THE HIGH-ENERGY LIGHT ACTION CONTROLLING PLANT RESPONSES AND DEVELOPMENT

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Abstract.—Evidence is advanced for a network of three photoreactions and five dark reactions in control of plant growth and development by phytochrome.

Introduction.—Plant responses potentiated by the photoreversible change of phytochrome (P) are often negated or modified by irradiation for a long time in the 680–760 nm region.[1] These responses to energies much higher than are required for reversible change of P are grouped under HER as an acronym. Various explanations of the HER have been advanced, but none is without question. Attempts by us and others to involve light absorption in the HER by pigments other than P have generally led back to P. The most satisfactory model for the HER is that of Hartmann.[2] It is shown in Figure 1, where X is the site of P_{fr} action φ_r, φ_{fr} are effectivenesses for photoconversion of P, k_{1d}, etc., are rate constants, and the subscript, d, indicates darkness. This model formulates the photoreversibility of P,[3] the dark reversion of P_{fr} to P_r,[4] the fact that P_{fr} is the active form of P,[5] the loss of P_{fr} to an inactive form, other than P_r,[6] and the logical necessity of P combining with something (symbolic X, irrespective of the nature of X) to lead to responses. Each of these components is well supported.

Hartmann's model accounts for many HER responses where P_{fr}/P is small and times of irradiation are long compared with the half-life of P_{fr}. It does not account for HER responses where P_{fr}/P approaches the maximum value of 0.8. Many of these responses, as well as those where P_{fr}/P is low, have action maxima near 720 nm. The model neither gives a convincing reason for the observed intensity dependence of the responses nor does it consider photochemical properties of $P_{fr}X$.

Recent findings of a pH form of P[7] in equilibrium with P_{fr} *in vitro*, and its possible occurrence *in vivo*[8] suggests an addition to the model. Displays where P_{fr}/P exceeds 0.4 require another addition concerned with the photochemical properties of $P_{fr}X$. A model which takes these facts into account is shown in Figure 2. Three photoreactions are involved, namely **1, 4,** and **5** with five dark reactions **1, 2, 3, 5,** and **6.**

We present some of the published work crucial to the extension of the model as well as unpublished work that was only appreciated as puzzling in terms of reaction **1** alone. Experimental work is reported on seed germination, flowering of long-day plants bearing on reaction **4** and confirmation of Hartmann's work relative to reaction $\mathbf{2}_d$ with seed of another type. We feel that the complex nature of the HER is best analyzed for detail in this multiplicity of displays where individual component reactions might be dominant in one of the displays.

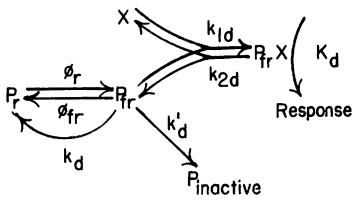

FIG. 1.—A scheme for the high-energy reaction (HER) after Hartmann.[2]

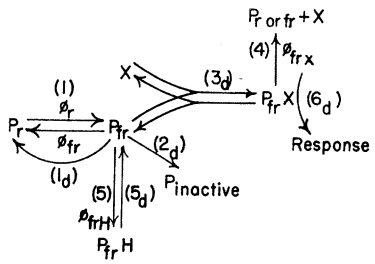

FIG. 2.—A proposed scheme for the HER. Photoreactions **1**, **4**, and **5** are accompanied by dark reactions 1_d, 2_d, 3_d, and 6_d. Reaction **5** is probably dark-reversible, but not photoreversible.

Responses to radiation <600 nm are not considered, as the blue region of the spectrum probably involves action of pigments other than phytochrome.

