

Action Spectrum and Characteristics of the Light Activated Disappearance of Phytochrome in Oat Seedlings¹

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Summary. The action spectrum for the light-activated destruction of phytochrome in etiolated *Avena* seedlings has been determined. There are 2 broad maxima, one between 380 and 440 m μ , the other between 600 and 700 m μ , peaking at about 660 m μ . On an incident energy basis, the red region of the spectrum is more efficient than the blue by about one order of magnitude in activating phytochrome disappearance. Both the red absorbing as well as the far-red absorbing forms of phytochrome are destroyed after exposure of *Avena* seedling to either red or blue light.

From the action spectrum and photoreversibility of pigment loss, we conclude that phytochrome acts as a photoreceptor for the photoactivation of its metabolically-based destruction. We suggest that another pigment might also be associated with the disappearance of phytochrome in oat seedlings exposed to blue light.

The plant pigment phytochrome appears to be the photoreceptor for many morphogenic and biochemical processes initiated by low irradiances of visible light (see 8, 16 for recent summaries). Phytochrome exists in 2 forms, one with an absorption maximum at 600 m μ , the other at 730 m μ (1). These 2 forms of the pigment are interconvertible most efficiently by wavelengths of light near their absorption maxima, a conversion that is the basis for a spectrophotometric assay for phytochrome (3, 4). The pigment has been isolated and partially purified. It is a chromoprotein with a molecular weight between 90,000 and 150,000 (13). The chromophore is probably a bilitriene-type hile pigment (17).

Phytochrome is found at relatively high concentrations in etiolated seedlings, where it occurs as the red absorbing form (P_{660}). Exposure to red light will convert some of the P_{660} to the far-red absorbing form (P_{730}). Butler et al. (4) reported that exposure of etiolated maize seedlings to red light causes a metabolically dependent disappearance of phytochrome, a loss ascribed wholly to destruction of the P_{730} formed by the exposure to red light.

Hillman and co-workers (6, 7, 10) working with both monocot and dicot tissues, have also found that red light causes a loss of phytochrome. They like-

wise attribute the loss solely to the lability of P_{730} . Butler et al. (4) stated that a reversion of P_{730} to P_{660} occurred in maize, but later (3) interpreted this P_{660} found after red irradiation and several hours of growth as residual pigment, i.e., that redlight converts only 80 % of the original P_{660} to P_{730} . Since the amount of P_{660} thereafter remained constant, they concluded that P_{660} is stable and that little, if any, dark reversion of P_{730} occurred. A similar conclusion was reached by Hopkins and Hillman (10).

We have followed the disappearance of phytochrome and the conversion of P_{660} to P_{730} in etiolated oat seedlings that had been exposed briefly to white light (5). Our results suggested that both the P_{660} and P_{730} forms of phytochrome were destroyed, and that wavelengths other than those in the red region of the spectrum may activate phytochrome destruction². This paper deals with the interconversion and disappearance of phytochrome after exposure of the etiolated oat seedling to light of various wavelengths in the visible spectrum.

Materials and Methods

Unhulled dry seed, 10 g. of *Avena sativa* cv. Clinton were planted in plastic boxes, 2.0 x 5.5 x 1.0 inches in width, length and height, respectively. The medium consisted of one-quarter inch cellulose paper moistened with 35 ml of tap water. After sowing, the seeds were covered with a galvanized wire screen, one-quarter inch mesh. The seedlings were grown for 5 days in light-tight containers of approximately 12 l. capacity through which moistened air at 23° was passed at a flow rate of about 2 l./minute. After experimental exposure to light, the plants were returned to the containers at 23°. The

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² We use the terms disappearance and destruction solely in the sense of an irreversible decrease in concentration of interconvertible pigment. It could well be that a drop in concentration represents a conversion of the holochrome to a nonreversible form, rather than actual disappearance of the complex. It could also reflect a curtailment of phytochrome biosynthesis.

phytochrome was stabilized by chilling the plants in ice water for about 60 seconds. The coleoptile, including the foliage leaf, was severed from the shoot and cut into 1 to 3 mm sections. A portion, 0.35 g. was packed to a depth of 5.0 mm, with a variation of no more than 0.5 mm, in a 1.0 cm diameter cell for spectral examination. The entire assay procedure was conducted at 2 to 5°.

