

## The Effect of Visible and Near-Visible Radiation on Sporangium Production by *Phytophthora cinnamomi*

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### ABSTRACT

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The effect of light quality and intensity on the production of *Phytophthora cinnamomi* sporangia was studied using cultures that were grown in a chemically-defined liquid medium. Cultures were irradiated at intensities ranging from  $3 \mu\text{W cm}^{-2}$  to  $200 \mu\text{W cm}^{-2}$ , and at wavelengths between 312 and 1,350 nm. Sporangia were produced at all wavelengths and intensities tested, and in darkness. Sporangium

production was stimulated in the near-UV by intensities up to  $100 \mu\text{W cm}^{-2}$ . At  $200 \mu\text{W cm}^{-2}$  there was no significant difference in sporangium production in wavelengths ranging from the near-UV to infrared (312 nm-1,350 nm). These results indicate that sporangium production by *P. cinnamomi* is light-variable, but not light-dependent.

*Additional key words:* soil extract, pea broth, sporangium morphology, light.

Production of sporangia by *Phytophthora cinnamomi* Rands has been the subject of many experiments, numerous publications, and considerable controversy. Two principal methods have been employed: use of a nonsterile soil extract (2, 3, 6, 11, 13, 14, 21, 22) and the axenic method developed by Chen and Zentmyer (7).

Varied effects of light on the production of sporangia by several other *Phytophthora* spp. have been reported (1, 4, 8, 9, 10, 18, 19), but the effect of light on sporangium production by *P. cinnamomi* has not been clarified. Zentmyer and Marshall (22) reported that sporangia were produced in continuous artificial light, in continuous darkness, and in alternating light and dark periods. Sporangium production was less in continuous light than in the other two systems; these tests were in nonsterile soil extract, using disks cut from PDA or V-8 agar cultures. Manning and Crossan (11), using a nonsterilized hemp seed decoction, found that sporangia were produced by *P. cinnamomi* only when cultures were exposed to continuous light or alternating light and darkness; intensity and wavelength were not indicated. Schoulties and Baker (20) recently reported that "uniform light conditions" are important for reducing variability in sporangium production by *P. cinnamomi*.

We examined sporangium production by *P. cinnamomi* as part of a current project on the effect of quality and quantity of light on spore production and germination in the genus *Phytophthora*. This paper summarizes these results.

### MATERIALS AND METHODS

The following 15-W fluorescent sources were used: Phillips and Sylvania BLB lamps (spectral emissions

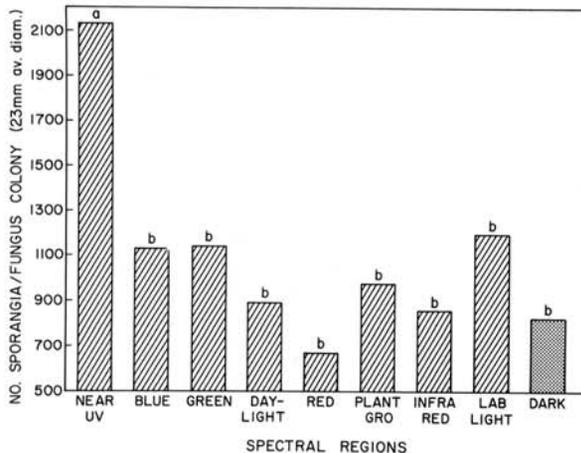
between 312-440 nm, peak intensities at 405 nm, 370 nm, and a broad peak between 356-344 nm) suspended above single-strength window-glass tanks that contained 5% (w/v)  $\text{CuSO}_4$  solution (6 cm deep) to remove the mercury line band at 1,025 nm in the infrared; Westinghouse blue (315-680 nm, peak intensities at 437 nm and 462 nm); Sylvania green (380-650 nm, peak intensity at 537 nm); Sylvania red (600-800 nm, peak intensity at 662 nm); G. E. Daylight (315 nm, 366 nm, 450 nm, 550 nm, and 625 nm); Westinghouse Plant-Gro (368-800 nm, peak intensity at 662 nm, with lesser peaks at 450 nm and 500 nm). The infrared source consisted of an Oscar 25-W aquarium lamp (700-1,500 nm, peak intensity at 950 nm). Emission of near-ultraviolet (UV) from this lamp was eliminated by placing a sheet of Dupont Mylar W between the lamp and the cultures. The apparatus used to irradiate cultures at a very low light intensity ( $3 \mu\text{W cm}^{-2}$ ), in the blue, red, and far-red regions of the spectrum was described previously (16).

