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Photocontrol of the Germination of *Onoclea* Spores

I. ACTION SPECTRUM

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LESLIE R. TOWILL[1] AND HIROSHI IKUMA
Department of Botany, University of Michigan, Ann Arbor, Michigan 48104

ABSTRACT

Light stimulates the germination of spores of the fern *Onoclea sensibilis* L. At high dosages, broad band red, far red, and blue light promote maximal germination. Maximal sensitivity to these spectral regions is attained from 6 to 48 hours of dark presoaking, and all induced rapid germination after a lag of 30 to 36 hours. Maximal germination is attained approximately 70 hours after irradiation. Dose response curves suggest log linearity. The action spectrum to cause 50% germination shows that spores are most sensitive to irradiation in the red region (620–680 nm) with an incident energy less than 1000 ergs cm^{-2}; sensitivity decreases towards both shorter and longer wavelengths. Although the action spectrum is suggestive of phytochrome involvement, photoreversibility of germination between red and far red light has not been demonstrated with *Onoclea* spores. An absorption spectrum of the intact spores reveals the presence of chlorophylls and carotenoids. Since the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea does not inhibit germination, it is concluded that photosynthesis does not play a role in the germination process.

Fern spore germination has been repeatedly shown to be stimulated by red light (22, 27, 30). Blue light and far red light have either no effect, delay germination, or inhibit it (18, 19, 29). The action spectrum for germination of *Dryopteris filix-mas* reveals a region of maximal promotion between 650 and 670 nm, whereas wavelengths below 500 nm and above 700 nm inhibit red induction of germination (18). Similarly, red irradiation stimulates spore germination of *Pteris vittata,* and this stimulation can be reversed by far red and blue light (29). In contrast to blue inhibition, far red inhibition could repeatedly be reversed by red light, thus indicating the phytochrome system as the photoreceptor for spore germination of *Dryopteris, Osmunda cinnamomea, O. claytoniana* (18, 19). In *Osmunda* species, exospore splitting is under phytochrome control, whereas the protrusion of the rhizoidal cell depends upon the reception of light by the photosynthetic pigments (19).

In our preliminary studies on spore germination of *Onoclea sensibilis,* it was shown that light stimulates germination (9), but red-far red reversibility could not be demonstrated for germination. The phytochrome system thus appears not to be implicated in the germination process. These observations prompted further investigations on the nature of photocontrol of *Onoclea* spore germination. In this paper, we report an action spectrum and general characteristics of photoinduced germination of these spores.

MATERIALS AND METHODS

Plant Material. Sporophylls of *Onoclea sensibilis* L. were collected from the swampy, southwestern margin of Pickerel Lake in the Pinckney State Recreational Area in southeastern Michigan in early December, 1970. They were stored in plastic bags in the cold room at about 4 C. The spores were removed from the sporophylls and sporangia by submerging the entire sporophylls in water overnight in the dark. Under diffuse light, the sporophylls were removed from the water, blotted between pieces of paper towel to remove excess moisture, and placed on wax paper in a vacuum desiccator containing silica gel desiccant. A vacuum was applied by means of a water aspirator, and the desiccators were placed in the dark. In approximately 1 to 2 weeks, the sporophylls opened and the sporangia dehisced, releasing the spores onto the wax paper. The spores were filtered through a single layer of lens paper and then stored in a desiccator at 4 C until needed for experimentation.

The dehiscing procedure described above gives rise to a population of spores homogeneous in size and in the ability to germinate, as compared with hand grinding method (14) for removing spores from the sporangia. Figure 1 illustrates the following: (*a*) the hand grinding method yields two populations of imbibed spores having widths of 39 and 45 μ (curve B, Fig. 1a); (*b*) the dehiscing method releases only one population of spores with a width of 48 μ (curve D, Fig. 1b), whereas grinding of the dehisced sporangia yields a second population of spores with a width of 45 μ (curve C, Fig. 1b); (*c*) nongerminated spores after 4 days of continuous white light, regardless of harvest method, were found to have a width of approximately 39 μ (curve A, Fig. 1a), which coincides with one of the populations of spores obtained by the hand grinding method. In addition, the hand-ground spores also contained a large proportion of contaminants (broken spores, sporangia walls, sporophyll tissue, *etc.*), whereas the dehisced spores contained very little contamination (less than 10% under microscopic observation).

Chemicals. The ferrous ammonium sulfate and sulfuric acid used as an infrared filter were of reagent grade chemicals. Clorox was purchased from a local supermarket.

The 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, from E. I. duPont de Nemours & Co.) used for the photosynthetic inhibitor experiments was dissolved in ethanolic ethylene glycol solution to make a 10^{-4} M stock solution. The stock solution was then diluted to 10^{-5} and 10^{-6} with ethanolic ethylene glycol. Distilled water and ethanolic ethylene glycol in water (v/v, 0.25% of both ethanol and ethylene glycol, Fisher certified reagent grade) served as controls for the experiments.

[1] National Defense Educational Act Fellow.

