Bot. Bull. Academia Sinica **14**: 35-40 (1973)

PHOTOTROPISM OF THE UREDOSPORE GERM TUBES OF *PUCCINIA GRAMINIS TRITICI*

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(Received for publication March 15, 1973)

Abstract

A tentative action spectrum of negative phototropism of *Puccinia graminis tritici* germ tubes was investigated; the maximal effective wavelengths of radiation were in the vicinity of 400 nm, the upper limit was 600 nm and the lower limit was 300 nm. Within the range of temperatures which permit germ tube growth they respond to the light stimulation.

The significance of the negative phototropism of germ tubes is still unknown. The penetration of germ tubes of *P. recondita* through stomata is independent of light and direction of incoming light.

Introduction

Phototropic reactions have been observed in most of the major groups of fungi, with the exception of the aquatic Phycomycetes. A voluminous literature on the subject has existed, particularly on the phototropic response of sporangiophore of *Phycomyces blackesleanus* and *Pilobolus keinii* (Carlile, 1970; Page, 1968).

With some species of rust fungi, the germ tubes are negatively phototropic to unilateral illumination. Rust fungi with a negative phototropic response of the germ tubes are: *Phragmidium subcarticium, Puccinia coronata, P. cornifera, P. dispersa, P. graminis tritici, P. malvacearum, P. methae, P. rhamni, P. siplex* and *P. triticina*. Rust fungi with no phototropic response of their germ tubes are: *Puccinia anthir rhini, P. glumarum, P. magnusian* and *P. suvaeolens* (March *et al.*, 1959). No positively phototropic response has been reported for germ tubes growth of rust fungi. With the exception of *Puccinia triticina* and *P. dispersa*, no action spectrum on the negative phototropism of uredospore germ tubes has been worked out. Present paper is to report the results of our investigation on action spectrum of uredospore germ tube growth of *P. graminis tritici*.

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Materials and Methods

Sources of Uredospores of Puccinia graminis tritici race 56

The race 56 of *P. graminis tritici* used in present experiment was typical of the race and had orange-red colored uredospores. The original cultures of the race was obtained from the Coorperative Rust Laboratory, USDA, St. Paul, Minnesota.

The production and preparation of uredospores for experiments were similar to the previous report (Calpouzos and Chang, 1991). Throughout the experiments, water agar (1.3 precent Difco agar power in distilled water and autoclaved for 15 min.) was used as the medium for studying phototropism of germ tubes. Test uredospores were dispersed on agar plates by using either a settling tower or the streaking method. Concentration of uredospores were about 50 spores per mm^2. At this concentration of uredospores no self-inhibition of germination occurred.

Light Source

White light was derived from a General Electric F 15 T8-B 15-watt blue lamp. The distance from the light source to test spore was 30 cm.

For light quality tests, a Bausch & Lomb Grating Monochromator was empolyed. Radiation energy was 800 ergs/cm^2/sec.

Procedures of the Experiments and Evaluation of the Experimental Results

Hydrated uredospores of *P. graminis tritici*, race 56 were seeded on water agar plates and exposed to unilateral light. The path of light was parallel to the plate surface. Observations on the direction of germ tube growth were made after 3 hours of continuous illumination. Check uredospores were in darkness on water agar plates wrapped with black tape and exposed to same unilateral light. The experiments were carried out in a dark space and at room temperatures (19° to 24°C). Two hundred germ tubes were examined in every trial of the experiment. Three trials were made in each test wavelength of radiation.

In another procedure hydrated uredospores on water were unilaterally illuminated with white light for 2 hr. Most of the germ tubes at this time had grown away from the incoming light. The agar plate was then turned 90° and exposed to unilateral illumination of different wavelengths for one hour. Thus most of the germ tubes were placed at a right angle to the incoming light. Observations on the tropism were made by counting the number of germ tubes that were L-shaped because they had changed their growth direction away from the incoming light. Two hundred germ tubes were observed in each trial of the experiments.

Significance of the phototropic reaction of germ tubes in the early stage of infection (penetration and substomatal vesicle formation)

About 2 cm long pieces of epidermis was stripped from the lower surface of 10-day old Little Club wheat primary leaves, and laid on water agar. Most of the stomata closed when the epidermis was removed from the leaf. Uredospores of stem rust race 56 and of leaf rust race UN 2 were dusted onto the stripped epidermis surface and placed in a Biotronette growth chamber equipped with four fluorescent lamps. The light intensity was 400 ft-c and the temperatcre was 20°C. The inoculated epidermal strips were incubated in darkness for 4 hours, then treated as follows: 1) illuminated from above the epidermal strips (400 ft-c), 2) illuminated from below the epidermal strips through the water agar. and 3) kept the inoculated epidermal strips in darkness. Observations on penetration and vesicle formation were taken 20 hours after the uredospores were placed of the epidermal strips. The percentage of appressoria that formed vesicles was determined. Experiments were repeated five times.

Results

Phototropism of germ tubes of Puccinia graminis tritici race 56

Hydrated uredospores of *P. graminis tritici* on water agar surface were unilaterally illuminated with white light from a blue fluorescent lamp at 300 ft-c for 3 hr. All the germ tubes grew away from the incoming light. The experiment was repeated numerous times and the same result was obtained each time.

