Blue Light Interference in the Phytochrome-controlled Germination of the Spores of *Cheilanthes farinosa*[1]

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ABSTRACT

Short exposure of the spores of *Cheilanthes farinosa* to low intensity red light promotes their germination, which is not reversed by a subsequent exposure to far red light. Germination is, however, inhibited by blue light administered before or after red light. Inhibition of germination by blue light is annulled by exposure to a higher intensity of red light, and germination of the repromoted spores is inhibited by far red light. Mutual photoreversibility of germination is also observed in repromoted spores irradiated successively with far red and red light. Although germination appears to be basically under phytochrome control, it is postulated that the presence of a blue light-absorbing pigment interferes with phytochrome transformations in the spores.

The spores of several species of ferns have a light requirement for germination (4, 6, 7, 13, 18). This is generally satisfied by red light, and the effect of red light can be reversed by a subsequent exposure to far red light, thus implicating phytochrome in the over-all control of germination (9, 11, 17, 20). Red light required for potentiation of germination may vary from a few seconds to a few hours of exposure at a low intensity.

Blue light has been known to inhibit germination of the spores of some species, either by interfering with phytochrome action or by activating a separate pigment system, although there are no clear notions regarding the mechanism of this light effect (6, 9, 13, 18). A recent analysis of blue light inhibition of germination of the spores of *Pteris vittata* (19, 21) has led to the interesting suggestion that blue light is absorbed by carotenoids, which release an inhibitor of germination.

In the course of screening work on the germination of the spores of several ferns, I encountered a striking example of blue light interference in the expression of red light effect and in the reversal of red light effect by a subsequent far red irradiation. The results of these studies are presented in this paper.

MATERIALS AND METHODS

Observations are reported on the spores of *Cheilanthes farinosa* (sexual diploid), a homosporous fern, belonging to the family Cheilanthaceae, order Schizaeales (12). The spores

were collected from Chandigarh, India in the fall of 1971 and held in storage at 4 C. They were sown on the surface of 10 ml of a mineral salt medium (15) contained in 5 cm diameter Petri dishes, and allowed to imbibe in the dark for 4 days before irradiation. Since the spores were easily damaged by sterilization in Clorox (a hypochlorite bleach), aseptic conditions were not employed; however, there were no fungal or bacterial contaminations in the cultures during the experimental period. Preliminary experiments indicated that responsiveness of spores to a given irradiance of red light increased as dark imbibition continued from 24 hr to 96 hr; thereafter the response was constant to 168 hr. Germinated spores were counted 4 days after the last light treatment. Breakage of the exine, followed by emergence of the rhizoid initial as determined by microscopic examination, was the minimum criterion of germination adopted, although by 4 days after an inductive light exposure, majority of the spores had both rhizoid and protonema. Except when irradiated, fully imbibed spores were held in complete darkness. Irradiations and all dark incubations were carried out in an air-conditioned dark room maintained at 25 to 27 C. Treatments were duplicated, and counts of about 100 spores from each dish were averaged to yield the percentage of germination for any treatment. All experiments have been repeated at least twice with essentially the same results.

Radiation was from fluorescent and incandescent sources filtered through Plexiglas filters. Red light (7 μw/cm^2) was obtained by filtering light from three 15-w fluorescent tubes through a 3 mm thick red filter (Rohm & Haas, No. 2444; cuts off all radiation below 540 nm and gives maximum emission at 650 nm) and 32 layers of cheesecloth. In some experiments where the cheesecloth filter was not used, light intensity was 67.4 μw/cm^2; this is designated arbitrarily in the text as "high intensity red light." Blue light (166 μw/cm^2) was provided by six 20-w General Electric blue fluorescent tubes placed over a 3 mm thick blue filter (Rohm & Haas, No. 2045; gives maximum emission at 454 nm and none above 516 nm); fractions of this irradiance were obtained by covering the dishes with cheesecloth. Far red source (410 μw/cm^2) consisted of a 500-w photoflood lamp filtered through 8 to 10 cm depth of distilled water and a 3 mm thick black filter (West Lake Plastics, Lennis Mills, Pa., No. FRF-700; transmits less than 1% below 705 nm and more than 50% between 730 nm and 740 nm). Measurements of light intensity were made with Model SR spectroradiometer (Instrumentation Specialities Co., Lincoln, Neb.). All manipulations in the dark were carried out at a distance of 2 feet from a dim green safelight.