If only total phytochrome content was to be determined, sampling was done under laboratory light. When assays were made for P_{730} as well as for total pigment, sampling was carried out under a green safe-light. This consisted of two 40-watt green fluorescent tubes behind one-eighth inch of Amber (2451) and one-eighth inch of Green (2092) Plexiglas G (Rohm and Haas). The transmittance of this assembly has a single maximum at 565 $m\mu$ and 0.1% cut offs at about 545 and 590 $m\mu$ (14). In addition, 1 layer of DuPont dark blue cellophane No. 3158 was added to the assembly.

The Argonne Spectrograph (11) was used to obtain narrow wavelength bands for the determination of the action spectrum. When necessary, irradiance was reduced by interposing wire screens in the undiffracted beam. Each band was reflected downward by a front-surfaced mirror mounted above the seedlings. The spectral distribution across the focal curve is such that approximately 95% of the energy incident to each box of seedlings fell within 5 $m\mu$ about any wavelength locus (9). An Eppley thermopile, calibrated against a standard lamp, and Liston-Becker chopper amplifier were used to determine irradiances with monochromatic sources. For white light a Weston Model 756 ft-c meter was used. The source of white light was a tungsten filament lamp operated at 115 v, 60 cycles; different irradiances were obtained by varying the distance between the lamp and the seedlings.

The source of red light was two 40 w General Electric red fluorescent tubes behind 2 layers of red-cellophane DuPont 3153, giving an incident energy to the plant of about 73 $\mu w \cdot cm^{-2}$. The far-red source was a 75 w, reflector-flood, tungsten filament lamp (operated at 115 v) behind 2 layers of the red and 2 layers of the dark blue cellophane described above, giving an incident energy of about 5.3 $mw \cdot cm^{-2}$ to the plant.

Phytochrome was assayed spectrophotometrically (3,4) in a dual beam spectrophotometer (Agricultural Research Specialty Company, Hyattsville, Maryland). Assay results are expressed as Δ (Δ Absorbancy) [Δ (Δ A.)] (4), a relative measure of the amount of reversible phytochrome present in the plant tissues. The Δ (Δ A.) readings for total phytochrome were corrected by multiplying by the factor 1.25 on the basis that saturating exposures of red light converts only 80% of P_{660} to P_{730} (3). Each mean Δ (Δ A.) value presented was derived from at least 2 experiments with at least 3 determinations in each. The coefficient of variation of these means ranged between 5 and 10%.

Results

Coleoptile Length and Phytochrome Content. Phytochrome contents of 5-day-old etiolated seedlings of 2 cultivars were measured. The results obtained for groups of different coleoptile lengths are given in table 1. The data indicate that the phytochrome content per unit weight decreases with increasing coleoptile length, a decrease that has also been shown to occur in barley seedlings (2). In all subsequent experiments only coleoptiles 20 to 25 mm in length were taken for assay.

Table 1. *Phytochrome Contents of 5-day-old Etiolated Oat Coleoptiles of Different Length*

Length mm	Δ (Δ A.) /0.35 g	
	Brighton (Hullless)	Clinton
10-15	0.109	0.083
15-20	0.086	...
20-25	0.061	0.070
30-40	0.049	...
35-45	...	0.043

Kinetics of Phytochrome Disappearance, White Light. Etiolated seedlings were exposed 1.0 minute to different irradiances of white light. They were then grown in the dark and assayed for phytochrome after various intervals up to 6 hours. Phytochrome contents of dark-grown controls were measured at the same intervals. The percent of the pigment that disappeared relative to the nonirradiated controls is plotted against radiant exposure in figure 1.