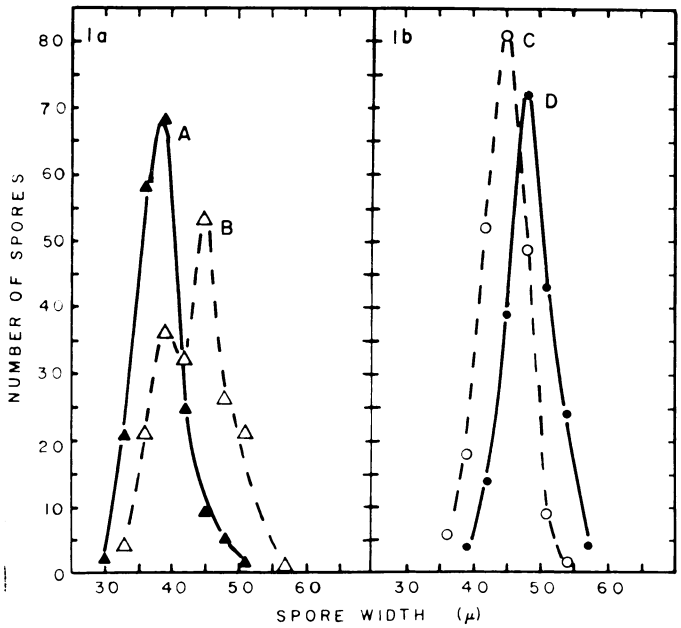


FIG. 1. Comparison of width distribution of spore populations. Curve A: spores not germinated after 4 days of continuous light (the same width distribution apply for non-germinated spores obtained with either the hand grinding or the dehiscing method); curve B: fully imbibed spores obtained with the hand grinding method; curve C: fully imbibed spores obtained by grinding the dehisced sporangia; curve D: fully imbibed spores harvested with the dehiscing method.

Germination Procedure. A small spatulaful of spores was sown under dim green light (see below) on the surface of 10 ml of distilled water in a 5-cm Petri dish. Short periods of exposure to this green light were shown not to affect germination significantly. Since *Onoclea* spores germinate equally well in one-fifth Knop's solution (14, 29) or in distilled water, the latter was preferred for this work because of its simplicity. The Petri dishes were incubated in a dark box at 27 ± 1 C in a dark room for a given time period prior to light treatment. After the light treatment, the dishes were returned to the dark room until germination was scored.

The criterion for germination was protrusion of either the rhizoidal cell or the protonematal cell as seen under 100 times magnification of a standard compound microscope. Each experiment consisted of duplicate Petri dishes. Two slides were prepared from each dish 6 days after light treatment, unless otherwise stated, and 200 spores per slide were counted to score germination. This procedure was derived from a series of preliminary experiments to ascertain sampling and counting errors. All experiments were repeated at least twice.

Germination was also scored in several experiments by the acetocarmine-chloral hydrate stain procedure of Edwards and Miller (6). Since the final germination percentages determined by the stain method were within experimental error of those obtained by the protrusion method, the latter procedure continued to be our method for scoring germination. Only the results of the time course pattern of germination differed between the two methods (see "Results").

Light Sources. The green safelight source was two 15-w white fluorescent lamps filtered through two sheets of green Plexiglas, two sheets of amber Plexiglas, and a single layer of green gelatin. These filters transmitted between 530 and 600 nm with an energy of less than 100 ergs cm^{-2} sec^{-1}.

Routine irradiation work was carried out with broad band red, far red, and blue light. The broad band red light source was four 15-w cool white fluorescent lights filtered through red Plexiglas (incident energy 600–800 ergs cm^{-2} sec^{-1}). The far red source was provided by four 150-w reflector flood lamps filtered through 3 cm of water and two sheets of red Plexiglas with two sheets of dark green gelatin sandwiched between the Plexiglas layers (1.1×10^5 ergs cm^{-2} sec^{-1}). The blue light source was a single 300-w incandescent bulb filtered through water (4 cm) and blue Plexiglas (1.1×10^4 ergs cm^{-2} sec^{-1}). The Plexiglas filters were purchased from the Cadilac Plastic & Chemical Co., Detroit, Michigan, and the gelatin filter was from National Theater Supply Co., Philadelphia, Pennsylvania. The far red filter transmitted light above 680 nm with 50% transmission at 710 nm; the red filter transmitted above 590 nm with 50% transmission at 606 nm; and the blue Plexiglas transmitted between 340 and 580 nm with 44% maximal transmission at 450 nm with practically no transmission in the far red region.

An interference filter monochromator system similar to Withrow's (32) and Mohr and Schoser's (20) was employed to determine the action spectrum of *Onoclea* spore germination. Second order interference filters of the Fabry-Perot type with auxiliary cut off components (Balzers obtained from Photovolt Corp., N. Y.) were used. Transmission through these filters ranged from 30 to 45% with half-peak widths of 8 to 12 nm. Since these filters transmitted some infrared light, a 5 cm thick solution of 0.2 M ferrous ammonium sulfate in 1% sulfuric acid was placed in front of the interference filter to minimize the interference of infrared light. The light source was either a 500-w or 1000-w projection lamp (GE Model DMX 115–120 V and Westinghouse Model DFT 115–120 V, respectively). To increase the intensities, one or two lenses (focal length about 7 cm) were placed between the light source and the solution filter.

