Comparative Effects of Monochromatic Radiation on the Germination of Oospores of Three Phytophthora spp.

O. K. Ribeiro, G. A. Zentmyer, and D. C. Erwin

Postgraduate Research Associate and Professors of Plant Pathology, respectively, Department of Plant Pathology, University of California, Riverside, California 92502.

Research supported in part by NSF Grant GB 29283.

Accepted for publication 20 March 1975.

ABSTRACT

A simple light apparatus was used to determine the effects of the regime and quality of light on oospore germination of *P. cinnamomi, P. megasperma* var. *sojae* (races 1 and 2), and *P. capsici*. A combination of light filters and aqueous salt solutions was used to obtain wavelengths with peaks at 450 nm, 545 nm, 650 nm, and 750 nm. Four light regimes were tested at a light intensity of 2.0 μW cm^{-2}, and one light regime at 0.1 μW cm^{-2}. Oospore germination was stimulated under the blue and far-red filters, regardless of the light regime. This system may offer a method for obtaining consistent germination of oospores in genetical studies of these species.

Phytopathology 65:904-907

Additional key words: photoresponse, reflector flood lamps.

The effects of light on several fungal species have been well documented (4, 12). A number of studies with the genus *Phytophthora* indicate that light is necessary in several phases of morphogenesis (9, 11). Development and germination of sexual structures (oospores) of *Phytophthora* is reported to be enhanced by irradiation in the blue region of the spectrum (400-480 nm), and in the far-red (700-1,000 nm) (2, 8), while irradiation of *Phytophthora* cultures with white light inhibited oospore formation (6, 8). Since erratic oospore germination appears to be a valid criticism of much of the genetical studies undertaken (5, 15, 16, 17, 18), we investigated the efficiency of four monochromatic wavelengths for consistent oospore germination in *Phytophthora megasperma* var. *sojae* (races 1 and 2), *P. cinnamomi* and *P. capsici*.

MATERIALS AND METHODS.—The apparatus employed to develop monochromatic sources has been previously described (14). Fungus cultures were placed in a 42×40×16 cm wooden box, the top of which had a 27×27 cm window, over which the primary filters (Carolina Biological Supply Co.) were placed. The red filter was modified by taping two primary red filters together, to give monochromatic radiation with a peak at 650 nm. The far-red filter was comprised of two primary far-red filters, a neutral-density filter, and a cinemoid component (from a blue filter). It transmitted monochromatic radiation

with a peak at 750 nm. The blue and green filters were not modified and transmitted radiation with peaks at 450 nm and 545 nm, respectively. Radiant energy was supplied by 75-W reflector flood lamps (General Electric), for the 545-nm, 650-nm, and 750-nm wavelengths. A 150-W reflector flood lamp was used for the 450-nm wavelength. The lamps were suspended directly above each filter system and the heights adjusted to give the desired intensities.

In addition to the primary filters, aqueous filters were used to screen out extraneous wavelengths and dissipate the heat from the reflector flood lamps. These aqueous filters consisted of 115 g acidified $CuSO_4 \cdot 5H_2O$ in 11.5 liters of distilled water for the 450-nm and 545-nm wavelengths, 126.5 g acidified $CuSO_4 \cdot 5H_2O$ in 11.5 liters distilled water for the 650-nm wavelength, and 2.0 kg $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ together with 220 ml concentrated H_2SO_4 in 11.5 liters for the 750-nm wavelength.

Each radiant energy source was connected to an automatic time switch. The lamp heights were adjusted to give an intensity of 2 μW cm^{-2} on the surface of the culture under each monochromatic filter. This level of radiation is the average that might be expected under dense vegetation over the range 400-750 nm (13). Light intensity measurements were made with a Kettering radiant power meter, Model 68, and an ISCO Spectroradiometer Model SR.

The experimental light regimes employed in this study were as follows: (i) duplicate *Phytophthora* cultures were grown in dark boxes for 20 days before exposure to irradiation for 12 hours (hr), followed by 12 hr darkness. This alternate 12-hr light/12-hr dark cycle was continued for 10 days. (ii) Cultures were grown in the dark for 20 days, followed by continuous irradiation for 10 days. (iii) Cultures were grown in the dark for 10 days followed by exposure to continuous irradiation for 10 days and then returned to dark conditions for an additional 10 days. (iv) Cultures were grown in the dark for 4 days before exposure to continuous irradiation for 10 days followed by 4 days in the dark. (v) Cultures were exposed immediately following inoculation to continuous irradiation for 10 hr, followed by 14 hr of darkness. This alternate light/dark cycle was continued for 30 days. (vi) Cultures incubated in the dark for 30 days were used as controls.

