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Pulvini as the Photoreceptors in the Phytochrome Effect on Nyctinasty in Albizzia julibrissin[1]

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Abstract. **The fact that far-red pretreatment slows the closing response of *Albizzia julibrissin* pinnules to darkness was used to locate the photoreceptor region for the role of phytochrome in nyctinasty, and to determine whether the effect is localized or translocated. Illumination of pinnule tissue alone induced no response, while illumination of an area as narrow as 1 mm, including only the tertiary pulvini and adjacent portions of rachilla and pinnules, was sufficient for a full response. This suggests that the pulvini themselves, the sites of the response, act as photoreceptors. In experiments with various shielding devices, pinnules on the same rachilla responded independently to local illumination, suggesting the absence of any translocatable effects.**

The participation of phytochrome in the nyctinastic response of *Mimosa pudica, Albizzia julibrissin,* and other legumes is evident in the fact that pairs of pinnules close rapidly when red illumination precedes darkening and more slowly if darkness is preceded by far-red light (2, 3). The objectives of this investigation were to locate the red, far-red photoreceptive regions for the phytochrome effect in nyctinasty, and to determine if the effect is translocated to non-illuminated regions.

Materials and Methods

Albizzia julibrissin plants, grown from seed and kept in the greenhouse for several months until well established, were transferred to growth chambers at least 3 days before being used, and maintained on a 16 hour photoperiod at about 24°. In previous studies (3) a 12 hour photoperiod was used, but plants maintained on this or a shorter photoperiod for extended lengths of time go dormant and stop producing new leaves, so the longer photoperiod was adopted for all purposes. All experiments were conducted during the first few hours of the daily photoperiod. The leaves were numbered consecutively from the apex starting with the first expanded leaf, designated leaf 0. Generally leaves 2 to 5 were selected, and, when necessary, pinnule pairs were cut from paired pinnae (3).

In experiments to locate the red, far-red photoreceptive region, pinnules were cut in pairs and placed on a 10 mm wide strip of polyurethane packing material mounted on plastic. The packing material on the bottom protected the tissues from excess pressure as well as shielding them from reflected light. Portions of the pinnule pairs were then covered with either a strip of clear plastic or a strip of plastic covered with black tape, and the bottom and top sections held in place by rubber bands (fig 1a). The unshielded portions of the pinnules were exposed to far-red light and the pinnule pairs then removed from the device and transferred to petri dishes containing water. The far-red light source used for most of the experiments consisted of a 500-W incandescent spot light located above a water bath and a piece of far-red plastic (Rohm and Haas V58015) mounted on or over a device containing plant material. Red illumination, when necessary, was obtained from a cool white fluorescent tube separated from the plant material with a piece of red plastic (3). Since, as expected, pinnules taken directly from the white light of the growth chamber responded to darkness as rapidly as those first exposed to red light, only pre-illumination with far-red light was used in many experiments. Thus the terms "red light" and "dark" may be used interchangeably to describe illumination pretreatments other than far-red.

To reduce further the area exposed to light, a slit (*ca.* 1 mm) was cut through a piece of one-fourth inch plywood and a section of rachilla possessing pinnule pairs was placed in the slit, so that the pinnules rested on top. The pinnules were then covered with packing material mounted on a thin piece of wood and the 2 sections of wood held together by a rubber band. Thus with this device the rachilla and pulvini could be exposed to red or far-red light through the slit.