These curves show that about 25 ft-c minutes virtually saturates the light-activated disappearance of phytochrome, irrespective of the subsequent growth period. With 4.0 hours of growth, saturating exposures led to a destruction of about 60% of the phytochrome. The same percent destruction was

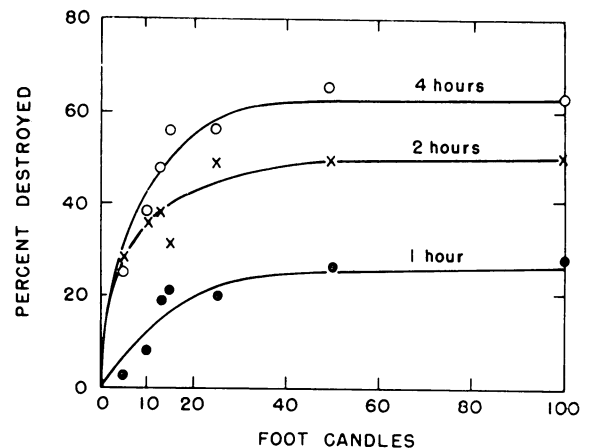


FIG. 1. Percent disappearance of phytochrome at 3 intervals after exposure of etiolated oat seedlings to various irradiances of white light for 1.0 minute.

observed when a post-irradiation growth period of 6 hours was tested.

In another experimental series with white light, the disappearance of P_{730} and P_{660} as well as total pigment was determined. Figure 2 shows the relative amounts of pigment after exposure to 15 ft-c minutes. Immediately after irradiation about 25% of the P_{660} was found converted to P_{730} . Both forms of phytochrome gradually disappeared. After 6 hours, no P_{730} was detected; the pigment concentration at this time was about one-third of the original level of phytochrome. The results with an exposure of 100 ft-c minutes were essentially similar.

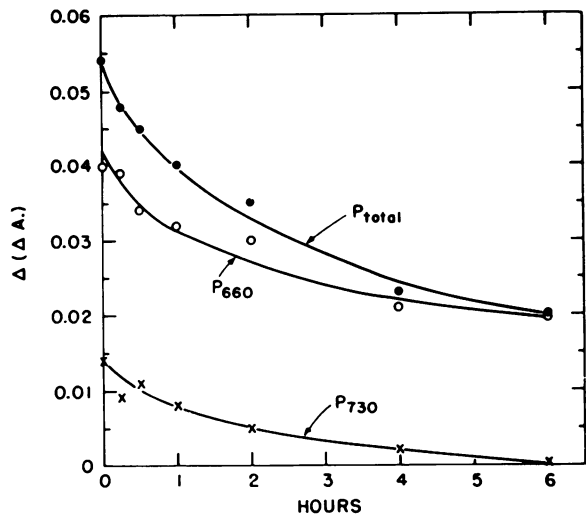


FIG. 2. Disappearance of phytochrome after 1.0 minute exposure of seedlings to white light, 15 ft-c.

Action Spectrum. Before determining an action spectrum for the light-activated destruction of phytochrome, irradiance and time were varied in a series of exposures at 660 $m\mu$ and 400 $m\mu$, wavelengths found to be relatively effective in preliminary experiments. At 660 $m\mu$ incident irradiances of 2.3 to 8.2 $\mu w \cdot cm^{-2}$, and at 400 $m\mu$ irradiances of 34.1 to 400 $\mu w \cdot cm^{-2}$, were within range of reciprocity up to the longest exposure time tested, 16 minutes at the lowest irradiances used. The action spectrum was based on the energy required to initiate a 30% (non-saturating) destruction of phytochrome with 4.0 hours of growth at 23° following the light exposure. Dose-response curves within the above incident energy ranges were run at each wavelength used; the energies required for 30% destruction were estimated by interpolation. Typical curves are shown in figure 3. For 660 $m\mu$ and 600 $m\mu$, 10 and 30 kiloergs $\cdot cm^{-2}$ were required, respectively, in order to obtain 30% disappearance of phytochrome.