Light action in germination of Lepidium virginicum seed indicative of photoreaction **5**: When establishing the P_{fr} requirement for germination of *L. virginicum* seeds,[9] we noted, but did not publish, that dark controls left in the diffuse light of the laboratory after an experiment came to full germination in a few days. The response was unexpected because the action spectra for interconversion of $P_r \rightleftarrows P_{fr}$ indicated that the radiation in the room would not promote more than 50 per cent germination. Detailed experiments indicated that seeds potentiated to high germination in red light were suppressed to the expected germination levels by short exposures to >600 nm radiation from *INC* of *SUN* (Table 1) adequate to approach equilibrium for photoreaction **1**. Exposures for longer periods, however, increased the germination to high levels.

Results of some experiments are shown in Figures 3 and 4. Lots of 100 *L. virginicum* seeds were held in darkness for 24 hours at 20° on filter paper moistened with 0.2 per cent KNO_3. They were then irradiated according to the schedules in the figures with sources listed in Table 1 and returned to darkness for two days before counting. Each point shows average results for four lots of seeds. The fractions of the $P_{fr} \rightleftarrows P_r$ steady state attained after t seconds, shown in the last column of Table 1, are calculated values for *P in vitro*[10] from the indicated starting condition.

Figure 3 shows that 1-second exposure to *SUN* or 20 seconds to *INC* gives the same minimum germination. The times are compatible with ratios of the 600–780 nm intensities from the two sources. Percentage of germination increases

TABLE 1. *Several radiation sources referred to in the text.*

Source	Region (nm)	Einsteins (cm^{-2} sec^{-1} $\times 10^{-8}$)	P_{fr}/P at steady state	Fraction of steady state in t sec
Sunlight *SUN*	600–780	10	0.6	0.8 (1 sec from P_r)
Inc. filament *INC*	600–780	1.0	0.6	0.8 (10 sec from P_r)
Inc. filament *FR*	710–750	0.2	0.01	0.8 (60 sec from P_{fr})
Fluorescent *R*	600–680	0.1	0.8	1.0 (240 sec from P_{fr})
Spectrograph	715–725	0.12	0.01	0.7 (60 sec from P_{fr})

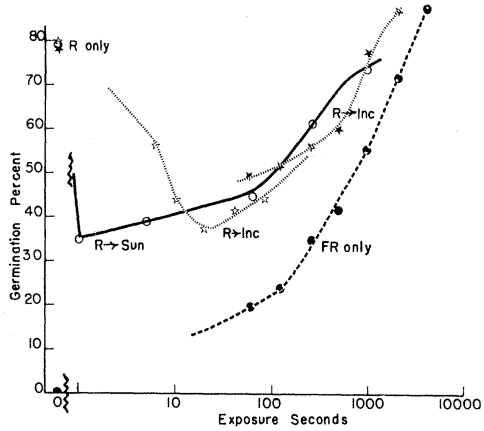

FIG. 3.—Germination of *L. virginicum* seed following exposure to various radiations in sequence. Radiations are listed in table 1.

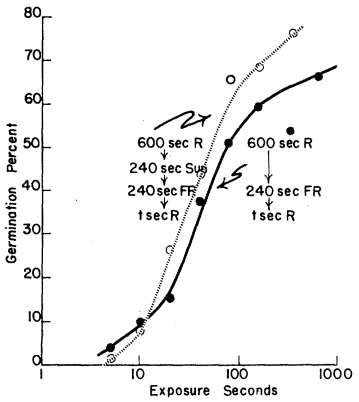

FIG. 4.—Germination of *L. virginicum* seed in response to red radiation (*R*) following the indicated radiation sequence.

from the minimum with increased exposure times. About 1000 seconds were required to attain the initial level of germination following *R*.

Seeds were tested for possible changes in sensitivity to radiation by sequences either including or omitting a 240-second exposure to *SUN* (Fig. 4). The irradiances for equal germinations below 50 percent differ by not more than 1.5 times. These results indicate that enhanced germination with prolonged radiation does not arise primarily from enhanced sensitivity of the germination process.

