

Effects of Light on Pycnidium Formation, Sporulation, and Tropism by *Septoria nodorum*

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ABSTRACT

Four isolates of *Septoria nodorum* formed fertile pycnidia under continuous light, or diurnal light-dark cycles, but not in constant darkness. Under light, the isolates formed pycnidia more frequently on potato-dextrose agar (PDA) than on Czapek's or malt agar, and more frequently at 15 than at 25 C. A single 24-hr exposure properly timed induced the cultures to form pycnidia; however, some of these pycnidia were sterile. As the duration of irradiation increased from 1 to 14 days, the total number of pycnidia increased as well as the percentage of fertile

pycnidia. Light intensities ranging from 80 to 8,000 ergs/cm² per sec were sufficient to induce pycnidia in two isolates; however, at 80 ergs/cm² per sec, one of the isolates formed only sterile pycnidia. Negative phototropism was exhibited by hyphae of all our isolates. Only wavelengths in the near ultraviolet shorter than 350 nm induced pycnidia. Phototropism was also induced by wavelengths shorter than 350 nm as well as those lying somewhere between 350 and 510 nm. Phytopathology 60:791-794.

Septoria nodorum Berk., the imperfect stage of *Leptosphaeria nodorum* Müller, causes lesions on stems, leaves, and glumes of wheat. This pathogen is present over a broad region of the midwestern USA, including Minnesota where field and laboratory studies on this fungus have been initiated. These studies require a constant supply of spores. *Septoria nodorum* grows readily on a wide variety of media, but it produces fertile pycnidia only occasionally under circumstances not well understood (1, 2, 3, 4, 7). Richards reported that light is required for sporulation (3). Our studies were made to determine the influence of light on sporulation in culture and to manipulate light for maximum spore production. The influence of various exposures, intensities, and wavelengths of radiation on pycnidium formation and sporulation was tested.

MATERIALS AND METHODS.—The single-spore isolates of *S. nodorum* originated from lesions on wheat leaves collected in Kansas, Nebraska, South Dakota, and Minnesota. The cylindrical spores measured 14-25 μ \times 2-4 μ , and usually had one septum. Mature pycnidia were globose, 150-250 μ in diam, and had a distinct ostiole. Wheat seedlings were inoculated with several isolates that proved to be pathogenic.

Two constant-temperature cabinets were set either at 15 \pm 1 or 25 \pm 1 C. The temperature inside the cabinet was monitored during each experiment by means of thermocouples and a recording thermograph. The light source consisted of cool-white fluorescent lamps (GE F20T12-CW) arranged along the ceiling of the cabinet. Light intensities were measured with a Model 60 radiometer, Yellow Springs Instrument Co. Normally the cultures were kept approximately 12 cm from the lamps, which provided an intensity of 8,000-10,000 ergs/cm² per sec at the colony surface. The lids were always on the culture dishes. The temperature inside a typical petri dish culture exposed to light was measured with a thin-wire thermocouple, and was found to remain within 0.5 C of the air in the cabinet. Dark-grown cultures were kept in the same cabinet, but they were loosely wrapped in two layers of black cloth or

were kept inside cans covered with black cloth. The effect of light intensity was studied by varying the distance between culture and light-source, or by covering the culture dish with layers of cheesecloth.

The nutrient media tested were potato-dextrose agar (PDA), malt agar, and Czapek's solution agar from the Difco Laboratories prepared according to the directions on the labels. The media in petri dishes were inoculated in the first series of experiments (Table 1) with an agar disc 5 mm in diam. on which the fungus was growing. In subsequent experiments, the petri dishes were seeded by flooding the agar surface with 0.5 ml of a spore suspension containing more than 2×10^6 spores/ml water. The latter method had the advantage of producing across the entire agar surface mycelial growth of uniform age, which is important for studying the effects of light at different growth stages of the fungus. Furthermore, by the flooding method it was easier to count the resulting pycnidia per unit area, as they tended to be more uniformly distributed than those formed in a dish originally inoculated with an agar disc.