Figure 4 shows the relative action spectrum for the light-activated destruction of phytochrome. There are 2 broad maxima, one lying between 370 and 450 $m\mu$, the other between 550 and 700 $m\mu$, peaking near 660 $m\mu$. A minimum appears in the neighborhood of 480 $m\mu$. The red optimum of the spectrum is

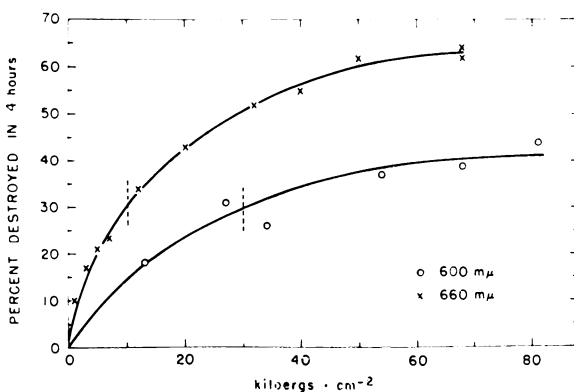


FIG. 3. Percent destruction of phytochrome 4 hours after exposure of seedlings to various radiant densities at 600 and 660 $m\mu$.

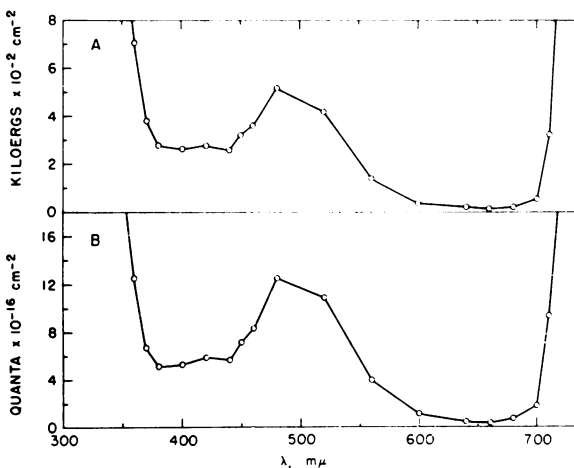
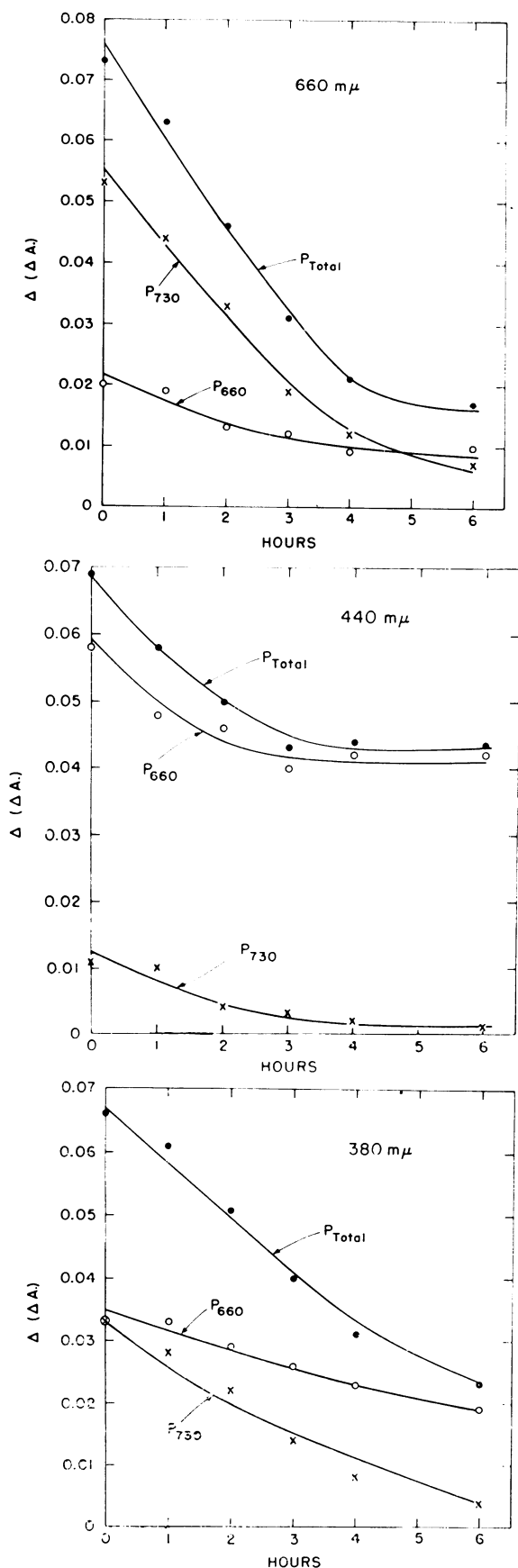


FIG. 4. Incident energies, expressed as kiloergs (A) or quanta (B) per unit area, required at various wavelengths for 30% destruction of phytochrome in 4 hours.

about 15 (on a quantum basis) and about 25 (on an energy basis) times more efficient than the blue in causing the disappearance of phytochrome. At wavelengths above 710 $m\mu$ and below 360 $m\mu$, with radiant densities up to 800 kiloergs $\cdot cm^{-2}$, we could not obtain a 30% disappearance of phytochrome within a 4-hour growth period.