Phototropic response of germ tubes to different wavelengths of light

Hydrated uredospores on a water agar surface were unilaterally illuminated with different wavelengths of light from a Bausch and Lomb Grating Monochromater for 3 hr at room temperatures. Table 1 shows that 400 nm

Table 1. *Effectiveness of different wavelengths of radiation on the phototropic response of germtubes of* ***Puccinia graminis tritici*** *race 56 at room temperatures*

Wavelength (nm)	% of germtubes showing negative phototropism*
300	24
400	93
500	59
600	22
700	36
Dark	23

* Average of three trials. 100 germ tubes were examined.

was the most effective wavelength of light for inducing negative phototropism in this fungus for more that 90 percent of the germ tubes were negatively phototropic at this wavelength of light. There was virtually no tropic response of the germ tubes at 300 nm and 600 nm of radiation. Blue-green light (500 nm) induced some tropic response of the germ tubes (56%). Radiation at 700 nm also appeared to cause a tropic response of germ tubes, however, the effect could have been due to a defect in the instrument which leaked some other wavelengths of light which was responsible for the result.

Effect of temperature on the tropic response of germ tubes to unilateral white light illumination

Hydrated uredospores of *P. graminis tritici* were dusted on a water agar surface and illuminated unilaterally with fluorescent light for 3 hr at different temperatures. Temperature of the water agar was maintained by placing it on a rectangular metal box filled with continuously circulated water of the desired temperature.

At 10°, 15°, and 25°C uredospores germinated and all germ tubes were negatively phototropic to the unilateral white light. At 30°C uredospores did not germinate.

When uredospores had germinated at 20°C for to hours on water agar under unilateral white light of 800 ergs/cm^2/sec, the agar plate was turned 90° so that the germ tubes were perpendicular to the light beam and then, the temperature was raised to 30°C. Two hours later the germ tubes were examined. The germ tubes did not grow, instead, the tips of germ tubes had became swollen. Temperature was then adjusted to 20°C and 4 to 6 hr later a fusiform structure emerged from the swollen tip of the germ tube. Hyphae formed from either side or one side of this structure, after about 6 hr at the same temperature either under light or darkness.

Significance of phototropism of germ tubes in the early stage of infection

With leaf rust uredospores dusted on epidermal strips appressoria, substomatal vesicles and infection hyphae formed in all three treatments. Appressoria formed on closed stomata 3 to 4 hr after inoculation; vesicles formed beneath closed stomata 6 to 8 hr afterward; and infection hyphae formed 20 hr after inoculation. Occasionally, vesicles and infection hyphae formed on the outside of closed stomata instead of benenth the closed stomata as usual. On the other hand, the germ tubes of the stem rust fungus formed appressoria on the closed stomata like leaf rust. But no substomatal vesicles formed beneath closed stomata, though a few formed beneath partial open stomata. No infection hyphae were found. The shape of the appressoria that formed on closed stomata differed with the rust fungi; stem rust appressoria in

general were fusiform fully covering the closed stomata but leaf rust appressoria were irregularly rectangular and formed on either end of closed stomata. With leaf rust, after vesicles formed beneath the closed stomata, empty appressoria were left on the stomata. No appressoria of stem rust or leaf fungus were observed on wide open stomata (Table 2).

In the present experiment, appressoria, vesicles and infection hyphae formed independently of light. The significance of phototropism of germ tubes of rust uredospores in infection is still questionable.

Table 2. *Direction of illumination in relation to the formation of substomatal vesicles and infection hyphae beneath the stomata of epidermal strips*

Direction of light from	% appressoria form vesicles and infection hyphae*	
	Stem rust	Leaf rust
Above epidermis	30**	80
Bleow epidermis	24**	84
Dark	27**	90

* 100 appressoria examined in each treatment.
** Only vesicles were found. No infection hyphae formed.

Discussion

An action spectrum for photoropism of germ tubes of stem rust fungus race 56 was obtained in this experiment, It is, in general, similar to previous reports on different fungi (Bergman *et al.*, 1969; carlile, 1970; Gottkandt, 1954; Page, 1968.) that the most effective wavelengths were about 400 nm. Wavelengths shorter than 300 nm and longer than 600 nm were not effective. With *Puccinia triticina* and *P. dispersa*, Gettkandt (1954) found that the most effective wavelengths to induce negative phototropism of uredospore germ tubes were 450–480 nm; the lower limit was 280 nm and upper limit was 500 nm.

Temperature did not affect the phototropic response of germ tubes of *P. graminis tritici*, within the range that permits germ tubes to grow. As long as germ tubes actively grew they were sensitive to light stimulation.

The significance of negative phototropism of germ tubes of the rust fungi in the infection process was not reached in the present work. Using epidermal strips of primary leaves of wheat seedlings uredospores of *P. recondia* germinated and formed appresoria on closed stomata, and subsequent formation of infection pegs, substomatal vesicles and infection hyphae were independent of light. Substomatal vesicles and infection hyphae formed heneath the closed stomata no matter which direction light came from or in darkness. The

positive phototropism of the necks of preithecia and asci in some groups of Ascomycetes and the stipes of Basidiomycetes leads the discharged spores into an open space from where they are dispersed by wind and so positve phototropism has a great significance in the survival of these species of organisms (Ingold, 1960).

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小麥黑銹病菌夏胞子發芽管生長的避光性

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本實驗是在探究小麥黑銹病病原菌的夏胞子發芽管避光性生長的作用光譜。我們發現引起發芽管避光生長的最有效波長是在400 nm左右，其上限波長是600 nm，下限是300 nm。發芽管避光性的反應，不受溫度的影響；在發芽管能够生長的範圍溫度，都發生這種反應。發芽管避光性對於生長的意義，本實驗結果沒得到肯定的結論。我們曾以小麥葉銹病之原菌做實驗，發現它的夏胞子侵入氣孔，並不受光質和光向的影響。