RESULTS

When spores were imbibed and held in total darkness for a period up to 4 weeks, maximum germination recorded was

about 8%. During the usual experimental period of 8 days trom sowing, dark germination was generally of the order of 2 to 5%. However, exposure of fully imbibed spores to even a flash of red light was sufficient to cause germination in significant numbers during the next 4 days. As shown in Figure 1, saturation of germination under the experimental conditions employed can be reached with as little as 20 to 30 sec of low intensity red light. Consequently, 30 sec of red light were used in all experiments, except when specified otherwise. Since the sample was invariably mixed with nonviable spores which were also counted, maximum germination recorded was about 80%. Irradiation of red potentiated spores with far red light for various periods of time ranging from 30 sec to 1 hr did not reverse the red light effect. This unexpected result, while discounting the involvement of a simple phytochrome-mediated reaction in spore germination, led to a further analysis of the germination process.

Data plotted in Figure 2 demonstrate that germination induced by red light was completely nullified if blue light was given either before or after red light. The interacting effects of blue light observed here are analogous to those reported in the germination of the spores of Pteris vittata (20). The extreme sensitivity of the spores to blue light is seen from the fact that exposure for 20 to 30 sec was sufficient to desensitize them to a subsequent red light or to reduce the percentage of red potentiated germination to the level of the dark control (Fig. 3).

Could the inhibitory effect of blue light on spore germination be attributed to an energy-dependent, so-called "high intensity light" reaction? To answer this question, spores were exposed to five different intensities of blue light for various periods of time following a saturating exposure to red light (Fig. 4). A nearly linear dependence of inhibition of germination on the logarithm of light energy was found to hold for all five intensities of blue light used. Therefore, a postulate of energy-dependent high intensity light reaction appears unnecessary to account for the inhibition of germination by blue light. Based on these data and those presented in Figure 2, unless otherwise indicated, 30 sec of blue light at an irradiance of 166 μw/cm^2 were used in all subsequent experiments.

If spores were exposed to blue light at various times during an intervening dark period following a red irradiation, they showed typical "escape" from inhibition with increasing duration of the interval between promotive and inhibitory irradiations (Fig. 5). If blue light is given 3 hr after red light, about 50% of the viable spores will germinate and 5 hr after red light, blue light is completely ineffective in negating the red light effect. Red light-sensitive processes essential for germination are apparently completed in darkness in about 3 to 5 hr.

Unlike typical red-far red effects observed in other photomorphogenetic systems, when cyclic treatments of red and blue irradiations were applied, the inhibitory effect of blue light on germination was not found to be reversed by a subsequent exposure to red light. Irrespective of the number of red-blue light cycles given, the percentage of germination remained close to the dark control level. Results of an experiment in which spores irradiated with blue light were exposed to different periods of red light revealed that increasing percentages of germination were potentiated as the period of red light exposure increased (Fig. 6). Red light exposure for 1 hr completely negated the inhibitory effect of blue light administered first or following an initial red light, and restored germination to the normal level. In contrast, when high intensity red light was used, exposure for 1 to 2 min was sufficient to repotentiate germination.