Inoculum consisted of mycelial plugs (4 mm) of each *Phytophthora* species, growing on V-8 juice agar (18). A mycelial plug of each mating type was placed in 7 ml of V-8 juice broth in 60-mm diameter plastic petri dishes, and incubated in dark boxes at 24 ± 1 C before exposure to radiation for different intervals of time. All experiments were terminated by 30 days. Experiments were repeated at least once.

The mycelial mats in each instance were comminuted in a Waring Blendor for a total of 2 minutes at 30-second intervals and filtered through a sterile nylon mesh (pore size, 53 μm). The filtrate was centrifuged at maximum speed in a clinical centrifuge for 1 minute and the supernatant discarded. Centrifugations were repeated as necessary to remove all mycelial fragments. Oospores were then spread on water agar in 90-mm diameter petri dishes, and individual oospores were transferred with a fine needle to petri dishes containing water agar (25 oospores/dish marked with a grid). A minimum of 200 single oospores were collected from each treatment and monochromatic light source. Petri dishes containing individual oospores were stored in darkness at 24 ± 1 C. Oospore germination was observed microscopically and recorded up to the tenth day.

RESULTS.—All three species of *Phytophthora* tested responded to monochromatic radiation. A significant increase in oospore germination occurred with radiation at 450 nm and 750 nm (Tables 1, 2, 3). There was a difference in response to these two wavelengths, depending on the *Phytophthora* species used. *P. megasperma* var. *sojae* (race 1) (P174), and *P. megasperma* var. *sojae* (race 2) (P406), exhibited a

TABLE 1. Effect of monochromatic radiation on germination of oospores of *Phytophthora megasperma* var. *sojae*, (race 1) (P174)

Light regime[a]	Monochromatic wavelengths				
	Blue (450 nm)	Green (545 nm)	Red (650 nm)	Far-red (750 nm)	Dark control
1. 20 days dk followed by 12 hr lt/12 hr dk for 10 days	35 ab xy	11 c y	18 bc xy	41 ab xy	20 bc yz
2. 20 days dk followed by 10 days lt	16 bc z	12 c y	24 bc xy	43 a xy	8 c z
3. 10 days dk/10 days lt/10 days dk	39 a xy	22 bc x	18 c xy	29 b y	13 c yz
4. 4 days dk/10 days lt/4 days dk	28 ab yz	14 c y	16 c yz	27 ab y	30 ab x
5. 10 hr lt/14 hr dk for 30 days	39 a xy	10 c y	9 c z	23 b y	19 b yz

[a]Light regimes 1-4 conducted at an intensity of 2 μW cm^{-2}. Regime 5 received light at 0.1 μW cm^{-2}. Values in the table represent % germination based on 200-300 individually picked oospores from two experiments with duplicate plates under each wavelength. abc, statistical comparison of percentage germination at the various wavelengths, and xyz indicates comparison of percentage germination under various light regimes. Values followed by the same letter are not significantly different from each other ($P = 0.05$). dk = dark; lt = light.

TABLE 2. Effect of monochromatic radiation on germination of oospores of *Phytophthora megasperma* var. *sojae*, (race 2) (P406)

Light regime[a]	Monochromatic wavelengths				
	Blue (450 nm)	Green (545 nm)	Red (650 nm)	Far-red (750 nm)	Dark control
1. 20 days dk followed by 12 hr lt/12 hr dk for 10 days	13 bc z	7 c x	8 bc x	27 a x	12 bc x
2. 20 days dk followed by 10 days lt	8 bc z	5 bc x	5 bc x	22 a x	10 bc x
3. 10 days dk/10 days lt/10 days dk	19 abc yz	5 d x	5 d x	22 ab x	10 cd x
4. 4 days dk/10 days lt/4 days dk	35 a x	5 c x	5 c x	21 bc x	15 bc x
5. 10 hr lt/14 hr dk for 30 days	21 a yz	5 b x	5 b x	5 b y	5 b y

[a]Light regimes 1-4 conducted at an intensity of 2 μW cm^{-2}. Regime 5 received light at 0.1 μW cm^{-2}. Values in table represent % germination based on 200-300 individually picked oospores from two experiments with duplicate plates under each wavelength. abc, statistical comparison of percentage germination at the various wavelengths and xyz, indicates comparison of percentage germination under various light regimes. Values followed by the same letter are not significantly different from each other ($P=0.05$). dk = dark; lt = light.