High light intensities were directly measured with a radiometer (YSI-Kettering Model 65, Yellow Springs Co., Yellow Springs, Oh.) placed at the position for spore irradiation. Because the radiometer was not sensitive enough to measure the lower intensities used, a SeO_2 photocell connected to a microvolt meter (150 A microvolt-ammeter, Keithley Instruments) was used after calibration against the radiometer for each light source.

Absorption Spectrum. Absorption spectra of intact spores with and without the brown perispore were taken with a Cary Model 13 recording spectrophotometer equipped with a light scattering attachment (Applied Physics Corp., Monrovia, Calif.). Since scattering by the spores is much higher than the light scattering attachment can accommodate, depigmented spores were used in the reference cuvette. The depigmented spores were prepared by soaking the spores in ethanol-glacial acetic acid solution (3:1) for 8 hr with stirring; during this time, the extracting solution was changed approximately five times. An absorption spectrum of spores was taken against depigmented spores in cuvettes with a 1-mm path length; both types of spores were suspended in 50% glycerol. The incubation in 50% glycerol and the narrowed optical path length can effectively prevent the spores from moving during measurement. The cuvettes were constructed with pieces of Plexiglas and a 1 mm thick rubber sheet used as a spacer. They were placed directly in front of an end-on photomultiplier. In order to minimize the light scattering problem further, a piece of opalescent polyethylene sheet was placed behind both sample and reference cuvettes (26). A similar procedure was used when the absorption spectrum of an intact spinach leaf was taken; the intercellular air spaces of the leaf were filled with distilled water by repeatedly applying and releasing a vacuum.

Table I. *Effect of Red, Far Red, or Blue Light on Germination of Onoclea sensibilis Spores*

Spores were irradiated for 5 min after presoaking in the dark from 6 to 17 hr. Germination was scored 6 days after irradiation.

Light Treatment		Germination				
Light Quality	Intensity	I	II	III	IV	Average
	ergs cm⁻² sec⁻¹	%				
Dark	...	28.5	10.9	1.9	6.5	12.0 ± 3.9
Red	0.5×10^3	58.4	71.4	62.5	60.7	63.2 ± 2.5
Far red	1.1×10^5	70.2	80.2	...	...	75.2 ± 3.1
Blue	1.1×10^4	58.8	59.1	44.0	44.9	51.7 ± 3.0

RESULTS

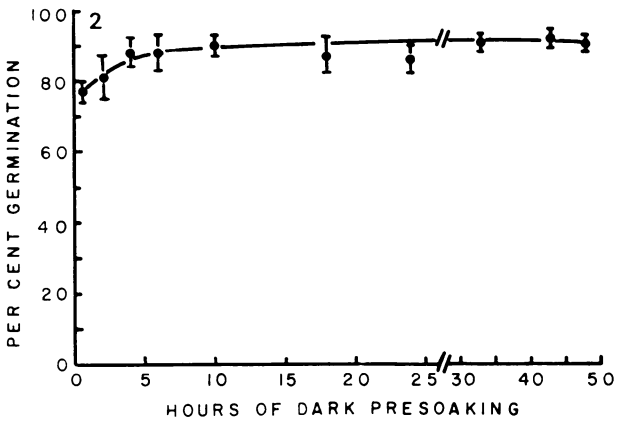
General Characteristics of Light-stimulated Germination. Similar to other fern spores, *Onoclea sensibilis* spores require light for germination. Table I illustrates the results from a number of experiments in which broad band red, far red, or blue light was given for 5 min after varying periods of dark presoaking. Germination percentages were determined after 6 days of dark soaking from irradiation. All colored light tested stimulated germination, whereas only a small percentage of spores were capable of germinating in the dark. It should be noted that there is noticeable variation in the per cent of germination attained. This is ascribed to spore lot-to-lot variation.

To determine the period of highest sensitivity to irradiation, spores were soaked in the dark for varying periods of time up to 48 hr prior to 5 min of red, far red, or blue light. The results (Fig. 2) for all colored lights at a given presoaking period were within an experimental error; the percentage of germination at a given presoaking time from the three spectral regions were averaged together. Although some variation exists in the extent of germination, as indicated by the standard error at each point, it is, nevertheless, clear that the maximal sensitivity is attained from 6 hr up to at least 48 hr of dark presoaking. It should also be noted that even with only a short period of soaking, 15 min, the spores are highly sensitive to irradiation, although irradiation to nonsoaked spores did not cause any greater germination than dark control. This rapid increase in photosensitivity strongly suggests rapid uptake of water by the spores.

In order to examine the time course pattern of germination, spores were presoaked for 19.5 hr in the dark, irradiated with monochromatic light through an interference filter at 470 nm, 665 nm, or 740 nm, and the percentage of germination was recorded after varying periods of time after irradiation. Figure 3 shows that all three wavelengths induce rapid germination after a lag period of approximately 30 to 36 hr. A maximal germination of 80 to 90% can be attained after 70 hr from irradiation. It should again be noted that all three wavelengths showed essentially the same pattern of germination with rates of approximately 2.0% germination per hr and a half maximal germination time of about 50 hr.