To see if spores exposed to blue light recovered their capacity to germinate with time, fully imbibed spores were ir-

radiated with blue light and then returned to darkness. After various periods in darkness, they were exposed to 30 sec of red light, and the percentage of germination determined after 4 days. It was seen that even after 10 days in darkness following blue light exposure, maximum percentage of germination was less than 50%. Similar results were obtained when spores were irradiated with red light followed by blue light before being returned to darkness. It thus appears that the effect of a short exposure to blue light persists for a long time in darkness, as a result of which the spores are unable to regain their sensitivity to a short exposure to red light. However, at any time following exposure to blue light, administration of red light for 1 hr or high intensity red light for 2 min potentiated full germination.

Previous experiments established the absence of far red reversibility and the presence of blue light sensitivity in spores initially irradiated with red light. The question follows whether these properties are still manifest in spores repromoted to germinate by red light following inhibition by blue light. An experiment was designed to answer this question in which the spores were successively irradiated with red light-blue light-high intensity red light (2 min) or blue light-high intensity red light (2 min). They were then exposed to different periods of far red or blue light. The results (Fig. 7) showed that spores whose germination was repromoted by red light could be completely reinhibited by 1 to 2 min of far red light administered immediately. In contrast, spores were no longer sensitive to
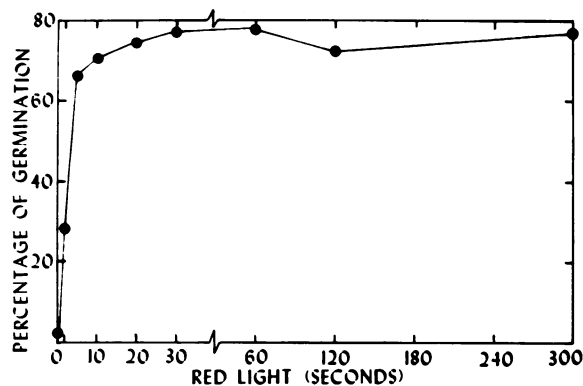

FIG. 1. Effect of dosage of red light (7 μw/cm^2) on the germination of spores. For each point in the graph, 200 to 220 spores were counted.
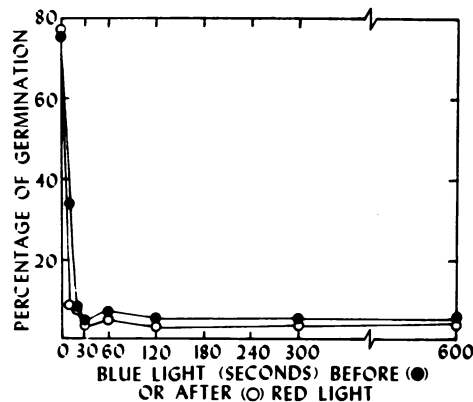

