far red treatments were selected; these initial measurements took about 10 min, and the maximum period between initial measurement and start of treatment was about 20 min. Observation on control leaves (Fig. 6) showed

that stomatal conductance changed little, on average, during this period. Thus, the initial measurements can be taken as estimates of stomatal conductance just prior to treatment with red or far red light for 4 minutes.

Experiments comparable to those which revealed a clear differential effect of brief exposures to red or far red light prior to darkness on the rate of closure of *A. julibrissin* pinnules failed to show any such effect on the rate of closure of *V. faba* stomata (Fig. 6). Transfer of the plants to darkness led to a rapid fall in stomatal conductance, but there was no significant difference between plants exposed to red and those exposed to far red light at any time. Control plants kept in light began to show stomatal closure after about 30 min, presumably because of damage caused by repeated attachment and removal of the porometer cup. This was not evident at the time of the second measurement, after 15 min, when stomatal conductance of the red and far red-treated plants was the same.

IV. DISCUSSION

The processes leading to the opening of the stomata of *V. faba* and of the pinnules of *A. julibrissin* have in common their dependence on the redistribution of potassium ions, and on irradiation with blue light to effect this. They differ in that opening of stomata is depressed by atmospheric CO₂ levels, which have no influence on pinnule opening. They differ also in that far red (720 nm) irradiation is ineffective in causing stomatal opening in *V. faba* (Hsiao, Allaway, and Evans 1972) although it is quite effective in opening the pinnules of *A. julibrissin*, but only after an initial lag period not evident in the response to blue light. Our action spectra for pinnule opening in *A. julibrissin* are similar to that measured by Fondeville *et al.* (1967) for the movement of leaves of *Mimosa pudica*, except that we observed maximum effectiveness at a wavelength of 440 nm, as with the *V. faba* stomata, whereas that with *M. pudica* was at 480 nm. The *A. julibrissin* action spectra are similar to those for many other 'high-energy' photomorphogenic responses, such as the inhibition of hypocotyl extension. With the latter reaction, in lettuce at least, the effectiveness of far red light diminishes as the seedling ages, until only the blue peak remains (Evans, Hendricks, and Borthwick 1965; Turner and Vince 1969). This suggests that two separate pigments may be involved, one absorbing mainly in the blue region, the other in the far-red region. The first could influence both stomatal and pinnule opening, through a light-driven potassium ion pump. The second pigment, which could be phytochrome, is apparently not involved in the opening of *V. faba* stomata, but may participate in the opening of *A. julibrissin* pinnules. Our results suggest, similarly, that although phytochrome influences the closing of *A. julibrissin* pinnules, it does not influence that of *V. faba* stomata. Thus, in spite of the dependence of both systems on a blue-light-driven potassium ion pump, only the *A. julibrissin* system provided evidence of phytochrome involvement in its opening and closing reactions.

The *A. julibrissin* pinnule response may prove to be a useful model for the analysis of comparable, but less convenient, responses such as the induction of flowering. During the day the phytochrome reaction tends to induce closure, but is overridden by the blue light reaction to give opening in white light, although more slowly than in blue light alone. After a short period in light, the rate and degree of

closing in darkness is particularly influenced by phytochrome, but a long period in light tends to mask the effect. A circadian rhythm also influences the response, causing some anticipatory opening of pinnules in old leaves towards the end of the dark period, and anticipatory closing especially in young leaves towards the end of the light period. The faster closing in darkness after a long period in light, however, did not appear to involve a rhythmic response to phytochrome.

V. ACKNOWLEDGMENTS

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