Observations were made 14-15 days after seeding the agar dishes. Only mature-looking pycnidia were counted, i.e., those having evident ostioles or spore masses. In the first series of experiments, the number of pycnidia was estimated according to four general categories, starting with 0 (no pycnidia) and progressively increasing to 4 (very many pycnidia). In subsequent experiments, the number of mature pycnidia per cm² of colony surface was counted in three random areas of each culture.

The percentage of fertile pycnidia was determined by removing 10 mature pycnidia at random from each culture and placing them in a drop of water on a glass slide. The pycnidia were squeezed gently with a glass cover slip and observed for the presence of spores.

RESULTS.—*Combinations of light, temperature, and media as they influence pycnidium formation.*—The formation of pycnidia was observed under continuous light (CL), diurnal cycles of light/darkness (L/D), and continuous darkness (CD) in four isolates growing

TABLE 1. Pycnidium formation by four isolates of *Septoria nodorum* growing on three media at two temperatures under continuous light or diurnal light-dark cycles

Temp	Isolate	Index of pycnidium formation ^a					
		Continuous light			Light-dark cycles		
		Agar medium			Agar medium		
		Czapek	Malt	PDA ^b	Czapek	Malt	PDA ^b
15 C	K	0	0	2	0	0	3
	N1	0	0	4	0	0	4
	N2	3	3	4	1	4	4
	SD	2	3	4	0	2	4
25 C	K	0	0	1	0	0	2
	N1	0	0	3	0	0	3
	N2	3	0	3	3	0	1
	SD	0	3	0	0	3	0

^a 0 = no pycnidia; 1 = few; 2 = moderate number; 3 = many; 4 = very many. Avg results from two experiments.

^b PDA = potato-dextrose agar.

on three media at two temperatures (Table 1). Whenever pycnidia were present, the majority were sporulating. Pycnidia formed in a number of instances under CL and L/D, but never under CD. With light, pycnidia formed in a greater number of combinations of conditions at 15 than at 25 C, and usually higher numbers occurred under 15 than at 25 C. On PDA, all four isolates produced pycnidia at 15 C, whereas only one or two isolates fruited on the other two media. As a result, 15 C and PDA are standard conditions in the subsequent experiments described in this paper unless noted otherwise.

Effects of duration of light exposure on formation of pycnidia and spores.—Tests were made to determine if a single 24-hr exposure to light would induce the fungus to form fertile pycnidia (Table 2). During a 2-week experiment, the largest number of pycnidia formed

TABLE 2. Pycnidium formation by four isolates of *Septoria nodorum* exposed to white light for one 24-hr period^a

Exposed to light at x days after seeding the agar plate ^b	Pycnidia/cm ² of colony surface per isolate ^c			
	K	N1	N2	SD
	<i>no.</i>	<i>no.</i>	<i>no.</i>	<i>no.</i>
0	0	0	0	0
1	0	0	0	114
2	17* ^d	124*	83*	134
3	156*	133	173*	271
4	179*	127	175*	285
5	15*	119	164*	271
6	14*	139	173*	281
7	3*	103	143*	267
8	0	101	11*	272
9	0	89	0	243
10	0	87*	0	181
11	0	0	0	149
12	0	0	0	0
13	0	0	0	0
Continuous light, check	141	146	245	383
Continuous darkness, check	0	0	0	0

^a All cultures on potato-dextrose agar at 15 C.

^b Light exposures began on the day indicated and ended 24 hr later.

^c Each figure is the avg of three areas sampled within a single culture plate.

^d * = no fertile pycnidia found in the culture.

when the 24-hr light exposure occurred on days 3 and 4 for isolate K, days 2-6 for N1, days 3-7 for N2, and days 3-9 for SD. Light exposure at an earlier or later age resulted in fewer or no pycnidia. After a 24-hr exposure to light, the resulting pycnidia formed by isolates K and N2 were sterile; N1 had sterile pycnidia only in the cultures irradiated on the 2nd or 10th day. Each culture of SD that had pycnidia showed sporulation.