FIG. 2. Effect of dosage of blue light (166 μw/cm^2) applied before or after a saturating exposure to red light (7 μw/cm^2, 30 sec) on the germination of spores. Counts of 196 to 219 and 192 to 260 spores were made to obtain data points for blue light applied before red light and after red light, respectively.
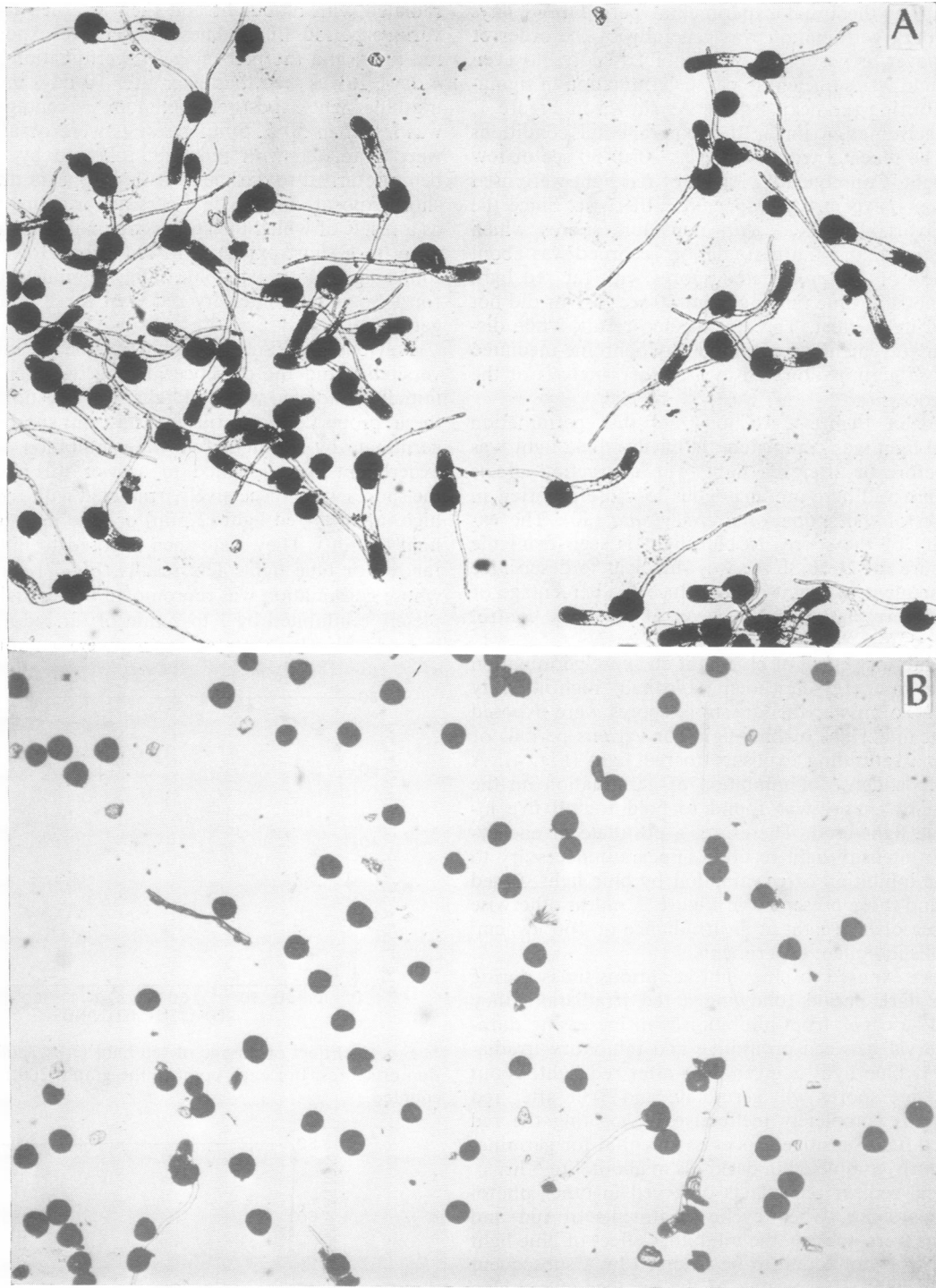
FIG. 3. A: Spores potentiated to germinate by exposure to red light (7 μw/cm², 30 sec); B: inhibition of spore germination when red light is followed by blue light (166 μw/cm², 30 sec).

short exposures of blue light, although longer exposures (1–2 hr) led to nearly complete inhibition of germination.

If repromoted spores were exposed to far red light at various times during an intervening dark period following a saturating exposure to high intensity red light, it was seen that far red light applied 5 hr following red light failed to inhibit germination (Fig. 5). Thus the curve for escape from far red inhibition was similar to that for escape from blue light inhibition. Irradiation of repromoted spores successively with far red (2 min) and high intensity red (2 min) light resulted in repeated

