

Effect of Monochromatic Light on Germination of Oospores and Formation of Sporangia of *Phytophthora citricola*

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ABSTRACT

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Oospore germination and sporangial formation of *Phytophthora citricola* in various bands of monochromatic light were studied. Acrylic filters with spectral transmission peaks at 450, 545, 650, and 750 nm were combined with aqueous filter solutions and various light sources to expose oospores to a radiant flux density of 20 ergs/cm²/sec. Oospore germination and sporangial formation occurred in the dark, but exposure to light greatly

enhanced both processes, especially in the blue (400-420 nm) and ultraviolet (350-400 nm) portions of the spectrum. Increasing periods of exposure to monochromatic light enhanced sporangial formation, but had little effect on oospore germination. When the intensity of far-red (750 nm) light was increased from 20 to 320 ergs/cm²/sec both oospore germination and sporangial formation decreased.

Phytophthora citricola Sawada causes a severe root rot of many hosts, including avocado (17), rhododendron (7), hops (16), and black walnut seedlings (4). The influence of various light conditions on the development of reproductive structures has been reported in studies of many *Phytophthora* species (2,3,5,15). These factors also may be important in the infection process. Initial studies of *P. citricola* (12) showed oospore germination to be lowest under red light, and highest under light passing through a yellow acetate filter. The filters, however, had unknown transmission characteristics. Investigations of other *Phytophthora* spp. (9,10,14) have shown that blue and ultraviolet (UV) light at low intensities enhanced oospore germination and production of sporangia.

The objectives of this study were to determine the effects of light quality, intensity, and duration on oospore germination and sporangia formation of *P. citricola*.

MATERIALS AND METHODS

A light exposure chamber 30 × 30 × 15 cm was constructed as shown in Fig. 1. CBS acrylic filters (Carolina Biological Supply, Burlington, NC 27315) were used singly as covers of the exposure chamber (13). The filters used were blue, green, red, and far-red with broad band transmission peaks at 450, 545, 650, and 750 nm, respectively. Blue, green, and red filters were used in conjunction with 10 cm of 1% CuSO₄ solution (w/v) in the aqueous filter tank to reduce infrared radiation and heat buildup in the exposure chamber (13). With the far-red filter, 10 cm of distilled water replaced the CuSO₄ solution, as suggested by Klein (11), since the chemical aqueous filters reduced the light intensity below the desired level. Therefore, the maximum transmission of the far-red filter system occurs over a range extending from 750 nm to an undetermined wavelength beyond the visible range. Actual transmission bands of the filters, shown in Fig. 2, were determined with an ISCO Model SR Spectroradiometer (Instrumentation Specialties Company, Lincoln, NE 68501).

Light sources were new 300, 150, and 75-W reflector flood type bulbs. The power source was through a variable voltage transformer to maintain a steady output at 120 V, since intensities can vary widely with slight changes in line voltage (13).

A Kahl Submarine Photometer (Kahl Scientific Instrument

Corp., El Cajon, CA 92020) was used to determine the correct heights of the light source so that a constant intensity was maintained through each filter. Temperature in the exposure chamber was maintained between 23 and 25 C by cooling the bulbs with fans.

Cultures of *P. citricola* were grown for 14 days on potato-dextrose agar (PDA) and used as inoculum for a glucose mineral salts medium as described by Honour and Tsao (8). Oospores were obtained by inoculating V-8 juice-CaCO₃ broth medium (8) with 1 ml of the 6-day-old liquid culture preparation and incubating the cultures in the dark at 25-26 C for 8 wk.

After incubation, oospores were prepared for exposure to monochromatic light by placing the contents of three bottles (80 ml each) of V-8 juice-CaCO₃ broth culture in a sterile metal blender and macerating at high speed for 2 min. The mycelium-oospore suspension was frozen in the blender at -3 C for 24-30 hr to inactivate the mycelial fragments.

The suspension of oospores was thawed and 1-ml aliquots were placed on 2% water agar (WA) in 100 × 15-mm sterile, plastic petri dishes (Falcon, Oxnard, CA 93030). Transfer of inoculum from the blender to the plates was done in the dark by using an adjustable pipette bulb that dispensed 1 ml of suspension (approximately 10⁴ oospores) to each plate. Nine plates were prepared for each light filter system tested.