Other tests were done to measure the formation of fertile pycnidia in cultures exposed to light for a progressively increasing period of 1-14 days. All the cultures were placed in the light immediately upon seeding with spores, then after each day a plate was shifted to darkness until the end of the 2-week period of the experiment. Table 3 summarizes the results in terms of total numbers of mature-looking pycnidia and percentage of these pycnidia-producing spores. With longer exposure to light, up to about 14 days, the fungus produced larger numbers of pycnidia, and the percentage of fertile pycnidia increased from 0-95%.

TABLE 3. The number of pycnidia formed and the percentage of fertile pycnidia in cultures of *Septoria nodorum* exposed to light for periods varying from 1-14 days^a

No. days in the light ^b	No. pycnidia/cm ² of colony	% Fertile pycnidia
1	0	
2	7	0
3	220	25
4	252	40
5	299	50
6	320	60
7	369	65
8	419	65
9	440	80
10	494	85
11	477	85
12	517	85
13	537	95
14	527	95
Continuous darkness, check	0	

^a Isolate SD on PDA at 15 C for 14 days total. Avg results of two experiments.

^b All light treatments were started on the day of seeding the agar plates.

TABLE 4. Pycnidium formation by two isolates of *Septoria nodorum* at two temperatures under different light intensities

Intensity ^b	Pycnidia/cm ² of colony surface per isolate ^a			
	N1		SD	
	15 C	25 C	15 C	25 C
ergs/cm ² per sec				
8,000	210	210	395	316
6,000	342	263	395	342
4,000	289	210	395	316
2,000	184	158	289	289
800	184	158	158	105
80	158	184	79* ^c	5*
Dark (check)	0	0	0	0

^a The avg results of two experiments.

^b With a foot-candle meter, the readings were 750, 520, 300, 100, 50, and 20 ft-c, respectively.

^c * = No fertile pycnidia found in the culture.

Effect of light intensity on formation of pycnidia and spores.—Continuous irradiation for 14 days resulted in pycnidia at all intensities tested from 80 to 8,000 ergs/cm² per sec at 15 and 25 C; however, for isolate SD, 80 ergs/cm² per sec appeared suboptimal, as the number of pycnidia diminished noticeably and the few pycnidia produced at low intensity were sterile (Table 4). Isolate N1, on the other hand, was still producing fertile pycnidia in abundance at the low intensity of 80 ergs/cm² per sec, showing that strain differences are important in determining the minimal polychromatic light intensity needed for sporulation.

Phototropism.—During the course of these studies, it was noticed that some of the hyphae grew away from the light source when it was placed to one side of the culture dish (Fig. 1). This was observed most readily in agar dishes seeded uniformly with a spore suspension. The negative phototropism was exhibited primarily by hyphae submerged in the agar medium, and to a lesser extent by aerial hyphae. All six isolates of *S. nodorum* grown at 15 and 25 C with the light source to one side of the dishes exhibited negative phototropism within 1 week's incubation period. In one

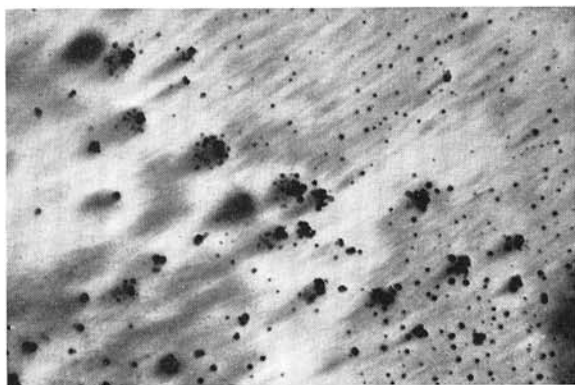


Fig. 1. Mycelium of *Septoria nodorum* growing away from the light coming from the upper right-hand corner of the photo (about $\times 4$). The dark points are pycnidia either singly or in groups.

test at 25 C, phototropism was faintly noticeable at a minimal light intensity of about 700 ergs/cm² per sec, and became very noticeable at 3000 ergs/cm² per sec.