photoreversals as in typical phytochrome-mediated responses (Fig. 8). The points in the histogram have nearly identical values for promotion and for inhibition of germination, and photoreversibility of induction of germination by red light by immediately following with far red light is complete or nearly complete. These data would be in good agreement with the notion of phytochrome control of germination. If spores were repromoted by exposure to 1 hr of red light, followed by successive exposures to far red (2 min) and red light (1 hr), there was a sharp increase in the percentage of germination
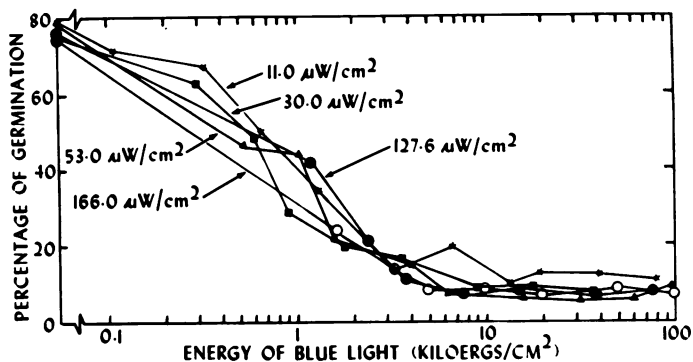
FIG. 4. Effect of different intensities of blue light on the inhibition of germination of red-promoted spores, plotted as a function of incident energy of blue light against the percentage of germination. Before exposure of the spores to blue light of different intensities shown in the figure, they were exposed to red light (7 μw/cm^2, 30 sec). For each point in the graph, 132 to 264 spores were counted.

less, it is recognized that in elucidating the photomorphogenesis of germination, it is important to study the different cellular events involved in the process separately.

One noteworthy feature of the present study is the insensitivity of the spores to far red light following an initial exposure to red light. Yet, following blue light inhibition, if the spores are repromoted to germinate by red light, far red nullifies this effect. Moreover, successive exposures of the spores at this stage to far red and red irradiations lead to mutual photoreversibility of germination. This fulfills the operational criterion for the existence in the spores of phytochrome, and control of germination may be ascribed to this pigment. No convincing explanation is at hand for the initial lack of photoreversibility of red light effect by far red light, although it seems probable that it is due to the presence of a blue light-absorbing pigment which prevents in some way the conversion of phytochrome from the active (Pfr) to the inactive (Pr) form. On the basis of this hypothesis, one would expect that, following exposure to blue light, this pigment may be removed in the ensuing
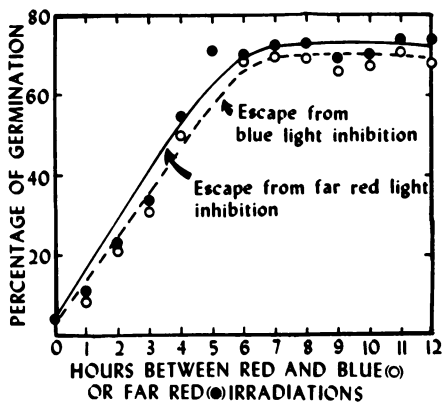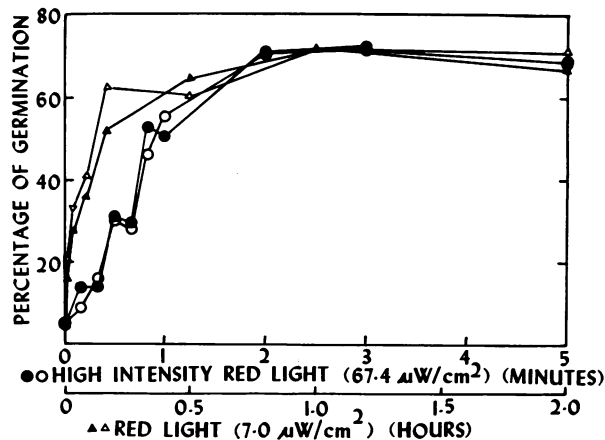

FIG. 5. Escape of red-potentiated spores from inhibition by blue light and far red light. Escape from blue light inhibition: spores were irradiated with red light (7 μw/cm^2, 30 sec) and at various times during an intervening dark period, they were exposed to blue light (166 μw/cm^2, 30 sec). For each point in the graph 190 to 210 spores were counted. Escape from far red light inhibition: spores irradiated with red light and blue light as above were repromoted to germinate by exposure to high intensity red light (67.4 μw/cm^2, 2 min) and at various times during an intervening dark period, they were exposed to far red light (410 μw/cm^2, 2 min). For each point in the graph 190 to 220 spores were counted.