Oospores of *P. citricola* were exposed to a constant radiant flux density of 20 ergs/cm²/sec for a 12-hr photoperiod for 5 days in the light column, using the filters described. After exposure, oospore germination and sporangium formation were determined for 100 oospores counted at random from each plate. Oospores were considered germinated or preparing to germinate if a germ tube was present or if conspicuous modifications of the inner oospore wall had occurred (6). The sporangia are easy to distinguish since they are a distinctive lemon-shape with papillae. One series of plates that served as controls was placed in the dark for 5 days while another series was exposed to light from a fluorescent lamp at 20 ergs/cm²/sec for 5 days on a 12-hr photoperiod. This low intensity was achieved by using an open container covered by several layers of cheesecloth and Miracloth (Chicopee Mills Inc., New York, NY 10018). Since the materials used do not act as a neutral-density filter, some undetermined spectral shift may have occurred. However, it was assumed that oospores were still exposed to a wide range of wavelengths from the lamp source representing the UV-visible spectrum. The intensity was determined with a photometer.

The blue (450 nm) and red (650 nm) filters were combined with a UV plastic film (Madico, Woburn, MA 01801) with a transmission

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cutoff below 390 nm, as determined with a Beckman ACTA III UV-Visible spectrophotometer. Oospores on WA plates were exposed in the light column under the blue and red filters combined with the UV film at the same constant radiant flux density described for the filters alone.

Oospores on WA were placed under a bank of 15-W, black light fluorescent lamps having maximum energy transmission at 366 nm and a spectral emission range of 310–400 nm. The plates were placed 16 cm from the light source to obtain an intensity of 20 ergs/cm²/sec. Oospore germination and sporangia production were recorded after 5 days at the 12-hr photoperiod.

To test the effects of varying light exposure times on germination, oospores of *P. citricola* on WA were exposed for 5 sec, 1 min, 6, 9, 12, 36, 48, and 60 hr at an intensity of 160 ergs/cm²/sec under fluorescent lights and then placed in darkness for the remainder of 5 days. After incubation, germination and sporangia formation were determined for 900 oospores counted at random for each test.

The effect of light intensity on oospore germination and sporangia formation also was studied. The far-red (750 nm) filter system was used to expose oospores for 5 days, on a 12-hr photoperiod, at intensities of 20, 80, 160, and 320 ergs/cm²/sec.

RESULTS

The effects of different monochromatic light sources at a constant intensity on oospore germination and sporangia

formation by *P. citricola* are summarized in Table 1. Oospore germination was greatest under blue, far-red, fluorescent, and UV light and under the blue plus UV filter combination, with values of 91.7, 91.1, 88.1, 94.4, and 88.8%, respectively. These values are not significantly different from each other. Oospore germination was lowest (49.6%) when exposed to red radiation and was significantly less than under all other filter systems, including the dark.

The production of sporangia by germinating oospores was greatest under blue and UV light sources (41.8 and 43.3%, respectively) and these values are not significantly different from each other. Some oospores that germinated in the dark produced sporangia and the percent was comparable to that in the red and red/UV filter systems (14.5, 13.3, and 16.8%, respectively). The values for the red, red/UV, and dark tests are not significantly different, but are much less than those for all other light sources. Numbers of sporangia produced under the green and far-red filters were similar, but were less than those for the blue and UV sources and greater than those of the red and dark tests.

Table 2 presents the percent oospore germination and sporangia formation when oospores were exposed to fluorescent light at an intensity of 160 ergs/cm²/sec for varying lengths of time. There was little difference in oospore germination over the various exposure periods. Germination was lowest at 5-sec exposure (82.6%), while the greatest germination occurred when oospores were exposed for 9, 36, 48, or 60 hr. By contrast, sporangia production increased significantly with each increment of exposure time (except between