Wavelengths which initiate fertile pycnidia and hyphal phototropism.—A series of Corning glass filters was used to isolate various regions of the near ultraviolet and visible portions of the electromagnetic spectrum. Pycnidia formed only when the fungus was irradiated with wavelengths in the near ultraviolet which were shorter than 350 nm (Table 5). Longer wavelengths in the near ultraviolet and those in the visible region were without effect regarding pycnidia. Negative phototropism was evoked by wavelengths shorter than 350, and also by wavelengths lying somewhere between 350 and 510 nm that include part of the near ultraviolet and the entire blue region of the spectrum.

DISCUSSION.—A single 24-hr exposure to light initiated only sterile pycnidia in some isolates and fertile pycnidia in others. Similarly, a low light intensity of 80 ergs/cm² per sec for 2 weeks initiated sterile pycnidia in one isolate, but fertile pycnidia in another. Furthermore, a progressively increasing exposure to light was paralleled by an increasing percentage of fertile pycnidia. Light is needed not only to initiate the formation of pycnidia but, at a slightly later stage of pycnidial development, an additional dose is needed to initiate spore production. Apparently isolates vary in the minimal light dosage they require for initiating both processes, and this could account for the several responses noted. Since by manipulating the light dosage one can separate sporulation from pycnidium formation, it is possible that different biochemical pathways are involved.

The results from the wavelength experiment are in accord with studies by other investigators who showed that several fungi require wavelengths shorter than 360 nm for fruiting and sporulation (6). Phototropism in other fungi and higher plants is caused by radiation in the blue and near ultraviolet regions of the spectrum (5); this again corresponds with our data. Since negative phototropism increased with increasing light intensity over the range of 700-3,000 ergs/cm² per sec, moderately bright light probably inhibits mycelial growth, and such a phenomenon may affect the pathogen's activities in the field.

TABLE 5. Pycnidium formation and phototropism by *Septoria nodorum* exposed to radiation of different wavelengths^a

Wavelength range, ^b nm	No. pycnidia/cm ² of colony	Negative phototropism
310-400 and IR ^c	79	Yes
310-IR	118	Yes
350-IR	0	Yes
510-IR	0	No
590-IR	0	No

^a The avg results of two experiments.

^b Resulting from the transmission characteristics of the Corning glass filter and the emission of the cool-white fluorescent lamp.

^c IR = infrared, wavelengths longer than 700 nm.

The light intensity study employed radiation consisting of a broad range of wavelengths, a few effective ones and many ineffective ones. Quite probably, if we had used a narrow radiation band containing only effective wavelengths, the fungus would have responded to lower doses than those demonstrated in this paper.

A knowledge of the range of conditions under which *S. nodorum* will sporulate is important to investigators who require a maximum supply of spores for studies on this pathogen. Richards pointed out that when the petri dishes with agar were seeded with a spore suspension, the fungus formed more pycnidia, and in a shorter time (5 days) than when dishes were inoculated with an agar disc, other conditions being optimal (3). Our own experiences confirm that report. Richards (3), who worked with one isolate, obtained sporulation only under one narrow set of conditions: CL, about 100 ft-c (equivalent to 2,000 ergs/cm² per sec in our experiment) and around 20 C. In contrast, our results with several isolates show that abundant fruiting and sporulation occurred under a broader range of conditions: either CL or L/D, 15 or 25 C, and 80-8,000 ergs/cm² per sec intensity. Since the isolates in this study came from widely separated areas of the Midwestern USA, they should be representative of this species. From the various combinations of conditions tested, *S. nodorum* appeared most likely to produce many fertile pycnidia

in about 1 week when PDA was seeded with a spore suspension and incubated at 15 C under CL or L/D at 8,000-10,000 ergs/cm² per sec from a cool-white fluorescent lamp.

Light has a number of important effects on *S. nodorum*. It stimulates pycnidium formation, it stimulates sporulation, and it affects hyphal growth. Although these studies were conducted in vitro, it is possible that light may affect the fungus similarly in nature and thus influence epidemiology.

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