A second photoreaction effective, in the 600–780 nm region, is evident shortly after the minimum germination is potentiated. This is within four seconds after the start of exposure to *SUN* and is within a factor of 2 of the minimum with *INC*. The second photoreaction is accordingly operative to some extent before the minimum is reached. We identify it as an HER and take it for evidence of photoreaction **5** in Figure 2 as will be discussed later.

Flowering control in Chenopodium rubrum[11] *as evidence for photoreaction* **5**: An effect of light on control of flowering of the short-day plant *C. rubrum* parallels the germination response described for *L. virginicum*. The pertinent results[11] are shown in Figure 5. Young *C. rubrum* plants with cotyledons fully spread were subjected to five 8-hr light and 16-hr dark cycles. Each dark period was interrupted at the midpoint by R (Table 1) for 240 seconds. Lots of plants were then placed across a spectrum and irradiated as indicated in Figure 5. They were returned to 16 light-8 dark cycles and, after seven days, were dissected to test flowering (stages 0 to 9). Controls, subject to R only, showed zero flowering and uninterrupted controls developed to stage 9.0.

An irradiance of 60 seconds in the 720–740 nm region, adequate to drive P_{fr} 0.7 of the way to P_r, promoted flowering to stage 6.8. Maximum flowering in the 680–780 nm region was attained in 480 seconds. Continued irradiation for 3840 seconds reduced flowering to stages of 0.1 and 1.1 at 722 and 737 nm, respectively.

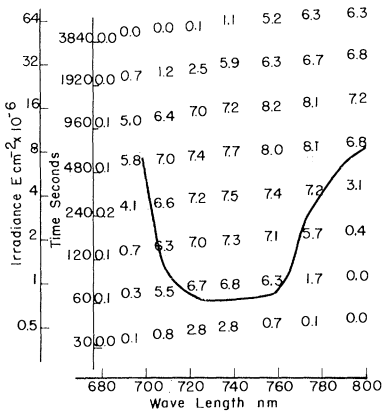

FIG. 5.—Effects of irradiation in the region of 680 to 795 nm on the flowering stage, on a scale of 0 to 9, of *C. rubrum* previously exposed to red radiation (*R*) adequate to prevent flowering. Exposure started at the 8th hour of 16-hr dark periods.

Changes of the degrees of flowering with periods of exposure to far-red radiation at various intensities are shown in Figure 6. The curve for an intensity of 1.2×10^{-9} E cm^{-2} sec^{-1} was at the 737 nm band on the spectrograph. The other curves were from a far-red source transmitting >700 nm. A significant feature of these curves is that flowering is depressed from the maximum more quickly at high than at low intensities. This is evidence for photoreaction **5,** but dark reaction **2,** that is loss of total P, as well as dark reactions **3** and **6** probably also affect the results. In a previous work,[12] the depression of flowering with the period of *FR* irradiation was interpreted only as attainment of the biological response (reaction **6**) through maintenance of low levels of P_{fr}. The higher average level of P_{fr} with irradiation time at low rather than high intensities (*Curves A* and *B*, Fig. 6) indicates participation of photoreaction **5.**

Action spectra for a possible photoreaction **5** are not reduced for *C. rubrum* flowering (or *L. virginicum* seed germination) control because of confounding with the photoreversible change of P by reaction **1** as well as with the simultaneous operation of the dark reactions.

Spectroscopic evidence for occurrence of $P_{fr}H$: Anderson *et al.*[7] in studying the

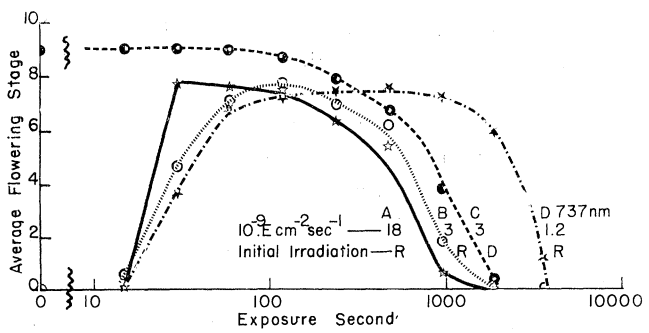

FIG. 6.—Flowering of *C. rubrum* after various times of irradiation with the two levels of far-red following *R*, C is for far-red only, and D is for 737 nm with the spectrograph as shown in Figure 5.