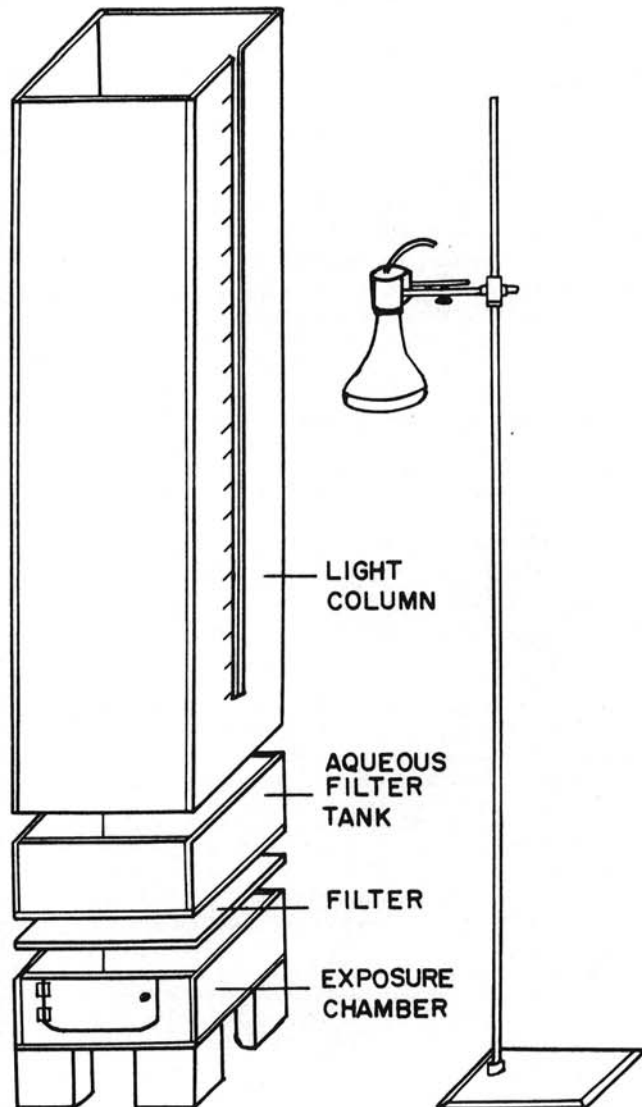


Fig. 1. Light chamber used to expose oospores of *Phytophthora citricola* to monochromatic light.

TABLE 1. Germination of oospores of *Phytophthora citricola* and sporangial production responses to monochromatic light^a

Illumination filter or source	Peak (nm)	Oospore germination ^v (%)	Sporangia formation ^v (%)
CBS ^w filters			
Blue ^x	450	91.7 ab ^z	41.8 a
Green ^x	545	82.1 bc	31.8 b
Red ^x	650	49.6 e	13.3 d
Far-red ^x	750+	91.1 ab	28.7 bc
Blue + UV ^y	450	88.8 ab	38.4 a
Red + UV ^y	650	76.0 cd	16.8 d
Fluorescent		88.1 ab	24.6 c
Ultraviolet (UV)	310–400	94.4 a	43.3 a
Dark		70.6 d	14.5 d

^a Total energy delivered, 20 ergs/cm²/sec.

^v From a random sample of 900 oospores per test.

^w CBS (Carolina Biological Supply, Burlington, NC 27315).

^x Plus 10 cm (light path) in 1% CuSO₄ (w/v).

^y Plus 10 cm (light path) in distilled water.

^z Values followed by different letters are significantly different according to Duncan's multiple range test, *P* = 0.05.

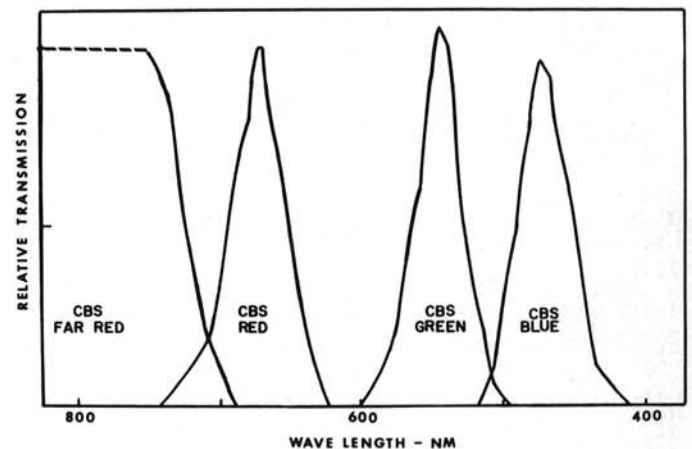


Fig. 2. Transmission curves of CBS (Carolina Biological Supply, Burlington, NC 27315) acrylic filters exposed to a single light source at an intensity of 200 ± 10 ergs/cm²/sec.

6–9 hr and 48–60 hr) and maximum sporangia production (43.4%) occurred under continuous exposure.

Oospores exposed to different intensities of far-red light responded with a decrease in both germination and sporangia production as the intensity increased (Table 3). Oospore germination decreased significantly when the intensity was increased from 20 to 80 ergs/cm²/sec, but there was no further decrease in oospore germination with increases from 80 to 320 ergs/cm²/sec. Sporangia production decreased significantly when the intensity was increased from 20 to 80 ergs/cm²/sec and from 160 to 320 ergs/cm²/sec. Thus, the data demonstrate that both oospore germination and sporangia production were reduced as the intensity of far-red light was increased.