The kinetics of germination were considerably faster when scored by the staining method of Edwards and Miller (6); after a lag of only 10 to 12 hr, germination was induced at a rate of 3% per hr with a half maximal germination time of 21 hr.

Action Spectrum for Germination of Onoclea Spores. The above results indicate that the spores of *Onoclea* are essentially equally responsive to all regions of the visible spectrum tested, at least at high dosages. In order to determine the most effective wavelength for germination, an attempt was next made to take an action spectrum for germination. For this purpose, spores were presoaked for 16 to 20 hr in the dark, given various doses of monochromatic lights, and incubated in darkness for 6 days for germination. Representative dose response curves are shown in Figure 4. The slopes of the curves for germination suggest log linearity rather than simple linearity (2, 28). Because of this, all dose response curves were plotted on the basis of log dose, and the dosage which gives 50% germination was determined for each of 16 wavelengths tested. However, during the course of these studies, it was noted that the spore response changed with the age of the spores; that is, spores stored in the cold for a long period after harvest tended to require less light energy to cause 50% germination. Figure 5 shows an action spectrum obtained with spores of the same storage age. It illustrates that the spores are mose sensitive to irradiation in the red region (620–680 nm) and increasingly insensitive towards both shorter and longer wavelengths. Despite the complication of increasing sensitivity with age, all action



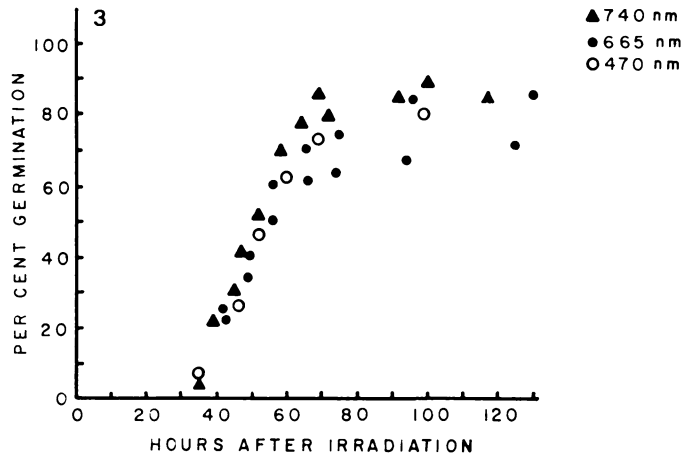
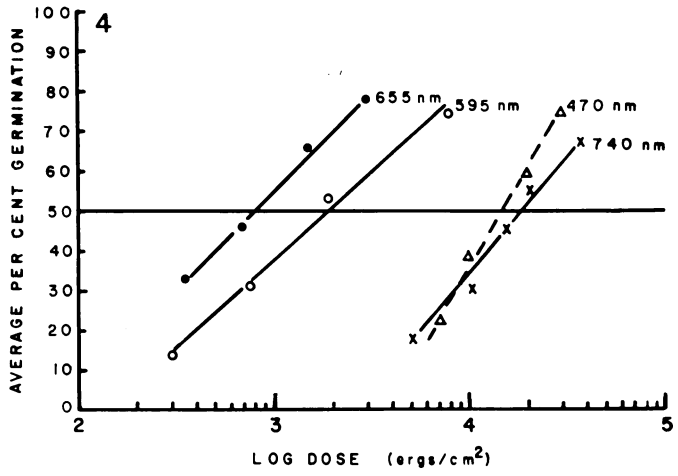


FIG. 2 (top). Effect of dark presoaking periods on the germination of *Onoclea* spores. After various periods of dark presoaking, spores were irradiated with 5 min of broad band red (600 ergs cm^{-2} sec^{-1}), far red (1.1×10^5 ergs cm^{-2} sec^{-1}), or blue (1.1×10^4 ergs cm^{-2} sec^{-1}) light. Germination was scored 6 days after irradiation. Each value with the standard error represents the average per cent germination of all three light treatments.

FIG. 3 (bottom). Time course of *Onoclea* spore germination after illumination with monochromatic light. Spores presoaked in the dark for 19.5 hr, irradiated with 740 nm (360×10^3 ergs cm^{-2}), 665 nm (7.5×10^3 ergs cm^{-2}), or 470 nm (4.8×10^3 ergs cm^{-2}), and dark incubated thereafter. Spore samples were removed from Petri dishes under dim green light at given intervals to score germination. Each point represents average percentage of germination for four slides of 200 spores per slide.