At higher intensities ( $200 \mu\text{W cm}^{-2}$ ), single-strength window-glass tanks that contained distilled water (6 cm deep) were suspended under the blue, green, red, Daylight, and Plant-Gro lamps to dissipate the heat and maintain the temperature at  $26 \pm 1$  C. The near-UV lamps had tanks that contained  $\text{CuSO}_4$  solutions as described above. The heat from the infrared source was dissipated by employing a clear plastic sheet (4-mm thick), combined with a sheet of Dupont Mirror Mylar® (E. I. DuPont de Nemours & Co., Wilmington, Delaware), suspended 22 cm below the lamp. There was no interference with the transmittance of infrared wavelengths (800-1,350 nm), through either the plastic or the mylar sheet. Radiation intensities and spectral distribution of all lamps used in this study were measured with an ISCO Model SR spectroradiometer using a remote probe attachment, calibrated against a 2,800° K spectral standard lamp. Measurements were made at 10-

25 nm (380-1,550 nm) wavelength intervals, by placing the remote probe directly under the lid of the petri dish. Since the spectroradiometer does not measure wavelengths shorter than 380 nm, a McPherson spectrophotometer was used in conjunction with a Hamamatsu type R106 photomultiplier to measure radiation in the UV and near-UV regions of the spectrum. Dark controls were placed in a blackened three-ply wooden box covered with two layers of heavy-duty aluminum foil.

The intensities chosen throughout this study are within the range of those recorded for the visible and near-visible spectrum in an avocado grove in San Diego County, California, over a period of 1 year.

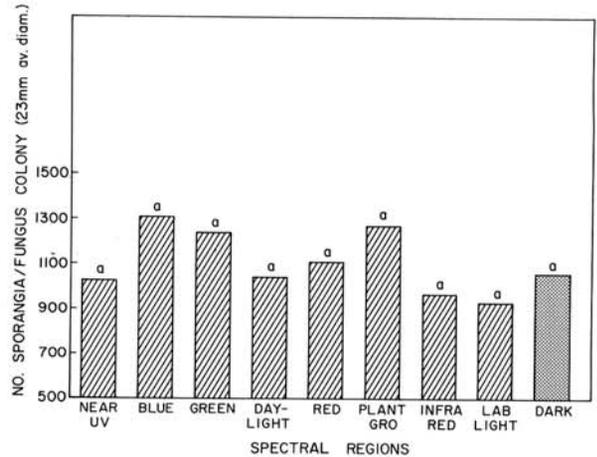
For sporangium production, cultures of *P. cinnamomi* (isolates Pc97 A<sup>1</sup> and Pc40 A<sup>2</sup>, University of California, Riverside, stock culture designations), were grown for 5 days on a synthetic agar medium (15). Preliminary tests indicated no interaction between this medium and the light sources employed. Disks 5 mm in diameter were transferred from the edge of the resulting colonies to 60-mm diameter plastic petri dishes that contained 6 ml of liquid synthetic medium. All dishes were incubated in darkness at  $25 \pm 1$  C until colony diameters were uniformly between 22-25 mm in diameter (48-60 hours). The medium was then decanted from each dish and replaced with 10 ml of salt solution (7). Four washings at one wash per hour were carried out, using 10 ml of salt solution per petri dish for each wash. After the final wash, 6 ml of salt solution (7) was dispensed into each dish and the cultures were placed (in quadruplicate) for 48 hours at  $26 \pm 1$  C under the different light sources described above. A continuous light cycle was employed. One replicate from each light condition was then removed and placed at 9 C for 20 minutes to check zoospore differentiation and release from sporangia, and the other replicates immediately were fixed in a solution of ethanol (50%),