DISCUSSION

Exposure of oospores of *P. citricola* to monochromatic bands of light enhanced both oospore germination and sporangial production, especially in the shorter wavelengths of the spectrum. Other investigators (1,3,10,14,15) of *Phytophthora* spp. have drawn similar conclusions.

Germination of oospores of *P. citricola* was increased by light, but light was not required. By contrast, *P. cactorum*, a closely related species, required light for germination (2,5). Also, germination percentages of oospores in this study were much higher than previously reported for *P. citricola* (12). This may be due to the use of more mature oospores (older than 7 wk) instead of younger, developing oospores. Counting only oospores with a defined germ tube could also account for the lower germination rates in the previous study.

Harnish (5) showed that oospores of *P. cactorum* and *P. capsici* were produced in greater numbers under red light and in the dark compared to white or blue light at 538–646 lux (50–60 ft-c). Sporangial production was stimulated by white and blue light while red light and dark were unfavorable. Although foot-candles and ergs/cm²/sec cannot, by definition, be directly compared, these

results support our findings that sporangial formation is enhanced by blue light. Also, the fact that oospore formation is favored by dark may also explain why oospore germination is low in the dark and is stimulated by light, especially the conditions which also favor sporangia production.

Ribiero (15) studied oospore and sporangia formation by *P. capsici*, *P. cinnamomi*, *P. palmivora*, and *P. megasperma* var. *sojae* with a filtered light system similar to the one used in this study. A diurnal cycle was used (12-hr photoperiod) at 8 μW/cm² (1 μW/cm² = 10 ergs/cm²/sec). Again, it was determined that near-UV and blue light stimulated sporangia production but, contrary to our findings, sporangia production increased in some species when the light intensity was increased from 8 μW/cm² to 100 μW/cm². However, at 100 μW/cm², oospore production in these species was significantly less than in the dark. This suggests that there is some intolerance to high light intensities and levels of sensitivity may differ between species. The processes of oospore germination and sporangia formation of *P. citricola* are favored under relatively lower intensities of light (below 320 ergs/cm²/sec). Ribiero (15) also showed that light quality was not a factor for oospore germination when the light was present during gametogenesis. However, when oospores are produced in the dark, light quality becomes important for germination.

The high rates of oospore germination observed in some light treatments are not necessarily correlated to high rates of sporangia production (Table 1). This suggests that light effects on sporulation are qualitative and germinating oospores do not necessarily produce sporangia. Experiments with increasing periods of light exposure (Table 2) indicated that oospores did not require long periods of light to maintain a high rate of germination. Conversely, sporangia formation increased as exposure periods increased until light was continuous. These results show that light has a quantitative effect on sporangia production but not on oospore germination, since the results were similar regardless of exposure period.

Both oospore germination and sporangia production were reduced when far-red light intensities were increased beyond 20 ergs/cm²/sec. Further investigation is necessary with *P. citricola* to determine whether the trends of decreased sporangia production occur with other light sources at different intensities.

TABLE 2. Germination of *Phytophthora citricola* oospores and sporangial production responses to various times of exposure to white light^w

Exposure time ^x	Oospore germination ^y (%)	Sporangia formation ^y (%)
5 sec	82.6 a ^z	3.3 a
1 min	86.3 b	6.4 ab
6 hr	89.3 bc	8.9 b
9 hr	90.6 c	10.9 b
12 hr	88.8 bc	18.5 c
24 hr	89.0 bc	31.2 d
36 hr	91.3 c	35.4 de
48 hr	90.8 c	37.2 e
60 hr	92.1 c	35.2 de
Continuous	86.0 b	43.4 f

^wTotal energy delivered, 160 ergs/cm²/sec.

^xAfter exposure, oospores were incubated for 120 hr (5 days) including the exposure period.

^yFrom a random sample of 900 oospores per test.

^zValues followed by different letters are significantly different according to Duncan's multiple range test, *P* = 0.05.

TABLE 3. Oospore germination and sporangial production of *Phytophthora citricola* exposed to different intensities of far-red (750 nm) light

Intensity (ergs/cm ² /sec)	Oospore germination ^y (%)	Sporangia formation ^y (%)
20	91.1 a ^z	28.7 a
80	73.0 b	19.3 b
160	71.1 b	20.2 b
320	71.2 b	4.7 c

^yFrom a random sample of 900 oospores per test.

^zValues followed by different letters are significantly different according to Duncan's multiple range test, *P* = 0.05.

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