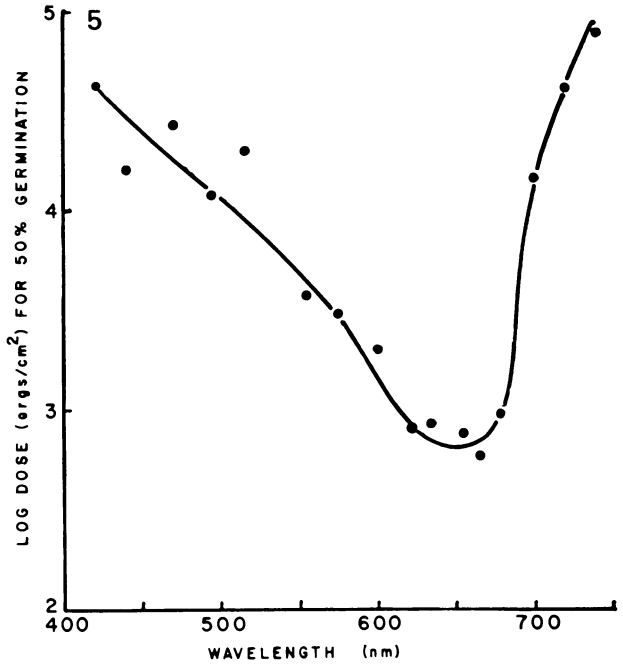


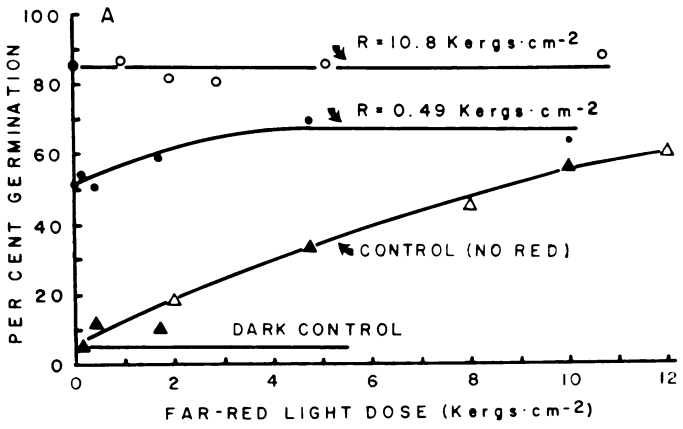
FIG. 4 (top). Representative dose response curves for promotion of *Onoclea* spore germination. Spores soaked in the dark for 16 to 20 hr, irradiated with monochromatic light, and returned to the dark for 6 days. Each point represents average percentage of germination for four slides of 200 spores per slide.

FIG. 5 (bottom). Action spectrum for photoinduced germination of *Oncoclea sensibilis* spores. Dose response curves were taken with spores of the same storage age at each wavelength, and dosages required for promotion of 50% germination plotted against wavelength.

spectra taken showed the same response with respect to wavelength; that is, the spores were always most sensitive to red irradiation and increasingly insensitive towards the blue and far red.

Participation of the Phytochrome System in Germination. The high effectiveness of the low energy red light for induction of germination as compared with light from other spectral regions suggested that the phytochrome system might be operating. Classically and most simply, a phytochrome-controlled response elicited by red light is reversed by far red light or *vice versa* (1, 3, 23, 24). Two series of experiments were thus designed to determine whether far red light could reverse the red effect or both lights act simply to stimulate the germination (Fig. 6). After 20 hr of dark presoaking, spores were irradiated with an incident red energy of 0, 0.49, or 10.8 kergs cm^{-2} which was immediately followed by various doses of far red light (Fig. 6A). In another series, spores were presoaked in the dark for 12 hr, irradiated with 0, 8, 12, or 60 kergs cm^{-2} of far red light first, then with various doses of red light (Fig. 6B). After irradiation the spores were returned to the dark, and germination scored 6 days later. There is, unfortunately, an unavoidable scatter in the final germination attained, ranging from 60 to 87%. Nevertheless, it is clear from the results in Figure 6 that no reversibility is observed at all combinations. In both series the second light acted to enhance the first nonsaturating irradiation for germination induction. No further stimulation of germination occurred by the second irradiation, when the first irradiation alone induced maximal germination (10.8 kergs cm^{-2} red light or 60 kergs cm^{-2} far red light). Since the reversibility by red and far red light is an important criterion for phytochrome participation in a biological response, the series of experiments were repeated several times: under our experimental conditions, reversibility was not observed. This lack of reversibility of red light induction of *Onoclea sensibilis* spore germination by far red irradiation is clearly different from the usual demonstration of the phytochrome system as the photoreceptor for germination (see "Discussion").

Absorption Spectra of Intact Spores with and without the



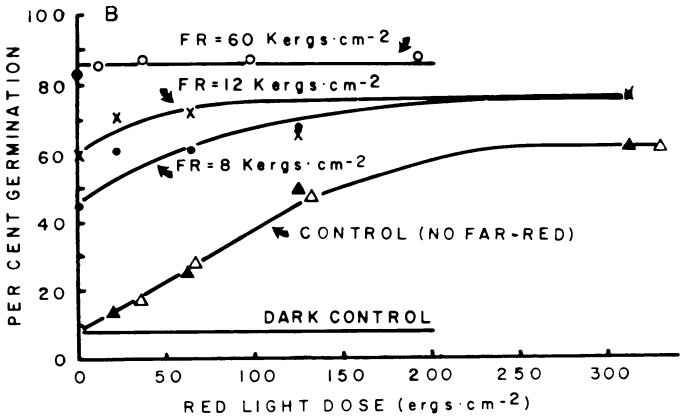


FIG. 6. Effect of varying doses of far red light on the germination of red pretreated spores (A), and effect of varying doses of red light on the germination of far red pretreated spores (B). The second light was given immediately after the first. Spores presoaked for 20 hr; germination scored 6 days after irradiation.