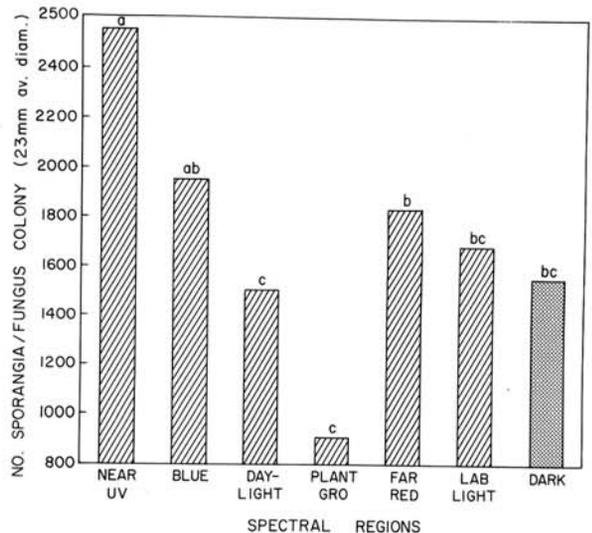
**Fig. 1.** Influence of light quality and intensity on sporangium production by *Phytophthora cinnamomi*: a number of sporangia produced in colonies initially grown in a chemically-defined medium and irradiated at equal intensities of  $100 \mu\text{W cm}^{-2}$  under specific regions of the spectrum. See text for details of spectral emission of each specific spectral region. Lab light (G. E. Daylight  $2 \times 40$ -W at  $1,200 \mu\text{W cm}^{-2}$ ), was included for comparison. Bars having the same letter do not differ significantly from each other ( $P = 0.05$ ).

acetic acid, and formalin (10:1:1). Numbers of sporangia per milliliter were counted using an eelworm counting slide (Gelman Hawksley Ltd., Sussex, England).

In some experiments, after the fungal colonies were washed with salt solution, 6 ml of a nonsterile soil extract was added to each petri dish before they were placed



**Fig. 2.** Influence of light quality and intensity on sporangium production by *Phytophthora cinnamomi*: number of sporangia produced in colonies initially grown in a chemically-defined medium and irradiated at equal intensities of  $200 \mu\text{W cm}^{-2}$  under specific regions of the spectrum. Lab light (G. E. Daylight  $2 \times 40$ -W at  $1,200 \mu\text{W cm}^{-2}$ ), was included for comparison. No significant differences were found among spectral regions ( $P = 0.05$ ).



**Fig. 3.** Influence of light quality and intensity on sporangium production by *Phytophthora cinnamomi*: number of sporangia produced in colonies initially grown in a chemically-defined medium and irradiated at  $45 \mu\text{W cm}^{-2}$  in the near-ultraviolet;  $3 \mu\text{W cm}^{-2}$  in the blue and far-red;  $20 \mu\text{W cm}^{-2}$  in the Daylight region;  $42 \mu\text{W cm}^{-2}$  in the Plant-Gro region; and  $1,200 \mu\text{W cm}^{-2}$  in laboratory light (G. E. Daylight  $2 \times 40$ -W). Bars having the same letters are not significantly different from each other ( $P = 0.05$ ).

under the different light sources. In other experiments, the fungus was grown in 6 ml of either a dilute pea broth or V-8 juice broth (7), before being washed with the salt solution (7).

### RESULTS

Sporangium production by *P. cinnamomi* irradiated with light intensities varying from  $3 \mu\text{W cm}^{-2}$  to  $200 \mu\text{W cm}^{-2}$  over a wide range of wavelengths from 312 nm to 1,350 nm was light-variable, but not light-dependent; significant numbers of sporangia also were produced in darkness (Fig. 1, 2, and 3). Most of the irradiation experiments were conducted at  $100 \mu\text{W cm}^{-2}$  (10 separate experiments). Data for this series are presented in Fig. 1. Production of sporangia in colonies exposed to near-UV at  $100 \mu\text{W cm}^{-2}$  were significantly greater than at lower wavelengths or in darkness. Sporangium production also was stimulated in the near-UV and blue regions of the spectrum at low light intensities ( $3 \mu\text{W cm}^{-2}$ ), but sporangia also were produced in significant numbers in

all other wavelengths and in darkness (Fig. 3). At the high intensity ( $200 \mu\text{W cm}^{-2}$ ), there were no significant differences in the production of sporangia at the different wavelengths ( $P = 0.05$ ). Production was remarkably uniform in all treatments (Fig. 2).