Kinetics of Phytochrome Disappearance, Monochromatic Light. The light activated disappearance of P_{730} , P_{660} and total phytochrome at 660, 440 and 380 $m\mu$, using saturating energies at each wavelength, was then examined. These data are shown in figures 5a-c. At each of the 3 wavelengths the P_{660} as well as P_{730} form of phytochrome disappears with time. The data in aggregate are suggestive of a slight lag in the disappearance of phytochrome immediately after irradiation (cf. 4); however the observation frequencies and variances in the present experiments do not permit rigorous inference about such lag.

The percentages of pigment interconversions immediately after irradiation, and the percentage of pigment disappearing in 6 hours associated with the sat-



urating radiant densities (fig 5a-c) are given in table II. Irradiation at 660 $m\mu$ converted three-quarters of the P_{660} to the P_{730} form, and about 80 % of the phytochrome that disappeared can be accounted for by the disappearance of the P_{730} so produced. Irradiation at 380 $m\mu$, which caused a 50 % conversion of P_{660} to P_{730} , resulted in the disappearance of 65 % of the phytochrome; one-third of this disappearance can be ascribed to the decrease in P_{660} . At 440 $m\mu$, where a conversion to P_{730} of only about 15 % of the pigment occurred, roughly two-thirds of the pigment disappearing is attributable to a loss of P_{660} .

The lability of P_{660} is also shown by the following experiment. Seedlings were exposed to far-red light for 2.0 hours. One-half of the seedlings were assayed immediately after exposure. The other half was returned to darkness and assayed 2.0 hours later. After 2.0 hours of the far-red, the phytochrome content had changed from a Δ ($\Delta A.$) of 0.070 to 0.044, a drop of about one-third. After 2 additional hours in the dark the phytochrome content dropped still further to a Δ ($\Delta A.$) of 0.027, 40 % of the original titer.

The conversion of P_{660} to P_{730} as well as the total phytochrome disappearance in 4 hours was then ascertained for various wavelengths beginning with non-saturating radiant densities. The results are plotted in figure 6. There is a gross correlation between the extent of pigment interconversion and the extent of pigment disappearance. Both phenomena are high at 660, progressively decreasing at 380, 400 and 440

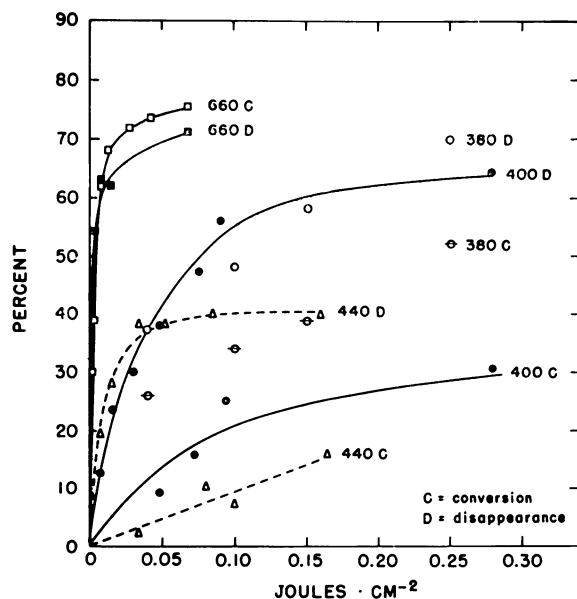


FIG. 6. Percent conversion of P_{660} to P_{730} and percent of phytochrome disappearing in 4 hours after exposure to various radiant densities at 4 wavelength bands.

