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PHOTOSENSITIVITY OF UREDINIOSPORE GERMINATION IN *PUCCINIA ARACHIDIS*

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Light inhibited germination of *Puccinia arachidis* urediniospores. The threshold value for complete inhibition of urediniospore germination was 5000 Wm^{-2}. Wavelengths of 430, 510 and 620 nm inhibited germination but had no adverse effect on germ-tube elongation of pregerminated urediniospores. Hydrated urediniospores were more sensitive to light than non-hydrated urediniospores. Inhibition was not permanent, and spores germinated when returned to the dark.

The effects of light on germination of urediniospores of various rust fungi have been studied by several workers. Most found that light inhibited germination (Lucas, Kendrick & Givan, 1975; Kochman & Brown, 1976; Stroede, 1933; Sood & Sackston, 1972; Givan & Bromfield, 1964*a*; Chang *et al.*, 1973; Cochrane, 1945; Nutman & Roberts, 1963; Wilson, 1958). McCracken & Burleigh (1962), however, reported that light promoted urediniospore germination in *P. striiformis* West. at 13·5 °C but not at 2°.

During examination of urediniospore viability in *Puccinia arachidis* Speg., the cause of groundnut (*Arachis hypogaea* L.) rust, we found inhibition of urediniospore germination on glass slides and on detached groundnut leaves incubated in an illuminated plant growth chamber. The same spores germinated rapidly in darkness. As little information was available on the effects of light on urediniospore germination in *P. arachidis* detailed investigations were carried out and the results are reported in this paper.

MATERIALS AND METHODS

Production and collection of spores

Fully developed leaves were detached from healthy, 30-day-old plants of cv. TMV 2 by cutting through the pulvinus, and the petioles were inserted into a layer of sterilized sand in plastic trays. The sand was moistened with Hoagland's solution (Hoagland & Arnon, 1938) and the trays covered with transparent polythene sheeting to maintain high humidity and incubated in a plant growth chamber (Percival MfG Co., Boone, Iowa, U.S.A.) at 25° and 12 h photoperiod. After 24 h incubation the leaves were sprayed with a suspension of *P. arachidis* spores. The polythene sheeting was replaced and the trays were returned to the growth chambers. Spores were collected two days after rupture of the urediniosori using a cyclone spore collecter. They were stored in screw-cap glass vials at −15° in the dark for up to 6 d before they were used.

Urediniospore germination

The regular test procedure, unless otherwise stated, was to make suspensions of urediniospores in sterile distilled water to a concentration of 1×10^5 spores ml^{-1}. A drop of the suspension was then placed on a glass slide in a glass Petri dish lined with moist filter paper and incubated at 25° in light (General Electric, U.S.A., cool white fluorescent lamps, 20 W each, 4000 Wm^{-2}) or in the dark for 4 h. A small drop of mercuric chloride solution (0·1 %) was then added to each spore suspension to prevent further germination and development of germ-tubes. Germination was assessed by direct microscopic examination. Spores were counted as germinated if the germ-tube length exceeded the spore diameter. No evidence of autoinhibition due to high spore density was observed but, as a precaution, all clumps of spores were ignored when counting. In each examination, ten replicate slides were used and 100 spores were observed on each slide. The experiments were conducted thrice. Percentage data were subjected to angular transformation and analysis of variance was carried out on pooled data from all experiments.

Light intensity and quality

Urediniospores were incubated for 4 h at 25° in plant growth chambers at different light intensities (Fig. 1). Spores were exposed to different portions of the spectrum by replacing the lids of the Petri dishes containing the spore suspensions with coloured cellophane filters. The approximate peak wavelength transmitted by each interference filter

was measured using a spectrophotometer. To estimate the effect on germ-tube growth, the urediniospores were pregerminated by incubating in the dark for 1 h, the initial length of germ-tube estimated, and the spores then exposed to different portions of the spectrum as described above. After 6 h incubation, the lengths of germ-tubes were again measured.

Hydration and temperature

Urediniospores were either hydrated overnight in an atmosphere of 100% r.h., hydrated and redried overnight in a plastic desiccator containing anhydrous calcium sulphate, dried without having been previously hydrated, or collected fresh. Suspensions were incubated for 4 h, in the dark or in light, at various temperatures (Fig. 2).

Time of illumination

Spore suspensions were incubated on glass slides at 25° and exposed to varying periods of light then darkness, or darkness then light (Fig. 3), the total incubation time being 4 h.

One-month-old groundnut plants of the susceptible cv. TMV 2 were inoculated with urediniospores. Plants were covered either with a transparent or black polythene sheet (light and dark treatments respectively). Both sets were placed under direct sunlight at 27±3°. Light intensity was measured every 5 min during the experimental period. The experiment was started at 09.00 h and the percentage urediniospore germination on leaf surfaces was estimated at 1 h intervals until 22.00 h. For each sampling, leaf pieces (1 cm^2) were taken from five plants from each treatment, the germinating urediniospores present on the leaf surfaces were stained with cotton blue–acid fuchsin, mounted in distilled water, and germination assessed.