Since the above data were obtained with cultures initially grown in dark incubators, it became necessary to ascertain if there might be a light effect on young actively growing mycelium which could in turn affect sporulation. Accordingly, after inoculation with 5-mm diameter mycelial disks, petri dishes containing synthetic medium immediately were placed under the different light sources at  $3 \mu\text{W cm}^{-2}$  and  $200 \mu\text{W cm}^{-2}$  for 48 hours. The colonies then were washed with salt solution as described above, and placed in darkness for an additional 48 hours before sporangia were counted. The results obtained were similar to those obtained with dark-grown cultures, again indicating that light is not an essential factor for sporangium production of *P. cinnamomi*.

Tests also were made with a nonsterile soil extract, pea broth, and V-8 juice broth, as compared to the chemical-

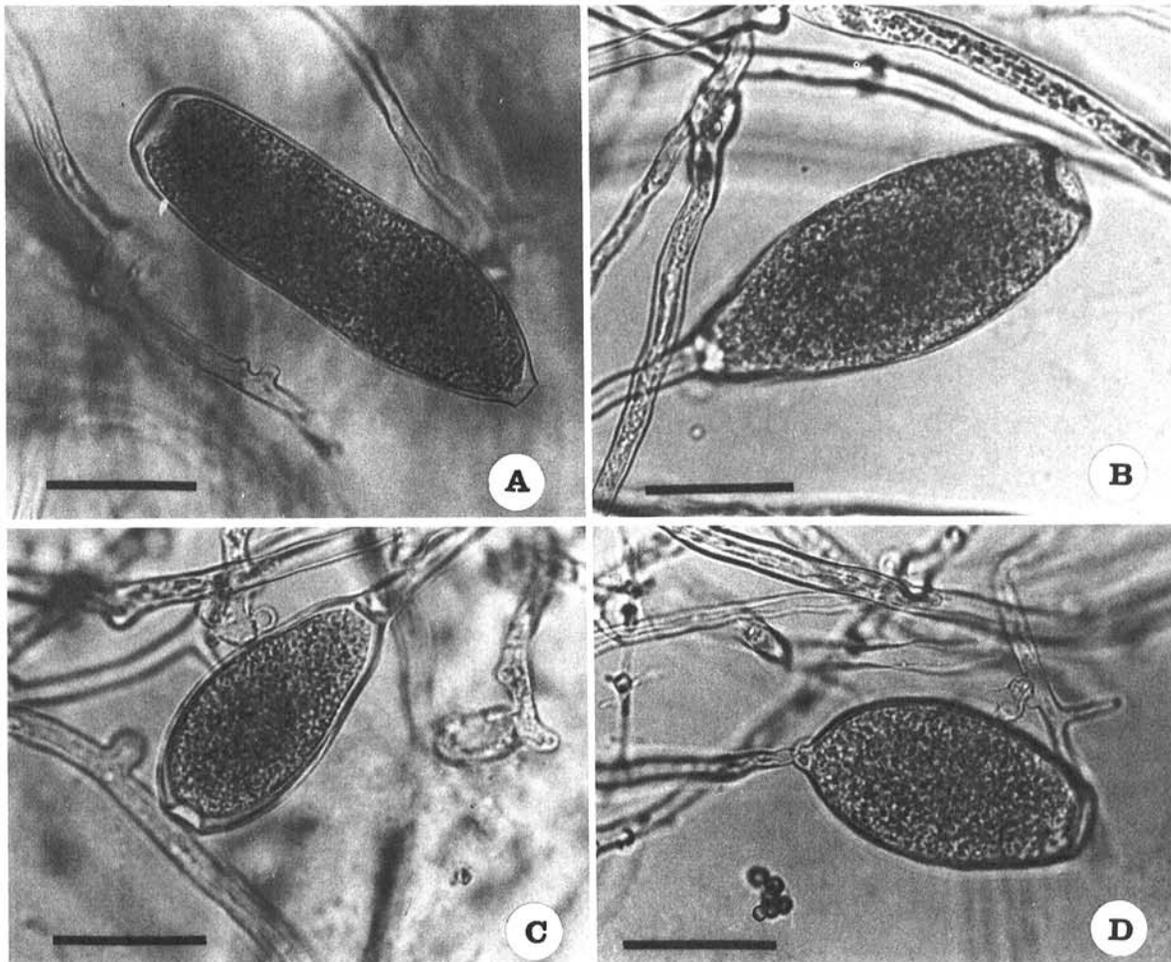


Fig. 4-(A to D). Morphology of *Phytophthora cinnamomi* sporangia produced under near-ultraviolet (UV) radiation and darkness; A, B, C) typical sporangia produced in the near-UV; D) typical sporangium produced in darkness. Bar = 50  $\mu\text{M}$ .