FIG. 5. Disappearance of phytochrome after exposure to saturating radiant densities at a) 660 $m\mu$, 0.068 joules· cm^{-2} ; b) 440 $m\mu$, 0.164 joules· cm^{-2} ; c) 380 $m\mu$, 0.25 joules· cm^{-2} .

Table II. Percent Conversion and Loss of Phytochrome after Exposure of the Seedling to Radiant Densities Causing Maximum Disappearance Rates at each Wavelength

λ m μ	joules·cm ⁻²	% Conversion		% Disappearing in 6 hours		% of the P total disappearance caused by loss of	
		P ₆₆₀ → P ₇₃₀	P ₇₃₀	P ₆₆₀	P Total	P ₇₃₀	P ₆₆₀
660	0.068	73	87	50	77	82	18
440	0.164	16	91	28	38	38	62
380	0.250	50	88	43	65	67	33

m μ . However, though the percentage conversion and disappearance are similar at 660 m μ , in the blue spectral region the disappearance is more sensitive and greater than is the conversion of the pigment to the P₇₃₀ form. The differences appear most marked at 440 m μ . At both 440 and 400 m μ at the higher radiant densities about twice as much pigment disappears as is converted (cf. also table II).

Photoreversibility of Phytochrome Disappearance. Seedlings were irradiated at both 400 and 660 m μ , each exposure being followed immediately with an exposure to 730 m μ . The P₇₃₀ formed at each of the first 2 wavelengths was converted (on a percentage basis) about equally well to P₆₆₀ by the far-red light (fig 7a and 7b). In other words, the pigment conversion induced by the blue light was, like that induced by the red light, reversible upon immediately subsequent exposure to far-red light. Furthermore, the disappearance of phytochrome elicited by the radiant densities used in the blue and red spectral regions are partially reversed by far-red light.

We found that 0.5 minutes of red light saturated the conversion of P₆₆₀ to P₇₃₀ and that 2.0 minutes of far-red light completed the photochemical conversion

of P₇₃₀ to P₆₆₀. Seedlings were therefore given 0.5 minute of red-light which was immediately followed by 3.0 minutes of the far-red to determine the photo-reversibility of pigment disappearance at initially saturating levels of both spectral bands. The plants were then returned to darkness and periodically assayed for phytochrome during the next 7.0 hours. After 1 hour of growth the phytochrome content dropped about 20 % and then remained essentially constant for the next 6 hours. The phytochrome content of seedlings that were irradiated only with the far-red light did not differ materially from the dark controls over the 7-hour period. Apparently destruction induced by exposure to red light at densities saturating pigment interconversion is not reversed completely by far-red light at analogous radiant densities.

Discussion

We find, that most, rather than all (4, 6, 10) of the loss of phytochrome in etiolated *Avena* seedlings exposed to red-light can be accounted for by loss of P₇₃₀. There also is a reduction in the amount of P₆₆₀ after irradiation with either white light (fig 2) or monochromatic bands in the red and blue (fig 5) and far-red spectral regions. We infer that both P₇₃₀ and P₆₆₀ are sensitive to light-activated metabolic destruction.

It was shown in table I that the phytochrome content per unit weight of coleoptile tissue decreases with increasing coleoptile length. Therefore, it could be suggested that the photoactivated decreases in pigment described in the present study are not real, but are concentration changes derived from an enhancement by light of coleoptile growth. If such occurred, it could only account for a relatively minor change in pigment concentration. The size of the coleoptiles taken for assay were of uniform length (20–25 mm), elongating at a rate of approximately one-half mm per hour. A red light-induced doubling of growth rate could thus account for at most a 5 % increase in weight in the next 2 hours, a period wherein pigment losses of 40 % occurred.

If there is a reversion of P₇₃₀ to P₆₆₀ in the dark, the reaction must proceed at a slower rate than the disappearance of P₆₆₀. If the back reaction were faster, and no destruction of the P₆₆₀ occurred, then the amount of P₆₆₀ should increase in darkness after a brief exposure to light. This was not observed.