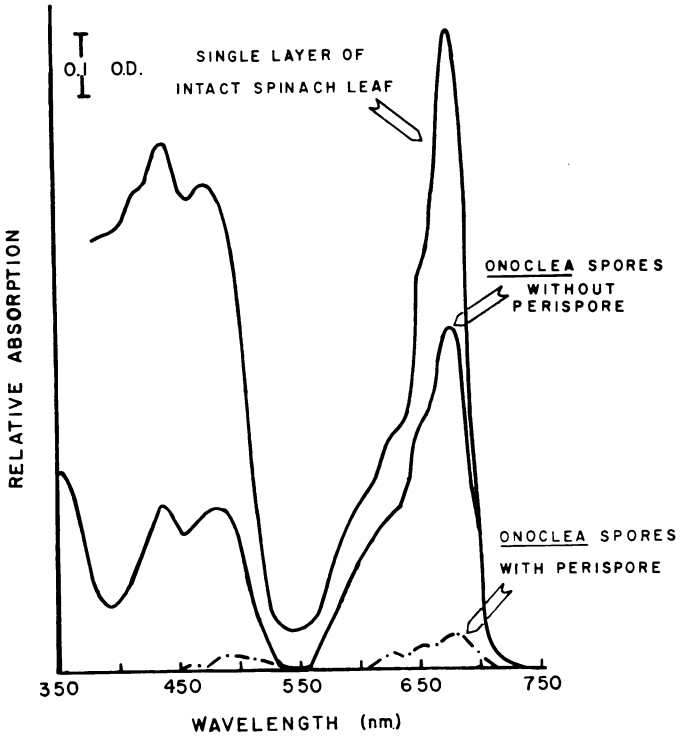


FIG. 7. Absorption spectra of intact *Oncoclea* spores with and without perispore and of an intact spinach leaf.

Perispore. Another possibility derived from the action spectrum is the involvement of chlorophylls (7, 19). For this purpose an absorption spectrum of the intact spores was taken. The intact spore is enveloped by a dark brown perispore which greatly hindered the measurements of the absorption spectrum. Nevertheless, after correction for baseline drift, a peak at 680 nm and shoulders at 650 nm and 625 nm are identifiable (broken line, Fig. 7). In order to alleviate the perispore problem, spores were treated for 1 min with full strength Clorox to remove the perispore. After washing with distilled water and centrifugation (1000*g*) three times, the pellet of green spores was resuspended in enough distilled water to approximate the density of the depigmented spores. Microscopic examination of the spores affirmed the removal of the brown outer coating. With perispore removed, three prominent peaks were observed (Fig. 7); one at 680 nm with shoulders at 650 nm and 620 to 625 nm, a second peak at about 485 nm, and a third at 435 nm. An absorption spectrum taken of an intact spinach leaf (Fig. 7) reveals identical peaks with those of the Clorox-treated spores.

From the comparison between the absorption spectrum of the spinach leaf and the absorption spectrum of the Clorox-treated spores, the 680 nm peak and 620 to 625 nm shoulder are identified as chlorophyll *a*, while the 650 nm shoulder is chlorophyll *b*. The blue peaks (435 and 485 nm) may be attributed to chlorophylls and carotenoids (25). It should be noted that absorption by the chlorophylls in the red region of the spectrum also occurs in spores having the brown perispores.

It should be added that we failed repeatedly to resolve phytochrome spectra of our spores with the available instrumentation, probably due to high scattering properties of the spores and also to high absorption by photosynthetic pigments.

Possible Participation of Photosynthetic Pigments as the Photoreceptors for Germination. The absorption spectra and also the green appearance of the spore indicate the presence of photosynthetic pigments. To test the possibility of participation of photosynthesis in the germination process, spores were incubated in 10^{-4}, 10^{-5}, and 10^{-6} M DCMU throughout the period of germination. After dark presoaking for 6 hr, the spores were irradiated with 5 min of red or blue light and then returned to the dark for 6 days. The results are given in Table II. Although ethanolic ethylene glycol alone inhibited germination slightly over the distilled water control, it is clear that DCMU did not cause any further inhibition of germination at any concentration, except 10^{-4} M in blue light.

To examine whether or not DCMU entered the spore, the effect of DCMU on oxygen evolution was determined with an oxygen electrode. The spores photosynthesized in intense white light from a microscope lamp. After addition of the inhibitor (final concentration in cuvette, 10^{-5} and 10^{-6} M), the spores no longer evolved oxygen, and only oxygen uptake could be observed. Thus, it was concluded that DCMU used in the above germination studies did enter the spores.

From the above evidence, in addition to the fact that blue light is nowhere near as effective as red light for stimulating germination at low energies, it can be concluded that photosynthesis is not involved in the germination process.