acidity and the temperature dependence of absorbance of isolated P, observed the appearance of a species with an absorbancy maximum near 650 nm. At pH 6.9, this form and P_{fr} varied reciprocally in the temperature range of -22 to $-41°$. Isosbestic points were observed at 400, 465, and 637 nm. Cross *et al.*[13] observed the same form upon warming P_r to about 35° after flash excitation. Anderson *et al.* interpreted their results as indicating that the absorbant was a $P_{fr}H$, or acid, form of P.

Boisard *et al.*[8] measured the levels of P_{fr}, by spectroscopic methods, in lettuce seed before irradiation with far-red and at various times after irradiation. They observed that absorbance lost in the region of 730 nm reappeared with time, and interpreted this in terms of reaction **1** with dark reversion of P_r to P_{fr}. Such a reversion is opposite to that observed for lettuce seed at 35°[4] and many other materials. The $P_{fr}H$ form, if present, would have absorbance properties noted by Boisard *et al.* and moreover could change in darkness to P_{fr}. A further interpretation would be that in the lettuce seed conditions *in vivo* stabilize $P_{fr}H$ compared with *in vitro* conditions.

A control of Amaranthus arenicola and A. retroflexus seed germination as evidence for dark reaction **2**: Hartmann[2] found that minimum germination of *Lactuca sativa* seeds under continuous radiation was attained with mixed red and far-red radiation sources leading to P_{fr}/P ratios near 0.10. We obtained a similar result with *A. arenicola* (Fig. 7) as will be described in detail elsewhere. Each point in Figure 7 is for two lots of 200 seeds continuously irradiated under mixed sources for three days at 27°. The ratios of intensities for 656/740 nm at the minimum points are near 0.08 for 60 and 300 pE cm^{-2} sec^{-1} with 740 nm radiation. The corresponding P_{fr}/P ratio is about 0.16. Hartmann[2] concluded that such a P_{fr} loss permitted maximum P_{fr} action relative to P_{fr} loss through reaction **2d**. Decrease in sensitivity of *L. sativa* seed germination to promotion by red-light with time of the irradiance supported the interpretation. *A. retroflexus*, which requires temperatures >32° for germination, gave results (not shown) at 35° similar to those in Figure 7. These seeds under continuous *FR* became markedly less sensitive to red radiation than ones held imbibed in darkness. This change is indicative of a lowering of total P.

Action spectra for Mimosa pudica leaflet closure at high P_{fr}/P ratios[14] *as evidence for photoreaction* **4**: Leaflets of *M. pudica* close within 20 minutes after darkening from normal growing conditions, which maintain high P_{fr}/P ratios[15] (>0.5). The leaflets remain open in darkness if the ratio is reduced to 0.02 by far-red radiation at the beginning of the dark period. The closing reaction is repeatedly reversible, corresponding to the phytochrome change by photoreaction **1**.

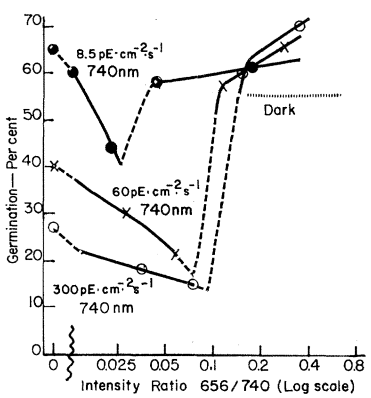

FIG. 7.—Germination of A. arenicola seed under continuous irradiation at 740 ± 5 nm at three intensity levels with accompanying 656 ± 5 nm radiation of various intensities.