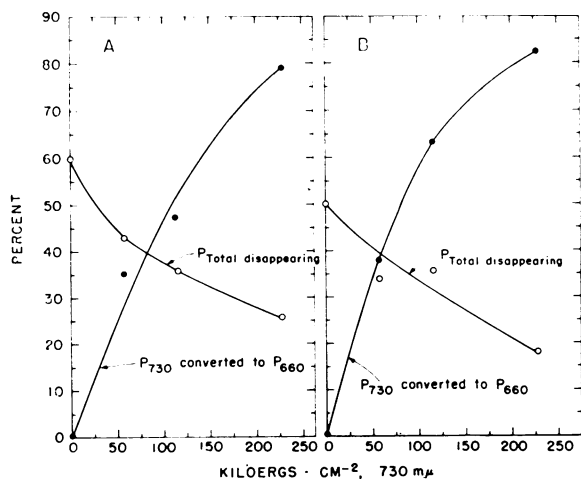


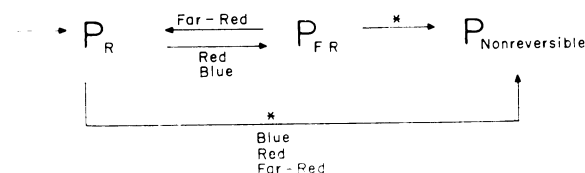
FIG. 7. Reversal by far-red of red induced conversion and disappearance of phytochrome. A) Seedlings irradiated at 660 m μ , 68 kiloergs·cm⁻², followed by the indicated radiant densities at 730 m μ . The \circ curve represents the percentage of the total pigment lost after 4 hours, the \bullet curve the percent of P₇₃₀ that is converted back to P₆₆₀ by 730 m μ light. B) As preceding, but initial irradiation at 400 m μ , 936 kiloergs·cm⁻².

We conclude that in etiolated *Avena* tissue the dark transformation of P_{730} to P_{660} either does not occur or occurs at a relatively low rate. Since P_{660} is metabolically destroyed after brief illumination, this type of etiolated tissue appears unsuitable for studying the dark conversion of P_{730} to P_{660} .

It is likely that phytochrome itself is a photoreceptor activating the metabolic self-destruction. First, the action spectrum for the process in the visible spectrum has a strong band at about 660 $m\mu$ and a weaker band in the region near 400 $m\mu$, with a minimum in the blue-green. This is the complement of the in vitro absorption spectrum of the P_{660} form (15). Below 350 $m\mu$ the efficiencies per incident photon decrease drastically (fig 4), and no destruction was observed at 300 $m\mu$. Second, the pigment decrease induced by irradiation at 400 $m\mu$ or 660 $m\mu$ can be partially reversed if these 2 irradiances are followed immediately by light at 730 $m\mu$.

Light at 660 $m\mu$ was most effective in causing, to an almost equal extent, both the photochemical conversion of the chromophore and its later disappearance. Light in the blue spectral region also elicits both effects, but, in contrast to red light, the energies appear to be channeled preferentially toward phytochrome disappearance (fig 6). For example, at 440 $m\mu$, with a radiant density of 33 millijoules \cdot cm $^{-2}$, the photoactivated disappearance of phytochrome is over 10 times that of chromophore conversion. If only the phytochrome system were involved in activating the disappearance of phytochrome, immediate post-irradiation with far-red light should completely reverse the effect of red light. Since this does not occur (fig 7), another pigment might also function as a photoreceptor for phytochrome destruction. The preferential destruction over conversion in the blue spectral region, particularly at 440 $m\mu$, is suggestive of a carotenoid or a flavin, though there is no band for phytochrome destruction in the near ultraviolet region. Participation of a porphyrin might also be considered since many of these pigments absorb in both the blue and red spectral regions. In this connection, the high-energy nonreversible photomorphogenic pigment described by Mohr (11, 12) which has action maxima at about 440 $m\mu$ and 730 $m\mu$, should be mentioned. It seems unlikely that such a pigment participates in phytochrome destruction: there is no band in the far-red in the action spectrum for phytochrome destruction, and the incident energies required for the metabolically-based destruction are low.

From the present study, we suggest the following association of the photoactivated changes of phytochrome in the etiolated *Avena* coleoptile:



The rates of the reactions marked by asterisk are relatively low and metabolically limited.

Acknowledgments

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