DISCUSSION

It was reaffirmed that light stimulates the germination of *O. sensibilis* spores, as had been previously demonstrated by Hartt (9). The action spectrum (Fig. 5) reveals maximal quantum effectiveness for germination in the red region of the spectrum, specifically between 620 and 680 nm. All wavelengths of light tested, however, are capable of stimulating germination when given at saturating dosages. Our action spectrum resembles the action spectrum for promotion of lettuce seed germination (1) and inhibition of flowering in *Xanthium* (23). In both these responses, phytochrome has been shown to be the photoreceptor. Thus, on the basis of the action spectrum alone, phytochrome could be involved in the germination process in *Onoclea*.

The second criterion for evoking photocontrol by phytochrome is the demonstration of photoreversibility by red and far red light. Photoreversibility has not been clearly demonstrated under our experimental conditions for the germination response of *O. sensibilis* spores (Fig. 6A). This lack of reversibility and the promotion of germination by far red light may be explained in the following four ways: (*a*) *Onoclea* spore germination is promoted by a very low level of Pfr which is

Table II. *Effect of DCMU on Germination of Onoclea sensibilis Spores Exposed to Red or Blue Light*

Spores were incubated in treatment solution throughout the period of germination. DCMU was dissolved in ethanolic ethylene glycol (0.25% ethanol and 0.25% ethylene glycol, v/v, in distilled water). Presoaking time, 6 hr; irradiation for 5 min with intensity as in Table I; germination scored 6 days after irradiation.

Treatment	Germation		
	Dark	Red	Blue
	%		
Distilled water control	4.2 ± 0.5	82.5 ± 0.8	69.1 ± 0.6
Ethanolic ethylene glycol control	5.8 ± 0.8	63.7 ± 6.8	60.8 ± 1.4
10^{-6} M DCMU	5.3 ± 0.7	66.5 ± 2.4	62.0 ± 8.1
10^{-5} M DCMU	2.7 ± 0	70.3 ± 5.0	72.1 ± 1.1
10^{-4} M DCMU	3.6 ± 0.9	60.8 ± 6.7	41.9 ± 2.8

produced from Pr by not only the red light but also the far red light sources used in these experiments; (*b*) once Pfr is formed it is very rapidly used by succeeding steps in the germination process, thus allowing *Onoclea* spores to escape quickly from inhibitory far red light; (*c*) phytochrome is masked by a second pigment which also absorbs in the far red region and which also stimulates germination in *Onoclea* spores; and (*d*) pigment or pigments other than phytochrome may be operating as the photoreceptor in our fern spores.

Far red-induced inhibition of flowering in *Lemna perpusilla* 6746 (10), *Pharbitis nil* (21), and *Chenopodium rubrum* (12, 13), and far red-induced promotion of seed germination of *Amaranthus retroflexus* (31) and some bromeliads (5) have been attributed to the maintenance of a low but effective level of Pfr (1–2%) by far red light for the time required to complete the initial process; *Onoclea* spore germination may perhaps respond to far red irradiation in a manner similar to these examples (possibility *a*). The possibility *b* is partially supported by observations indicating rapid disappearance of reversibility by far red light in flowering responses of *Xanthium* and Biloxi soybean (4) and *Pharbitis nil* (8). Since the escape from far red reversal is affected by temperature (4, 11), we have tested whether red-far red reversibility of *Onoclea* spore germination be exhibited at a low temperature of 0 to 4 C. The results from low temperature experiments were, however, essentially the same as those at 27 C (Fig. 6) and did not show any reversibility between red and far red light in various combinations of light energies. In accordance with the possibility *b*, these results may suggest that Pfr, once formed, is made unresponsive to far red reversal. The presence of a masking pigment (possibility *c*) was proposed by Miller and Miller (16) for elongation of *Onoclea* protonemata, but it has not been detected in our absorption spectra (Fig. 7). Since the major detectable absorbing species are photosynthetic pigments (Fig. 7), pigments such as chlorophylls may be involved in the light reception for *Onoclea* spore germination (possibility *d*, see also ref. 19). The observations that DCMU could not inhibit spore germination (Table II), however, allow us to conclude that the photosynthetic processes *per se* are not involved in germination.

Although our data does not allow us to choose among the above four possibilities, the preponderance of evidence in the literature of similar phenomenon seem to support the first two possibilities. Further work is clearly needed for the identification of the photoreceptor for *Onoclea* spore germination.