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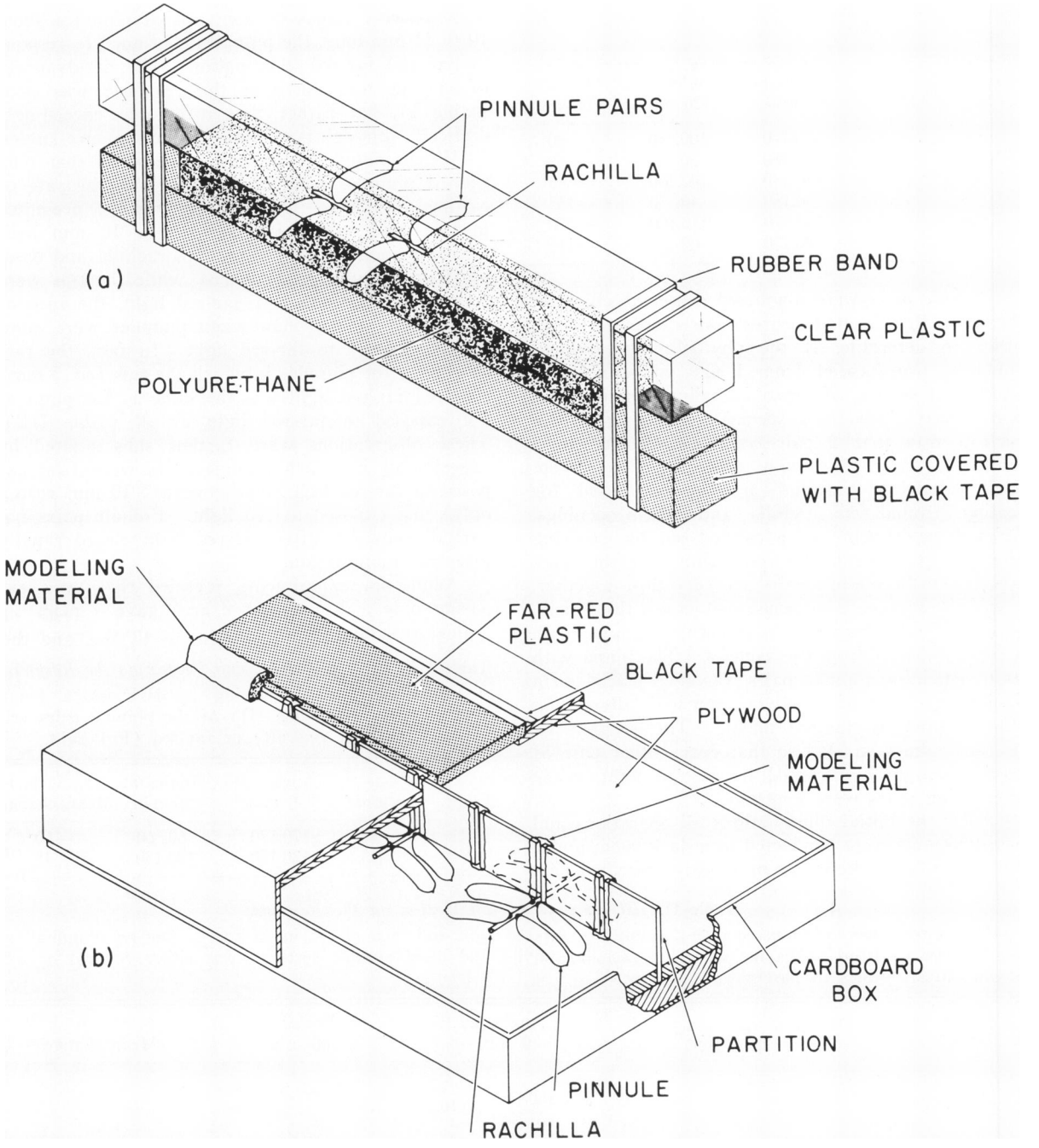

FIGURE 1

FIG. 1. Devices used to shield certain portions of pinnae.

Table I. *Response of Pinnule Pairs with Tips Removed*

Pinnule pairs, either intact or with 5 or 7 mm segments removed from the tips, were exposed to red (R) or far-red (Fr) light and then placed in darkness.

Amount cut	Light	Angles at 0 min	Angles at 120 min	Mean change
None	Fr	170,170	100, 80	— 80
None	R	180,180	50, 50	—130
5 mm	Fr	180,180	100,100	— 80
5 mm	R	180,180	40, 50	—135
7 mm	Fr	180,170	110,100	— 70
7 mm	R	180,170	60, 60	—115

Pinnule pairs were numbered consecutively, with the first pair located nearest the rachis (3). In order to determine if the phytochrome response could be translocated from 1 pair of pinnules to another, a section of pinna including pinnule pairs 5 to 8 was placed in a special device (fig 1b) in such a way that 2 pair could be exposed to 2 minutes of far-red light, while the other 2 were kept dark. Modeling material or clay was used for sealing around the rachilla and other openings. Related experiments were carried out by exposing alternate pinnules to far-red light. For this purpose, since the pinnule pairs are very close together, it was advantageous to remove all except the fourth, seventh, tenth, thirteenth, sixteenth, and nineteenth pair of pinnules from the rachilla. The pinna with its 6 remaining pinnule pairs was then placed on a device consisting of a plywood top with alternating slits covered by far-red plastic. The sections of the top were separated so that every other pair of pinnules could be exposed to far-red light, while alternating pairs were kept dark.