The leaflets, however, remain open in *SUN* or *INC* filament light giving P_{fr}/P ratios adequately high for closure as is displayed on darkening. It follows that the closing is opposed by a photoreaction other than reaction **1**. The action spectrum[14] is a typical HER with maximum effectiveness near 720 nm. This spectrum was determined after prolonged periods in light with the period for measurement only a small part of the total irradiation time. Reaction **2** thus cannot be a factor in the response.

We interpret these results in terms of photoreaction **4**, where $P_{fr}X$ has a high absorbancy in the region of 720 nm and a much lower one in the 600–680 nm region than does P_{fr}. The dark reactions **3**, moreover, are slow compared with the photoreaction **4** at moderate irradiation intensities in the 700–750 nm region; that is, $P_{fr}X$ remains at a low level under steady-state light conditions.

Action spectra for control of A. arenicola and Poa pratensis seed germination,[16] *flowering of Hyoscyamus niger,*[17] *Spinach olereacea and Beta vulgaris at high P_{fr}/P ratios as evidence for photoreaction* **4**: The action spectra were measured, under continuous irradiation, for *A. arenicola* and *P. pratensis* seed germination. P_{fr}/P ratios were maintained at high values by the presence of radiation in the 600–680 nm region. Action maxima under these conditions were observed in the region 720 nm. The spectra, although requiring a long time to be displayed are strictly comparable to those obtained in short times for *M. pudica* leaflet closure. Thus, photoreaction **4** is displayed even though it might be accompanied by reaction **2**.

Flowering and stem-lengthening responses were measured for the 700–760 nm region for the long-day plants *H. niger,*[17] *S. olereacea,* and *B. vulgaris* at both high and low P_{fr}/P ratios (that is, with and without supplementary 600–680 nm radiation) and in the 600–700 nm region at high ratios. Plants on 8 L–16 D cycles were irradiated for eight hours across the midpoint of each dark period on 21 consecutive days at 4×10^{-9} E cm^{-2} sec^{-1} in the individual wave bands, and then dissected (note, ref. 17 for technique). Maximum effectiveness was displayed near 720 nm irrespective of the P_{fr}/P ratio. *S. olereacea* and *H. niger* responded somewhat to radiation in the 600–700 nm region (Fig. 8) while *B. vulgaris* did not respond.

The responses observed with the low irradiancies of Figure 8 were paralleled by the three species under high-fluorescent light intensities (radiation chiefly <680 nm) for 16-hour days with a low intensity gradient of incandescent filament radiation.

These several results are indicative of a photoreaction with maximum effectiveness in the 720 nm region irrespective of the P_{fr}/P ratio. The photoreaction is effective when radiation intensity in the region of 600–680 nm is appreciable. Both P_{fr} and P_r have high absorbancies in this region. The response accordingly does not arise from continued excitation of P. The effective pigment, moreover, has a very low absorbancy in the 600–680 nm compared with P_{fr} as shown by the responsiveness of beet (*B. vulgaris*). These are interpreted as properties of $P_{fr}X$.

General Discussion.—Reasons for including each component of Figure 2 are perhaps evident from study of the several sections. Some elaboration, however, might be useful.

