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Short Communication

Photocontrol of Fungal Spore Germination[1]

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ABSTRACT

Germination of *Puccinia graminis* f. sp. *tritici* uredospores is inhibited by continuous irradiation. Prehydration of spores enhances both dark germination and photoinhibition. Simultaneous irradiation with ineffective red (653 nanometers) and inhibitory far red light (720 nanometers) results in partial nullification of the inhibition brought about by far red light alone. This result would be consistent with the involvement of a photoreversible pigment system similar to phytochrome, operating via the high irradiance reaction.

Light influences many aspects of fungal growth and development (7, 15). In the majority of cases the most effective wavelengths of light controlling photoresponses in fungi lie in the blue and UV regions of the spectrum (3, 15). The action spectra for these responses suggest that the photoreceptor is either a carotenoid or flavoprotein (9, 18), although recent evidence favors the latter (19). A number of other fungal photoresponses are mediated by red and far red light (1, 4, 5, 8, 10, 14, 16, 20), implicating an alternative photoreceptor. It has been suggested that a phytochrome type of system may be operative in these fungi (6, 10, 22). Only a definite red/far red reversibility provides unequivocal evidence for the involvement of phytochrome. This has been reported in two cases (10, 22), but in one (22) the reversibility required relatively long periods of irradiation, which is at variance with the usual situation in higher plants. Only in one case have direct red/far red reversible absorbance changes been detected by spectrophotometry in fungi (10).

Inhibition of uredospore germination in the wheat stem-rust fungus, *Puccinia graminis* f. sp. *tritici*, by continuous high intensity blue and far red light also shows some similarities to the phytochrome system of higher plants, operating in this case via the high irradiance reaction (17). Calpouzos and Chang (6) determined a preliminary action spectrum for the photo-inhibition consistent with this view. Involvement of phytochrome in the high irradiance reaction can only be definitely concluded from dual wavelength experiments of the type designed by Hartmann (13). The present paper reports the results of such a dual wavelength experiment.

MATERIALS AND METHODS

Uredospores of *P. graminis tritici* race 21 were collected from infected wheat seedlings cv. Opal grown under uniform conditions (14-hr photoperiod at 20 C). Particular attention was paid to conditions during spore production, as these may affect subsequent germination behavior (23). Relative homogeneity of spore batches was attained by tapping off spores matured during a 24-hr period. Spore samples were then stored in the dark at 4 C over silica gel. Spore viability declines fairly rapidly during storage and therefore spores were used within 3 weeks of collection. Prior to experimental use spores were hydrated in darkness for 16 hr in a chamber at 100% relative humidity. This pretreatment induces or enhances photosensitivity and eliminates much of the variability found in nonhydrated samples (11). For determination of percentage germination under various treatments, spores were sown on 2% tap water agar blocks which were maintained in an atmosphere of 100% relative humidity. At the end of the experimental period, blocks were transferred to a desiccator containing formalin to prevent any further germination prior to counting. On average 500 spores were counted for each treatment. Spores were scored as germinated when the length of the germ tube equalled the width of the spore. Although spore density was not quantified, no evidence of autoinhibition (2) was observed at the densities employed. Germination of dark controls was greater than 60% in all experiments.

White light was provided by a bank of four fluorescent tubes (Universal White, 80w) irradiance 10.5 w m^{-2}. Seeded agar blocks were placed on slides in plastic Petri dishes containing damp filter paper and positioned 50 cm below the light source. Dark controls were wrapped in aluminum foil. The temperature throughout experiments was maintained at 25 C. Light sources for dual wavelength experiments were: red light (653 nm) obtained from a slide projector in combination with an interference filter (band width at 50% of maximum transmission 12 nm, irradiance 1.6 w m^{-2}); far red light (720 nm) obtained from a Bausch and Lomb high intensity monochromator (band pass 19 nm), irradiance 2.6 w m^{-2}. Agar blocks were irradiated on a temperature-controlled microscope stage (25 C) with either one or both wavelengths simultaneously. Dark controls were incubated on the same microscope stage but outside the irradiated area. One hundred per cent humidity was maintained throughout the experiment. Omitting this precaution led to wide variations in percentage germination of controls, due to dehydration effects. Unless otherwise stated, the experimental period in all cases was 2.5 hr.