TABLE 3. Effect of monochromatic radiation on germination of oospores of *Phytophthora cinnamomi* (P97 × P40)

Light regime[a]	Monochromatic wavelengths				
	Blue (450 nm)	Green (545 nm)	Red (650 nm)	Far-red (750 nm)	Dark control
1. 20 days dk followed by 12 hr lt/12 hr dk for 10 days	19 a yz	6 bc x	1 c y	9 b y	9 b yz
2. 20 days dk followed by 10 days lt	2 b z	3 b x	4 b y	11 a xy	2 b z
3. 10 days dk/10 days lt/10 days dk	23 a yz	5 b x	1 c y	6 b y	6 b z
4. 4 days dk/10 days lt/4 days dk	45 a x	3 c x	10 b x	7 b y	12 b xy
5. 10 hr lt/14 hr dk for 30 days	9 b z	5 b x	4 b x	15 ab xy	16 ab xy

[a]Light regimes 1-4 conducted at an intensity of 2 μW cm^{-2}. Regime 5 received light at 0.1 μW cm^{-2}. Values in table represent % germination based on 200-300 individually picked oospores from two experiments with duplicate plates under each wavelength. abc, statistical comparison of percentage germination at the various wavelengths and xyz, indicates comparison of percentage germination under various light regimes. Values followed by the same letter are not significantly different from each other ($P=0.05$). dk = dark; lt = light.

response to both the blue (450 nm), and the far-red (750 nm) wavelengths, while *P. cinnamomi* (P97 × P40) was stimulated more by the blue than the far-red wavelength (Tables 1, 2, and 3). This stimulation to either/or both blue and far-red radiation was obtained regardless of the light regime employed. Highest percentage germination of oospores was obtained in light regime 2 in the far-red region for P174 while for P406 and P97 × P40, the highest percentage germination was obtained in light regime 4 in the blue region of the spectrum (Tables 2 and 3). With *P. capsici* (P504 × P505s), oospore germination was greatest with regime 3 for both the 450-nm and the 750-nm wavelengths. However, the percentage germination did not exceed 10%.

The effect of laboratory light (cool white fluorescent lamps), on oospore germination was anomalous. When compared to monochromatic radiation, laboratory light had no positive effect on oospore germination of *P. megasperma* var. *sojae* (race 2), at $P = 0.05$. With *P. cinnamomi, P. capsici,* and *P. megasperma* var. *sojae* (race 1), the data were not consistent in several repeats when compared to monochromatic radiation in the blue and far-red regions of the spectrum.

DISCUSSION AND CONCLUSIONS.—The

increase in oospore germination under differing light intensities and regimes employed by several investigators (2, 8, 10), can be explained by our data which would suggest that there is a response to irradiation by the blue and far-red regions of the spectrum regardless of the light regime employed. Our data also indicate that this response is obtained even with irradiation at low light intensities. The considerable variation in percentage germination of oospores (Tables 1, 2 and 3), of dark-grown cultures is attributed to the uneven exposure of the cultures to light during the separation of the oospores from mycelial mats. This inconsistency in percentage oospore germination is to a large extent overcome by previous exposure of the cultures during gametangial formation to monochromatic radiation in the blue or far-red regions of the spectrum (Tables 1, 2, and 3). The variation in percentage germination of oospores between the species tested is of some interest. It may be that *P. capsici* (a stem and foliar pathogen) requires a higher level of radiation than *P. megasperma* var. *sojae,* or *P. cinnamomi* (both root pathogens), to elicit a light response. We are presently investigating several *Phytophthora* species to ascertain if there are evolutionary or ecological significances to these phenomena.

Little is known about the action of the photoresponse in *Phytophthora.* Photoreceptor pigments have been proposed for several fungi (4, 7, 8, 12, 19), but little direct evidence to support most of these hypotheses is available, and the nature of the photoreceptor pigments remains largely unknown. With *Phytophthora,* an insight into the mode of action of the photoresponse may be possible by the use of compounds such as cyclic AMP, acetylcholine, or indoleacetic acid which interrupt or enhance cellular events, as suggested by Bergman (3) for *Phycomyces.* It should also be noted that certain flavins and dimers of the semi-quinone forms of flavin have been reported to absorb in the 450-nm and 700- to 1,000-nm regions (1, 20).