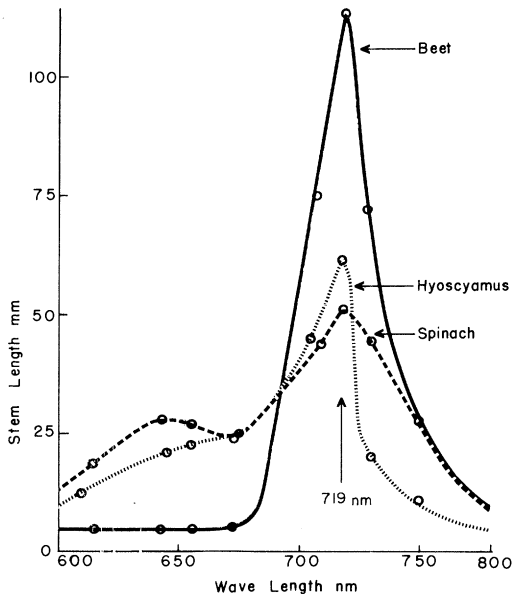

FIG. 8.—Response spectrum for stem lengthening accompanying flowering of beet, *H. niger*, and spinach. Note text for details.

The HER suppressions of *L. virginicum* seed germination and *C. rubrum* flowering appear to be displays of the same phenomenon. They are interpreted as involving the buildup of some form of P in the light from which P_{fr} can reappear in darkness. The build-up is light-energy dependent in the 700–780 nm region and, thus, probably involves excitation of P_{fr}. The effectiveness of light, however, is about an order of magnitude lower than the conversion of P_{fr} to P_r by reaction **1**. A time-intensity interaction would be expected because of the dark reaction, **5d**. These features are adequately expressed by reaction **5**.

Recognition of $P_{fr}H$ at low temperatures by Anderson *et al.*,[7] affords the model if not the reality for reaction **5**. Boisard's *et al.*[8] observation of spontaneous reappearance of P_{fr} at room temperature in lettuce seed after irradiation with far-red is in accord with the dark reversion of $P_{fr}H$ by reaction **5**. It is direct evidence, in any case, for the appearance of P_{fr} from something that is not P_{fr}, which is the only formal requirement of reaction **5**.

The effect of continuous irradiation on P_{fr} levels in imbibed seeds is in accord with Hartmann's model (Fig. 1). It is included as reaction **2** in the elaborated model (Fig. 2). Such loss of P when in the P_{fr} form has been shown for several objects by spectroscopic measurements.[6] If irradiations of seeds are for short periods followed by continuous darkness, germination percentage is higher than from continuous irradiation. This is evidence for countering of the germination process by light other than through reaction **2**. We interpret it as arising from reaction **4**, but reaction **5** could also contribute by withdrawal of P_{fr} in light while permitting its reappearance in darkness.

That P_{fr} must have a site of action for biological display is logically necessary. This is formalized as reactions **3** and **6** in Figure 2, where **6** is the first step in a spreading sequence. In a formal sense, multiple displays arise from branchings in **6**. P_{fr} at the site of action is indicated as $P_{fr}X$. The questions are: What might be the absorbance features of $P_{fr}X$ and what are the results of its excita-

tion? Maintenance of an open *M. pudica* leaf in sunlight and the requirement of a far-red radiation component for stem lengthening and flowering of *B. vulgaris*, under otherwise intense irradiation, answer the questions. Response spectra for the two phenomena are alike in showing maximum effectiveness near 720 nm and in being intensity dependent. Red light is markedly less effective than far-red in maintaining the open leaflet. Thus, we recognize $P_{fr}X$ as having about the same relative absorbancies as of the *B. vulgaris* action spectrum in Figure 8.

The view that $P_{fr}X$ is photodissociable, possibly leading to P_r and the free site, requires a steady state to be set up in continued radiation in the region of 600 to 800 nm. At high intensities, as in sunlight, dark reaction **3** would be expected to be rate limiting so that attainable levels of $P_{fr}X$ would be too low, because of photoreaction **4**, for effectiveness of reaction **6**. In short, the HER would prevent P_{fr} from acting during daylight, but would poise it for action when night comes. This, in fact, is how the leaves of *M. pudica* and many other legumes respond in quick display. This situation for P also is evidence that reaction **2** is not very effective for destruction of P in these fully developed plants in contrast to seeds and young seedlings.

The paradox in nature is—sunlight poises phytochrome for action, but prevents it from acting.

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