RESULTS AND DISCUSSION

The time course of spore germination at 25 C in the dark and under white light is shown in Figure 1. In the dark, germ tubes first emerged from equatorial pores at around 50 min. Dark germination showed a high degree of synchrony, maximum germination being recorded within 2 hr. At the same time, no germination had occurred in light-treated spores. Spore samples maintained in the light eventually began to germinate after 5 hr with subsequent recovery to within 10% of dark control levels after 14 hr. This confirms the observation of previous authors (*e.g.*, 11)

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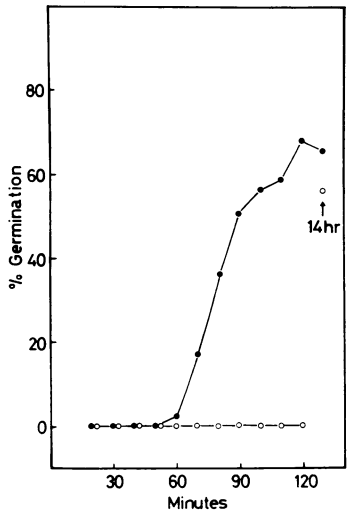

FIG. 1. Time course of germination in the dark (●) and under white light (○) at 25 C.

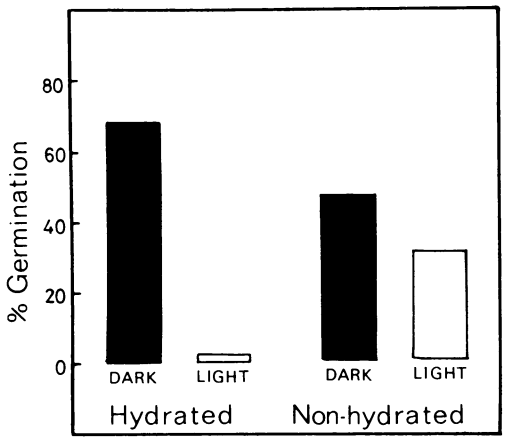

FIG. 2. Effect of prehydration on per cent spore germination after 2 hr at 25 C.

that the photoinhibition represents a delaying of germination rather than an absolute inhibition. At higher temperatures the photosensitivity of spore samples is substantially reduced (12). In this case, delay in initiation of germination brought about by this light source was reduced to 30 min by raising the temperature to 28.5 C, although dark germination was also reduced to less than 40%. The effect of hydration on light sensitivity is shown in Figure 2. Both the level of dark germination and the degree of photosensitivity in *P. graminis tritici* are enhanced by pre-hydration.

Results of a representative dual wavelength experiment carried out with prehydrated spores are presented in Figure 3. At the irradiances used red light (653 nm) proved virtually ineffective, whereas strong inhibition was obtained with monochromatic far red light (720 nm). Simultaneous irradiation with the ineffective red (653 nm) and inhibitory far red light (720 nm) resulted in partial nullification of the inhibition brought about by far red light alone. This result has been obtained in several replicate experiments and would be consistent with the involvement of a photoreversible pigment similar to phytochrome.

Although the present results and other data on fungal photoresponses mediated by red and far red light (6, 8, 10, 22) can be interpreted in terms of a phytochrome type of system, the lack of information on *in vivo* spectrophotometry and absence of accurate action spectra have to date prevented definite conclusions. The absorption spectrum of uredospores demonstrated that they are relatively transparent in the red and far red regions of the spectrum. This transparency enabled dual wavelength difference spectrophotometric measurements to be made at the peak absorbances characteristic of phytochrome in higher plants. These measurements were made by C. J. P. Spruit using a sensitive dual wavelength spectrophotometer (21). No detectable red/far red reversible changes in absorbance were observed. We are at present attempting to optimize conditions for this photoresponse with a view to obtaining dose response curves and an accurate action spectrum.

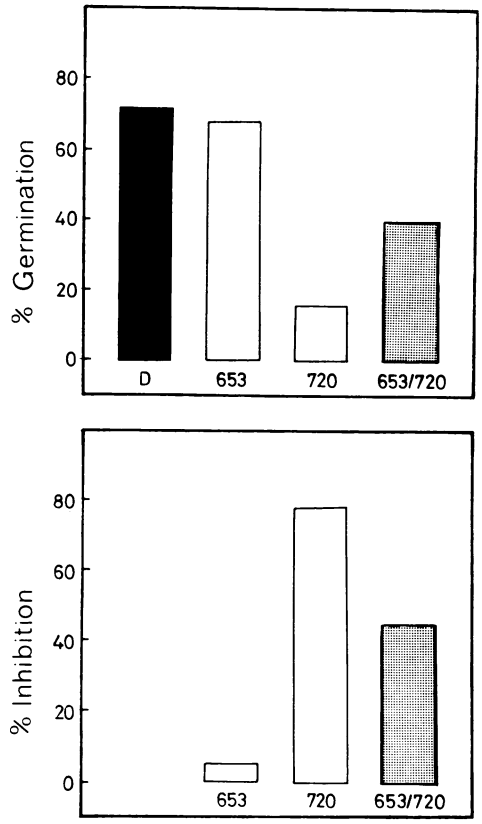

FIG. 3. Effect of red (653 nm) and far red (720 nm) light alone or given in combination on germination after 2.5 hr at 25 C. Results expressed both as per cent germination and per cent inhibition.

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