ly-defined medium. In these cultures also production of sporangia was independent of light, since significant numbers of sporangia were produced in darkness. Investigations with an A<sup>1</sup> isolate from camellia (Pc97), with varying light intensities, indicated that the response to visible and near-visible radiation was similar to that of the A<sup>2</sup> isolate (Pc40), from avocado.

Tests of zoospore differentiation and release from sporangia produced at varying intensities in the visible and near-visible spectrum showed no observable differences, except at 200  $\mu\text{W cm}^{-2}$  in the near-UV; some elongated sporangia in this shorter wavelength differentiated, but did not release zoospores.

#### DISCUSSION

Results with tests conducted in the visible and near-visible spectrum show that sporangium production by *P. cinnamomi* is independent of light; sporangia can be produced in any wavelength or in darkness. These findings are in agreement with earlier data reported by Zentmyer and Marshall (22), in which they observed that sporangia were produced in both light and dark conditions. The only significant differences in production were stimulation in the near-UV up to 100  $\mu\text{W cm}^{-2}$  and in the blue region of the spectrum at very low light intensity (3  $\mu\text{W cm}^{-2}$ ). *Phytophthora cinnamomi* thus differs from several other species of *Phytophthora* that exhibit a dependence on light for sporulation. Our tests with *P. capsici*, for example, show that this species forms sporangia in the near-UV-blue regions of the spectrum, and none in darkness (17). Some isolates of *P. palmivora* formed very few sporangia in darkness and were greatly stimulated by radiation in the near-UV and blue regions of the spectrum at intensities similar to those used for the *P. cinnamomi* tests (17). Many other fungi (5, 12), rarely produce spores in darkness, but respond to irradiation at specific wavelengths. *Phytophthora cinnamomi* does not appear to have any specific photoreceptor for asexual reproduction, as has been indicated with several other fungi (5).

Light measurements were taken with an ISCO Model SR spectroradiometer at the soil surface in an avocado grove. The total intensities between various bands in the visible spectrum (380-750 nm), varied between 12-500  $\mu\text{W cm}^{-2}$ , whereas in the near-infrared (800-1,500 nm), the intensity of radiation varied between 480-6,200  $\mu\text{W cm}^{-2}$ . These data indicate that the amount of radiation in the visible and near-visible spectrum under field conditions is not incompatible with that under which *P. cinnamomi* can produce sporangia. Inasmuch as *P. cinnamomi* is a soilborne pathogen that invades small roots primarily, it would seem that its exposure to light at any depth in the soil would be minimal under ordinary conditions. *Phytophthora cinnamomi* thus appears to be well adapted to sporangium production at any level of light or in the absence of light. Aerial pathogens would be expected to be more light-dependent than soilborne pathogens in relation to spore production.

The change in sporangium morphology noted under near-UV radiation (Fig. 4), confirms other observations (4, 10), with *P. capsici*, *P. heveae*, and *P. palmivora*. The use of sporangia length-breadth (l:b) ratios often used as a taxonomic criterion in identifying *Phytophthora* spp.

appears to have little validity under these circumstances.

Nutritional differences in culture media (e.g., whether one-fifth, one-half, or full-strength), and particularly the age of the fungus colony when tested for sporulation, may be responsible for some of the differences between our results and those of others (11, 20), who found uniform light conditions to be important for production of sporangia by *P. cinnamomi*. Also, the other research did not involve a complete range of wavelengths and intensities, so it is difficult to compare the results directly. The chemically defined medium used in this study for growth and sporangia production of *P. cinnamomi* has obvious advantages over previously described natural media for physiological studies involving in vitro sporangium production by that fungus species.

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