FIG. 6. Effect of dosage of red light (7 μw/cm^2 and 67.4 μw/cm^2) on the repromotion of germination of spores exposed to blue light (166 μw/cm^2, 30 sec). Before exposure to red light dosages given in the figure, spores were irradiated with red light (7 μw/cm^2, 30 sec) followed by blue light (166 μw/cm^2, 30 sec) (O, △) or with blue light only (●, ▲). Each point in the graph is based on a count of 190 to 220 spores.

following the third cyclic exposure to far red light, indicating an escape from inhibition by some members of the population, and the effectiveness of far red completely diminished in further cyclic irradiations.

DISCUSSION

The over-all goal of this study, which is part of a continuing investigation on the physiology of fern spore germination, is to define the photomorphogenetic systems operating in the germination of the spores of *Cheilanthes farinosa*. An earlier study (17) on *Asplenium nidus* spores which require prolonged red irradiation for germination suggested the existence of separate photoreactions controlling the initiation of rhizoid and the initiation of protonema. In the dark-germinating spores of *Pteridium aquilinum* (16), phases of rhizoid initiation and protonema initiation were separated by giving brief periods of red light to fully imbibed spores. Due to their extreme sensitivity to red light, a clear separation of the stages in the germination of the spores of *Cheilanthes* was not possible. Neverthe-
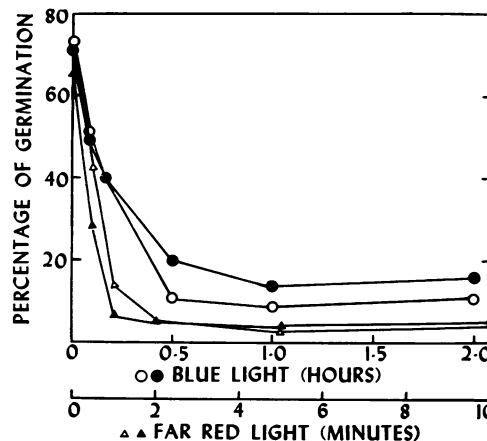

FIG. 7. Effect of dosage of far red (410 μw/cm^2) and blue (166 μw/cm^2) irradiations on the inhibition of germination of repromoted spores. For inhibition and repromotion, spores were previously exposed to red light (7 μw/cm^2, 30 sec), blue light (166 μw/ cm^2, 30 sec), and high intensity red light (67.4 μw/cm^2, 2 min) (O, △) or to blue light and high intensity red light (●, ▲). Each point in the graph is based on a count of 192 to 230 spores.