After the initial illuminations all operations and measurements were conducted in a dark room under dim green "safe" lights, as previously described (3).

Results and Discussion

Photoreceptive Regions. When 5 and 7 mm sections were removed from tips of pinnules about 10 to 11 mm long, the pinnules continued to respond to red and far-red illumination. The disadvantage of cutting was not that the response was poor (table I), but rather that it became exceedingly difficult, although not impossible, to measure angles.

When pinnule pairs were covered so that only tips of pinnules received far-red light, the rate of closing was very rapid. These results, presented in table IIA, showed that when a 10 mm area (vertex) including the 2 pulvini, rachilla and basal parts of pinnules was covered, while the tips were exposed to 2 minutes of far-red light, the rate of closing was faster than when pinnules were completely exposed to far-red light. In fact, the rate of closing for pinnules in which the tips (*ca.* 5 mm) received far-red light was the same as for pinnules not exposed to far-red light at all (table IIB). These observations were further substantiated by experiments in which pinnule pairs were first exposed to far-red light, then covered (10 mm vertex only) and exposed to red light. Pinnule pairs not partially covered after far-red light closed rapidly after red illumination.

Additional experiments revealed that even exposing only a 1 mm vertex area gave a perfectly typical response (table III). The most reasonable

Table III. *Effect of Exposing Vertex on the Response*

Immediately before placing in darkness, a 1-mm vertex region (see table III) of the pinnule pairs was exposed to either red (R) or far-red (Fr) light.

Light	Pinna	Angles at 0 min	Angles at 90 min	Mean change
R	B	170,170	60, 60	—110
Fr	B′	180,180	140,130	— 45

Table II. *Effect of Covering Vertex on the Response*

Pinnule pairs were illuminated with 2 minutes of far-red light and then placed in darkness. During illumination, the "vertex", a 10-mm area including the rachilla, pulvini and basal pinnule sections, was either covered or exposed. Mean values in parentheses.

"Vertex"	Pinna	Angles at 0 min		Angles at 60 min		Mean change
A						
Exposed	E	160,170 170,170	(167.5)	70, 80 110,120	(90)	—77.5
Covered	E′	160,170 170,170	(167.5)	30, 30 30, 40	(32.5)	—135.0
Exposed	F	160,170 170,170	(167.5)	80, 80 80, 80	(80)	— 87.5
Covered	F′	160,160 170,170	(165.0)	20, 30 30, 40	(30)	—135.0
B						
Covered	I	130,140 140,140	(137.5)	30, 30 30, 40	(32.5)	—105
Covered; no Fr treatment	I′	130,140 140,150	(140)	30, 30 40, 40	(35)	—105