RESULTS AND DISCUSSION

Effect of light intensity

Germination decreased with increasing light intensity (Fig. 1). Cochrane (1945), Zadoks & Groenewegen (1967), Chang, Calpouzos & Wilcoxson, (1973), Kochman & Brown (1976) and Knights & Lucas (1980) found similar inverse relationships between urediniospore germination and light intensity while working with other rust fungi.

The threshold value for complete inhibition of urediniospore germination by light was 5000 Wm^{-2} (Fig. 1), a much lower level than the 8000 Wm^{-2} reported by Zhou *et al.* (1980) as the threshold value for complete inhibition of germination of urediniospores in the People's Republic of China.

Although there was no germination of spores

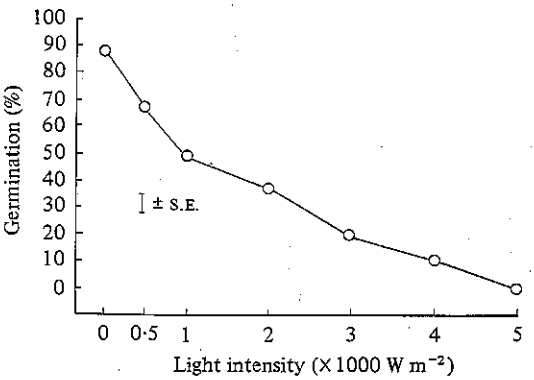

Fig. 1. Effect of light intensity on urediniospore germination.

even after prolonged incubation (24 h) at 5000 Wm^{-2}, once they were transferred to the dark 70% germinated rapidly. These results confirm that light-induced inhibition of urediniospore germination is reversible, as observed by Dillon-Weston (1932). Givan & Bromfield (1964*b*) and Lucas *et al.* (1975), however, reported that light inhibits urediniospore germination only during the first few hours of incubation, since illuminated and non-illuminated spores germinate to similar levels after prolonged incubation. Knights & Lucas (1980) observed only a partial recovery following 24 h continuous illumination.

Effect of light quality

There was a marked inhibitory effect of radiation of different wavelengths on urediniospore germination, especially at 430, 510 and 620 nm (Table 1), confirming reports by other workers (Dillon-Weston, 1932; Calpouzos & Chang, 1971; Chang *et al.*, 1973). In the present study, however, there was no precise quantitative relationship between different portions of the light spectrum and urediniospore germination. Determining this re-

Table 1. *Effect of radiation of different wavelengths on urediniospore germination and germ-tube elongation in* Puccinia arachidis

	Germination (%)	Germ-tube elongation (μm)
Blue (650 nm)	62	11·0
Green (620 nm)	42	9·7
Red (510 nm)	15	10·4
Yellow (430 nm)	19	10·1
White light	7	9·9
Dark	78	10·0
S.E.±	9·00	1·31
Coeff. var. (%)	26·00	7·10

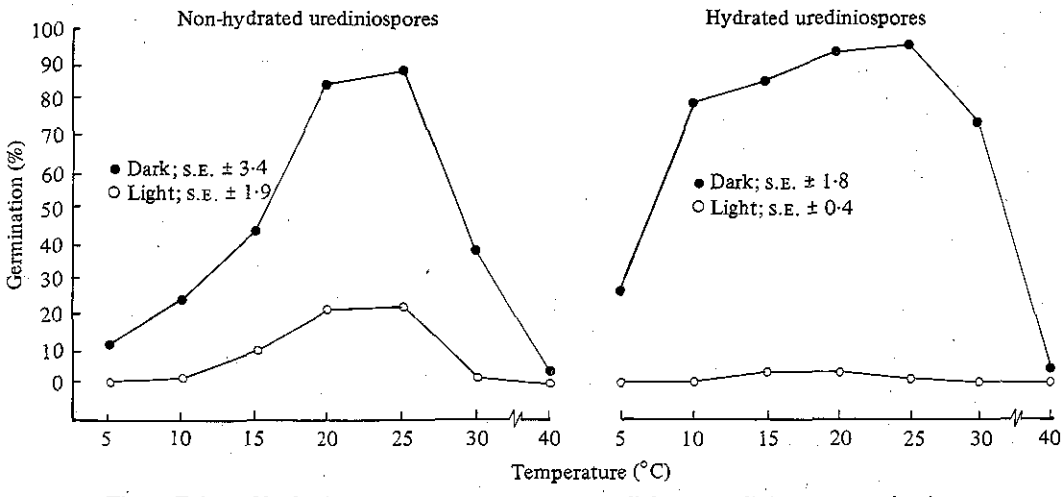

Fig. 2. Effect of hydration treatment, temperature and light on urediniospore germination.

lationship would require a more detailed study resulting in a true action spectrum.