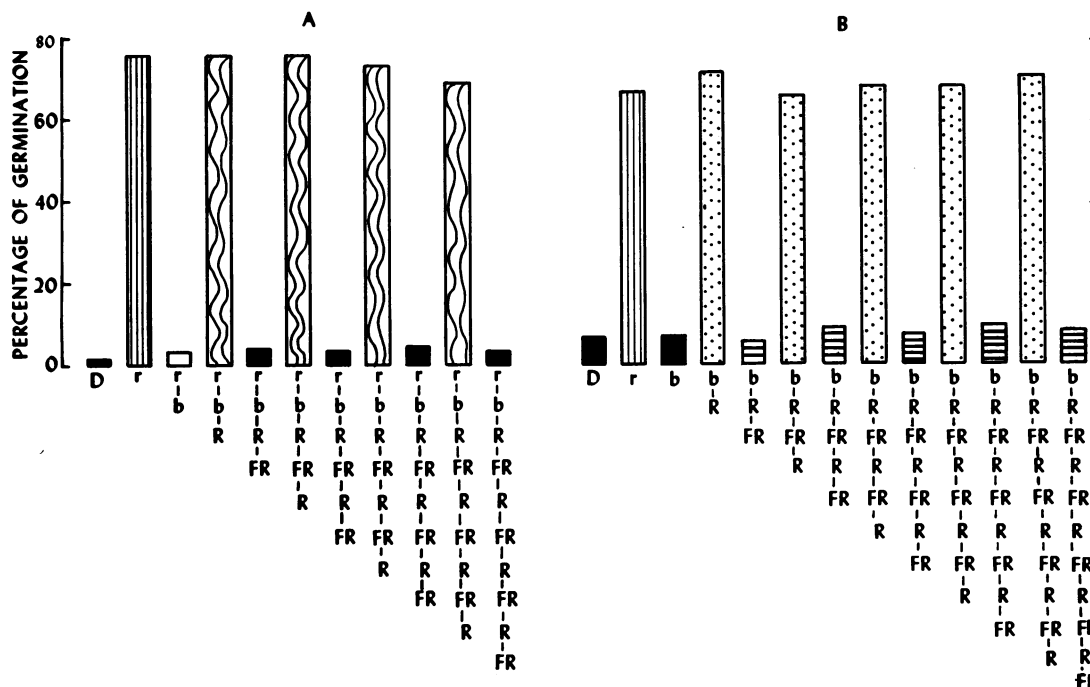FIG. 8. Effect of exposure of blue light inhibited spores to red (R, 67.4 μw/cm^2, 2 min) and far red (FR: 410 μw/cm^2, 2 min) light in sequence. Before subjecting them to sequential irradiation, spores were exposed to (A): red light (7 μw/cm^2, 30 sec, r) followed by blue light (166 μw/cm^2, 30 sec, b); (B); blue light (b) only. D is dark control. Data in A based on a count of 190 to 220 spores; those in B based on a count of 198 to 230 spores.

photochemical reaction and any additional Pfr formed during a subsequent saturating exposure to red light may be available for reversal by far red light. Although light energy requirement may not exactly reflect the concentration of phytochrome in the spore, the dramatic increase in the energy of red light required to repromote germination and the increase in response of the spores with time, might be taken to mean that there is little phytochrome present in the spore after exposure to blue light and that it is regenerated anew in the following red light.

Equally noteworthy is the fact that the spores become insensitive to red light if they are previously exposed to blue light. If we accept the validity of the presence of a blue light-absorbing pigment, it might appear that while the pigment in its native state prevents the Pfr → Pr transformation, in its "oxidized" state, it inhibits the reverse (Pr → Pfr) conversion. Recent physiological analyses have indicated that blue light-absorbing pigments are not uncommon in fern spores (19, 22).

The inhibition of spore germination by blue light calls for further characterization. The involvement of blue light in spore germination is not surprising, since this light quality has been shown to participate in other developmental phenomena in ferns such as elongation of the protonema (8), direction of its development (3), and its transition to biplanar morphology (10). Blue light interactions with phytochrome-mediated responses such as seed germination (2, 23), coleoptile elongation (14), stem growth (1), and flowering (5) have also been recorded. It appears unlikely that the blue light effect reported here is mediated through the phytochrome system, but the possibility is by no means completely eliminated. Whether or not blue light inhibition of spore germination is due to the production of an inhibitor following a photoreaction as suggested by Sugai (19) in the spores of *Pteris vittata* remains to be demonstrated.

The implication of these results in the wider context suggests that deductions on the photomorphogenesis of germination of fern spores based on the direct effects of light quality on promotion and inhibition of germination reported in the

older literature may be open to question. Examination of additional species will undoubtedly aid in our understanding of the primary regulatory mechanisms of spore germination in the pteridophytes and lead to the formulation of principles that may be generally applicable.

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