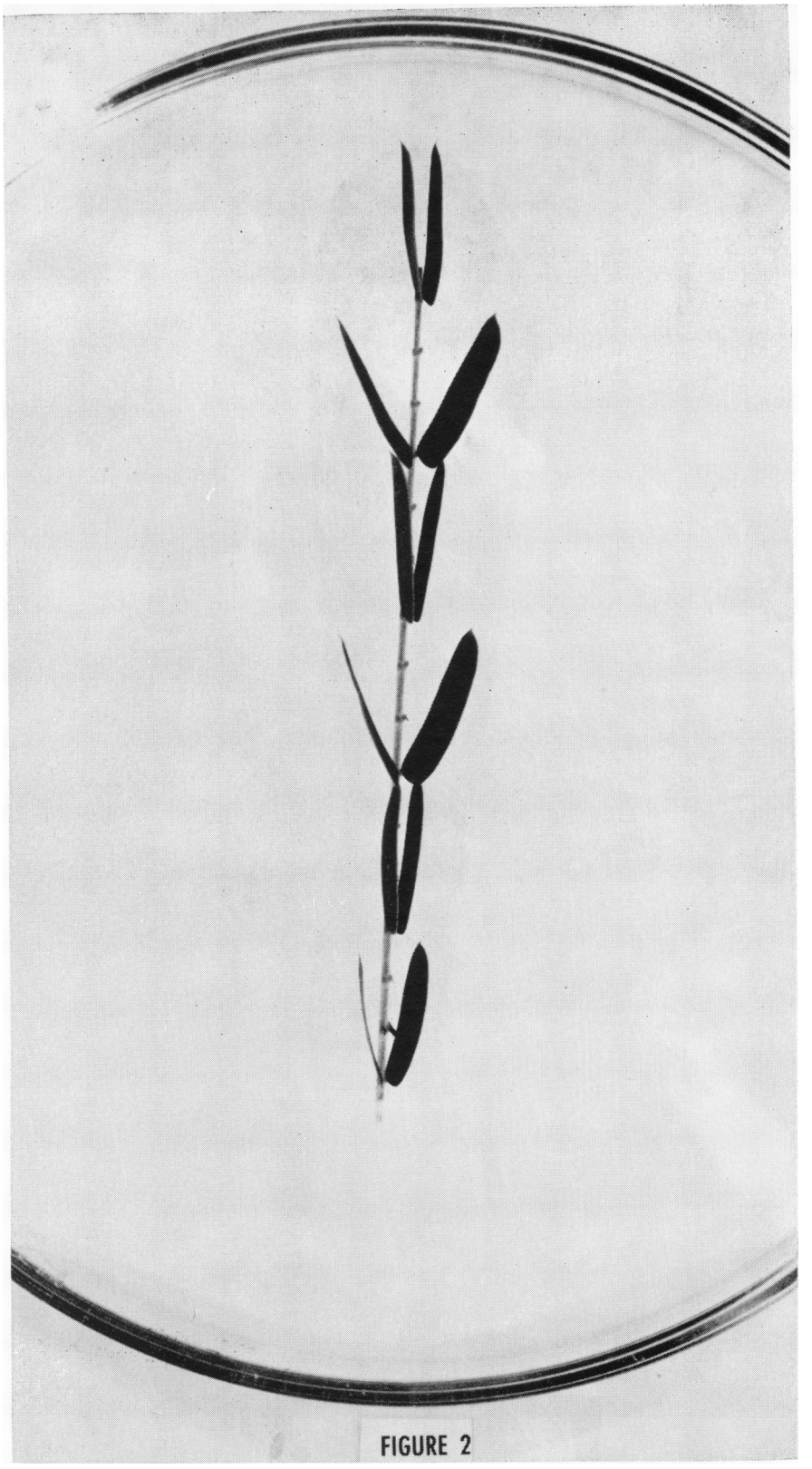

FIG. 2. Section of a pinna after approximately 80 minutes of darkness. The fourth, tenth, and sixteenth pair of pinnules have been exposed to 2 minutes of far-red light and the seventh, thirteenth, and nineteenth kept dark. Numbering starts from the base. Insert

interpretation of all these results is that the tertiary (pinnule) pulvini themselves, the photoresponsive regions, are also the photoreceptive regions. However, the data cannot exclude the possibility that the portions of rachilla and pinnule immediately adjacent to the pulvini also play a role. Nevertheless, preliminary experiments with the slight nyctinastic response of the secondary pulvini (joining the rachilla to the rachis) completely stripped of pinnule tissue also suggest that the pulvinus acts as its own photoreceptor. Bunning and Moser (1) reached a similar conclusion in their study on red light effects on leaf movements in *Phaseolus*.

Exposing 2 Pinnule Pairs to Far-Red While

Table IV. *Response to Darkness of 4 Attached Pinnule-pairs of Which 2 Have Been Pre-treated with Far-red*[1]

Pinna, pinnule pair		Light	Angle at 0 min	Angle at 120 min	Mean change	Pinna, pinnule pair		Light	Angle at 0 min	Angle at 120 min	Mean change
			(A)[1]						(B)[1]		
H′	5	0	190	20	—170	F′	5	0	170	10	—160
H′	6	0	190	30	—160	F′	6	0	180	15	—165
H′	7	0	180	20	—160	F′	7	0	180	15	—165
H′	8	0	180	30	—150	F′	8	0	180	15	—165
H	5	Fr	190	70	—120	F	5	0	180	35	—145
H	6	Fr	190	70	—120	F	6	0	180	40	—140
H	7	0	190	30	—160	F	7	Fr	170	60	—110
H	8	0	190	30	—160	F	8	Fr	170	70	—100
I	5	0	170	30	—140	G	5	0	170	15	—155
I	6	0	170	20	—150	G	6	0	180	15	—165
I	7	0	170	30	—140	G	7	0	170	15	—155
I	8	0	180	30	—150	G	8	0	170	15	—155
I′	5	Fr	190	110	— 80	G′	5	0	180	20	—160
I′	6	Fr	190	90	—100	G′	6	0	180	45	—135
I′	7	0	180	30	—150	G′	7	Fr	180	65	—115
I′	8	0	180	30	—150	G′	8	Fr	180	80	—100