The advantage of the monochromatic light system we have described is in its convenience and ease of construction. The entire apparatus can also be fitted into standard incubators if precise temperature control is desired. Our results indicate that, depending on the *Phytophthora* spp. used, oospore germination requires irradiation in either the 450-nm or 750-nm regions of the spectrum during the maturation process. Germination is possible with these two monochromatic sources, regardless of the irradiation regime employed, although some regimes increased the percentage germination of oospores (Tables 1, 2, and 3). The apparatus thus appears to offer a practical method for physiological and genetic studies requiring consistent germination of oospores of *Phytophthora* spp.

LITERATURE CITED

1. BEINERT, H. 1960. Flavin coenzymes. Pages 340-416 *in* P. D. Boyer, H. Lary, and K. Myrback, eds. The enzymes, Vol. 2. Academic Press, New York. 479 p.
2. BERG, L. A., and M. E. GALLEGLY. 1966. Effect of light on oospore germination in species of Phytophthora. Phytopathology 56:583 (Abstr.).
3. BERGMAN, K. 1972. Blue-light control of sporangiophore initiation in Phycomyces. Planta (Berl.) 107:53-67.
4. CARLILE, M. J. 1965. The photobiology of fungi. Annu. Rev. Plant Physiol. 16:175-202.
5. GALINDO, J. A., and G. A. ZENTMYER. 1967. Genetical and cytological studies of Phytophthora strains pathogenic to pepper plants. Phytopathology 57:1300-1304.
6. HARNISH, W. N. 1965. Effect of light on production of oospores and sporangia in species of Phytophthora. Mycologia 57:85-90.
7. HUGUENIN, B. 1972. Influence de la lumiere blanche sur les phases precoces de la gametogenese chez le Phytophthora palmivora. C. R. Acad. Sci. (Paris) Serie D., 274:3214-3217.
8. HUGUENIN, B., and B. BOCCAS. 1971. Role de quelques facteurs dans la formation et la germination des oospores chez le Phytophthora palmivora Butl. Ann. Phytopathol. 3:353-371.
9. HUGUENIN, B., and R. JACQUES. 1973. Etablissement du spectre d'action de la lumiere sur la sporogenese et l'oogenese de souches du Phytophthora palmivora Butl. C. R. Acad. Sci. Paris, Serie D, 276:725-728.
10. LEAL, J. A., and B. GOMEZ-MIRANDA. 1965. The effect of light and darkness on the germination of the oospores of certain species of Phytophthora on some synthetic media. Trans. Br. Mycol. Soc. 48:491-494.
11. LILLY, V. G. 1966. The effect of sterols and light on the production and germination of Phytophthora spores. Pages 259-272 *in* M. F. Madelin, ed. The fungus spore. Butterworths, London. 338 p.
12. MARSH, P. B., E. E. TAYLOR, and L. M. BASSLER. 1959. A guide to the literature on certain effects of light on fungi: reproduction, morphology, pigmentation and phototrophic phenomena. Plant Dis. Rep. Suppl. 261. 61 p.
13. POFF, K. L. 1968. Effect of visible and near-visible radiation on morphogenesis and growth of Syzygites megalocarpus. Ph.D. Dissertation West Va. University, Morgantown. 179 p.
14. POFF, K. L., and K. H. NORRIS. 1967. Four low-cost monochromatic sources of known equal intensities. Plant Physiol. 42:1155-1157.
15. POLACH, F. J., and R. K. WEBSTER. 1972. Identification of strains and inheritance of pathogenicity in Phytophthora capsici. Phytopathology 62:20-26.
16. ROMERO, S., and D. C. ERWIN. 1969. Variation in pathogenicity of progeny from germinated oospores of Phytophthora infestans (Mont.) de Bary. Phytopathology 59:1310-1317.
17. SATOUR, M. M., and E. E. BUTLER. 1968. Comparative morphological and physiological studies of the progenies from intraspecific matings of Phytophthora capsici. Phytopathology 58:183-192.
18. TIMMER, L. W., J. CASTRO, D. C. ERWIN, W. L. BELSER, and G. A. ZENTMYER. 1970. Genetic evidence for zygotic meiosis in Phytophthora capsici. Am. J. Bot. 57:1211-1218.
19. TRIONE, E. J., and C. M. LEACH. 1969. Light-induced sporulation and sporogenic substances in fungi. Phytopathology 59:1077-1083.
20. YAGI, K. 1968. Flavins and flavoproteins. University Park Press. Baltimore, Maryland. 286 p.