There was no adverse effect of radiation on germ-tube elongation of pregerminated urediniospores (Table 1). These results show that light does not inhibit the growth of germ-tubes. This agrees with the findings of Chang *et al.* (1973), working with *P. recondita* Rob. ex Desm. Kocham & Brown (1976), working with *P. graminis* Pers. f. sp. *avenae* Eriks. & Henn. and *P. coronata* Corda f. sp. *avenae* Eriks. & Henn., showed that various light intensities reduced germ-tube elongation.

Effect of hydration treatments and temperature

In the light treatment there was no germination of non-hydrated spores at 5 and 40°, and the maximum germination (21 %) was at 25° (Fig. 2). In the dark, non-hydrated urediniospores germinated at all test temperatures, with the maximum (87·5 %) at 25°. Hydrated spores only germinated in light at 15, 20, 25° but levels were significantly lower than the non-hydrated urediniospores incubated at these temperatures. Hydrated spores germinated in the dark at all temperatures, and at significantly higher levels than the non-hydrated ones (Fig. 2). Hydrated urediniospores of *P. arachidis* are, therefore, more sensitive to light than non-hydrated ones. This agrees with the observations of workers investigating various other rust fungi (Givan & Bromfield, 1964*a*; Chang *et al.*, 1973; Zadoks & Groenewegen, 1967; Lucas *et al.*, 1975). The results also show that light inhibition of germination of urediniospores of *P. arachidis* resembles that of other rust fungi in being temperature sensitive (Givan & Bromfield, 1964*a*,*b*; Nutman & Roberts, 1963; Stroede, 1933).

The initial water content of the urediniospores may be an important consideration in investigating the effects of light on urediniospore germination as suggested by Knights & Lucas (1980), since no dried or redried spores germinated in light or dark.

Effect of irradiation upon germination

When urediniospore suspensions incubated on glass slides at 25° were exposed to various combinations of light then darkness, germination was very high regardless of time of exposure to light (Fig. 3). When spores were initially incubated in the dark and then transferred to light there was some inhibition during the first 40 min, but subsequently the germination percentage was high (Fig. 3). These results suggest that germination was inhibited only during the illumination period and there was no residual effect of light. This finding agrees with those of Chang *et al.* (1973) and Knights & Lucas (1980) working with other rust fungi.

When groundnut plants inoculated with rust urediniospores were incubated under transparent polythene sheeting in direct sunlight no germination took place until sunset (18.00 h). After sunset, spore germination was rapid (Fig. 4). Similar results were obtained by Knights & Lucas (1981) for *P. graminis* Pers. f. sp. *tritici* Eriks. & Henn. in field studies, when no germination took place until darkness. Urediniospores on leaves kept under black polythene sheeting over the same period germinated from 11.00 h onwards (Fig. 4). These data support those from slide germination tests indicating reversible light inhibition.

Post-inoculation incubation of plants in the dark for about 2 h at 25° is suggested to obtain maximum

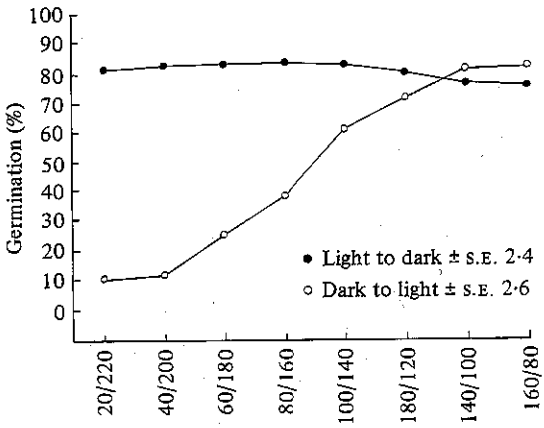

Fig. 3. Effect of pretreatment in light or dark on urediniospore germination.

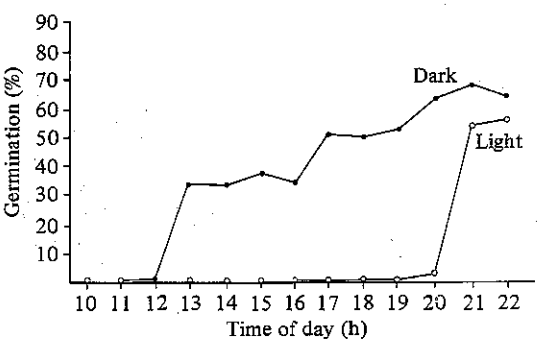

Fig. 4. Effect of sunlight on urediniospore germination.

germination. Hydrated urediniospores (placed overnight in an atmosphere of 100% r.h.) germinate better than freshly collected urediniospores and could be used for inoculation of plants in resistance screening. At ICRISAT, good rust disease development has been achieved by sprinkler irrigation of groundnut crops late in the afternoon followed by inoculation with urediniospore suspensions after sunset. Inoculations earlier in the day were less successful.

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