[1] A) effect of exposing fifth and sixth pairs to far-red, and B) effects of exposing seventh and eighth pairs. Far-red treatment, 2 minutes.

Table V. *Effects of Exposing Some But Not All Pinnule Pairs on a Rachilla to Far-red Light*

Materials were placed in darkness after initial treatments, as indicated, with none or 2 minutes of far-red (Fr) light. Pinnules are numbered from base to tip of pinnae. See figure 2.

		Experiment A Pinnae G, G′, H′			Experiment B Pinnae F, G, G′		
Pinuule pair	Light	Angles at 0 min	Angles at 90 min	Mean change	Angles at 0 min	Angles at 90 min	Mean change
4	Fr	120	70	— 50	150	60	— 90
7	0	120	40	— 80	150	10	—140
10	Fr	110	80	— 30	140	60	— 80
13	0	100	30	— 70	140	20	—120
16	Fr	100	70	— 30	140	70	— 70
19	0	100	40	— 60	130	20	—110
4	Fr	130	60	— 70	160	80	— 80
7	Fr	120	70	— 50	150	80	— 70
10	Fr	110	70	— 40	150	90	— 60
13	Fr	110	50	— 60	140	90	— 50
16	Fr	110	70	— 40	140	100	— 40
19	Fr	90	60	— 30	130	100	— 30
4	0	140	20	—120	160	30	—130
7	0	140	30	—110	150	20	—130
10	0	130	20	—110	150	30	—120
13	0	130	30	—100	140	20	—120
16	0	120	30	— 90	140	40	—100
19	0	120	30	— 90	130	30	—100

Keeping the Immediately Adjacent Pair Dark. It proved to be quite difficult to place a partition between 2 pairs of pinnules without exerting some pressure on 1 of the pairs, and such pressure has a variable effect on the response. After much testing, the simple device illustrated in figure 1b proved to be the most reliable.

When 4 successive pair of pinnules were oriented so that the fifth and sixth pairs received 2 minutes of far-red light while the seventh and eighth were kept dark, the fifth and sixth closed more slowly in darkness than the seventh and eighth. Conversely, when the seventh and eighth pair received far-red light they closed more slowly than the fifth and sixth pair that were kept dark (table IV). The fifth and sixth pinnules often applied pressure on the seventh and eighth pinnules making it more difficult to obtain quantitative measurements. Such results, however, seemed to indicate that each pinnule pair responds only to the illumination it has received, and that the effects of illumination are not translocated.

Illuminating Alternate Sections of Pinna. Individual pinnule pairs can be made to close independently along the intact rachilla in response to red and far-red light. For example, when the fourth, tenth, and sixteenth pinnule pairs were exposed to far-red light while the seventh, thirteenth, and nineteenth were kept dark, the latter started to close rapidly while the former remained relatively open. Results of such experiments are presented in table V and illustrated in figure 2, and show the independent responses quite clearly. Of the 2 experiments in table V, A might conceivably be interpreted as indicating a slight translocation of an effect induced by far-red, since the mean change of the untreated (O) pinnules on the same rachilla with the far-red-treated pinnules is somewhat less than that of untreated pinnules on a separate rachilla. However, no such effect is evident in B. Aside from the improbability of a far-red effect translocatable to red-treated regions—the reasonable assumption that P_{FR} is the active form of phytochrome would predict that translocation, if any, should occur in the opposite direction—the simplest explanation for results such as those in A, obtained a few other times as well, is light leakage due to technical difficulties. Taken all together, results of these experiments and those described above strongly suggest that there is no translocation of the phytochrome effect between pinnules on a given rachilla.

Acknowledgments

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