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LITERATURE CITED

1. Borthwick, H. A., S. B. Hendricks, E. H. Toole, and V. K. Toole. 1954. Action of light on lettuce seed germination. Bot. Gaz. 115: 205–225.
2. Briggs, W. R. 1964. Phototropism in higher plants. *In:* A. C. Giese, ed., Photophysiology, Vol. 1. Academic Press, New York. pp. 223–271.
3. Butler, W. L., S. B. Hendricks, and H. W. Siegelman. 1964. Action spectra of phytochrome *in vitro*. Photochem. Photobiol. 3: 521–528.
4. Downs, R. J. 1956. Photoreversibility of flower initiation. Plant Physiol. 31: 279–284.
5. Downs, R. J. 1964. Photocontrol of germination of seeds of the *Bromeliaceae*. Phyton 21: 1–6.
6. Edwards, M. E. and J. H. Miller. 1972. Growth regulation by ethylene in fern gametophytes. III. Inhibition of spore germination. Amer. J. Bot. 59: 458–465.
7. Flint, L. M. and E. D. McAlister. 1937. Wavelength of radiation in the visible spectrum promoting the germination of light-sensitive lettuce seed. Smithson. Misc. Collect. 96: 1–8.
8. Fredericq, H. 1964. Conditions determining effects of far-red and red irradiation on flowering response of *Pharbitis nil*. Plant Physiol. 39: 812–816.
9. Hartt, C. 1925. Conditions for germination of spores of *Onoclea sensibilis*. Bot. Gaz. 78: 427–440.
10. Hillman, W. S. 1966. Photoperiodism in *Lemna:* reversal of night-interruption depends on color of the main photoperiod. Science 154: 1360–1362.
11. Ikuma, H. and K. V. Thimann. 1964. Analysis of germination processes of lettuce seed by means of temperature and anaerobiosis. Plant Physiol. 39: 756–767.
12. Kasperbauer, M. J., H. A. Borthwick, and S. B. Hendricks. 1963. Inhibition of flowering of *Chenopodium rubrum* by prolonged far-red radiation. Bot. Gaz. 124: 444–451.
13. Kasperbauer, M. J., H. A. Borthwick, and S. B. Hendricks. 1964. Reversion of phytochrome 730 (Pfr) to P_{660} (Pr) assayed by flowering in *Chenopodium rubrum*. Bot. Gaz. 125: 75–80.
14. Miller, J. H. and P. M. Miller. 1961. The effect of different light conditions and sucrose on growth and development of the gametophyte *Onoclea sensibilis*. Amer. J. Bot. 48: 154–159.
15. Miller, J. H. and P. M. Miller. 1963. Effects of red and far-red illumination on the elongation of fern protonemata and rhizoids. Plant Cell Physiol. 4: 65–72.
16. Miller, J. H. and P. M. Miller. 1967. Interaction of photomorphogenetic pigments in fern gametophytes: phytochrome and a yellow absorbing pigment. Plant Cell Physiol. 8: 765–769.
17. Miller, J. H. and P. M. Miller. 1967. Action spectra for light-induced elongation in fern protonemata. Physiol. Plant. 20: 128–138.
18. Mohr, H. 1956. Die Beeinflussung der Keimung von Farnsporen durch Licht und andere Faktoren. Planta 46: 534–551.
19. Mohr, H., U. Meyer, and K. Hartman. 1964. Die Beeinflussung der Farnsporen-Keimung (*Osmunda cinnamomea* L. und *O. claytoniana* L.) über das Phytochromsystem und die Photosynthese. Planta 60: 483–496.
20. Mohr, H. and G. Schoser. 1959. Eine Interferenzfiltermonochromatoranlage für Photobiologische Zwecke. Planta 53: 1–17.
21. Nakayama, S., H. A. Borthwick, and S. B. Hendricks. 1960. Failure of photoreversible control of flowering in *Pharbitis nil*. Bot. Gaz. 121: 237–243.
22. Orth, H. 1937. Zur Keimungsphysiologie der Farnsporen in verschiedenen Spektralbezirke. Jb. wiss. Bot. 84: 353–426.
23. Parker, M. W., S. B. Hendricks, H. A. Borthwick, and N. J. Scully. 1946. Action spectrum for the photoperiodic control of floral initiation of short-day plants. Bot. Gaz. 108: 1–26.
24. Pratt, L. H. and W. R. Briggs. 1966. Photochemical and non-photochemical reactions of phytochrome *in vivo*. Plant Physiol. 41: 467–472.
25. Rabinowitch, E. I. 1956. Photosynthesis and Related Processes. Vol. II, Part 2. Interscience Publications, Inc., New York. pp. 1841–1849.
26. Shibata, K., A. A. Benson, and M. Calvin. 1954. The absorption spectra of suspensions of living microorganisms. Biochim. Biophys. Acta 15: 461–470.
27. Stephan, J. 1928. Untersuchungen über die Lichtwirkung bestimmter Spektralbezirke und bekannter Strahlungsintensitäten auf die Keimung und das Wachstum einiger Farne und Moose. Planta 5: 381–443.
28. Sugai, M. 1971. Photomorphogenesis in *Pteris vittata*. IV. Action spectra for inhibition of phytochrome-dependent spore germination. Plant Cell Physiol. 12: 103–109.
29. Sugai, M. and M. Furuya. 1967. Photomorphogenesis in *Pteris vittata*. I. Phytochrome-mediated spore germination and blue light interaction. Plant Cell Physiol. 8: 737–748.
30. Sussman, A. S. 1965. Physiology of dormancy and germination in the propagules of cryptogamic plants. *In:* W. Ruhland, ed., Encyclopedia of Plant Physiology, Vol. 15, Springer-Verlag, Heidelberg. pp. 933–1025.
31. Taylorson, R. B. and S. B. Hendricks. 1971. Changes in phytochrome expressed by germination of *Amaranthus retroflexus* L. seeds. Plant Physiol. 47: 619–622.
32. Withrow, R. B. 1957. An interference-filter monochromator system for the irradiation of biological material. Plant Physiol. 32: 355–360.