

NOTES

Light and temperature induced sporocarp formation of *Phellinus weirii*¹

CHING Y. LI

*Pacific Northwest Forest and Range Experiment Station, Forest Service, United States Department of Agriculture, Corvallis, OR, U.S.A. 97331*Received January 2, 1979²

Accepted June 4, 1979

LI, C. Y. 1979. Light and temperature induced sporocarp formation of *Phellinus weirii*. *Can. J. For. Res.* 9: 535-538.

Eight isolates of *Phellinus weirii* (Murr.) Gilb. (*Poria weirii* (Murr.) Murr.), a serious pathogen of conifer roots in northwestern United States and southern British Columbia, formed sporocarps on malt agar after exposure to continuous near-ultraviolet light for 4 days at certain temperatures followed by incubation at normal laboratory temperature (22-24°C) and lighting conditions (118 lx for 8-9 h). Isolates G7312 and T-55 formed sporocarps when cultures were placed in normal or inverted positions; other isolates produced sporocarps only in an inverted position. Effective light-temperature combinations varied among isolates. Of all isolates, only G7312 and T-55 were induced to form sporocarps by fluorescent white light.

LI, C. Y. 1979. Light and temperature induced sporocarp formation of *Phellinus weirii*. *Can. J. For. Res.* 9: 535-538.

Huit isolats de *Phellinus weirii* (Murr.) Gilb. (*Poria weirii* (Murr.) Murr.), un important pathogène qui s'attaque aux racines des conifères dans le nord-ouest des États-Unis et le sud de la Colombie-Britannique, ont produit des sporophores sur un milieu gélosé contenant de l'extrait de malt. Les fructifications sont apparues après une exposition continue, pendant 4 jours, à une source lumineuse émettant dans une zone du spectre voisine de l'ultraviolet, suivie d'une période d'incubation dans des conditions normales de lumière (118 lx pendant 8-9 h) et de température (22-24°C). Les isolats G7312 et T-55 ont fructifié en position normale ou inversée alors que tous les autres isolats ont produit des sporophores seulement lorsque les plats de petri étaient placés à l'envers. Les conditions de lumière et de température nécessaires pour la formation de sporophores variaient selon l'isolat. Seulement les isolats G7312 et T-55 ont produit des sporophores sous l'influence d'une lumière blanche fluorescente.

[Traduit par le journal]

Introduction

Phellinus weirii (Murr.) Gilb. (*Poria weirii* (Murr.) Murr.) is a Basidiomycete that causes a widespread and highly destructive root disease on conifer roots in northwestern United States and southern British Columbia. Even though most infection centers results from root contact with infected roots and stumps from the preceding stands, basidiospores are probable sources of some new infection centers. The fungus forms sporocarps on the undersides of fallen trees and upturned roots, but in many years, sporocarps are rare. The fungus does not sporulate readily in culture, although this phenomenon has been reported (Gillette and Driver 1974).

The infrequent, unreliable sporulation of *P. weirii*

in nature has hampered studies of its sexuality and disease epidemiology. Development of a technique to induce sporulation would make these studies more feasible.

Light induces sporulation of many fungi, especially Fungi Imperfecti and Ascomycetes (Carlile 1970; Leach 1971). Results of this study demonstrate some relationships of light and temperature to sporocarp formation by *P. weirii*.

Materials and Methods

A temperature-gradient plate (91.4 cm × 91.4 cm × 1.5 cm), as constructed by Leach (1967), was used for this study. It was heated at one end with thermostatically controlled circulating warm water and cooled on the other end with thermostatically controlled refrigerated water. The temperature gradient obtained (5-26°C) was not completely linear but was adequate for this study. Under constant light, the temperature variation at any single point on the gradient plate was less than 0.5°C. Under conditions of alternating light and darkness, however, radiant heat from the lamps

¹This article was written and prepared by United States government employees on official time, and it is therefore in the public domain.

²Revised manuscript received April 12, 1979.

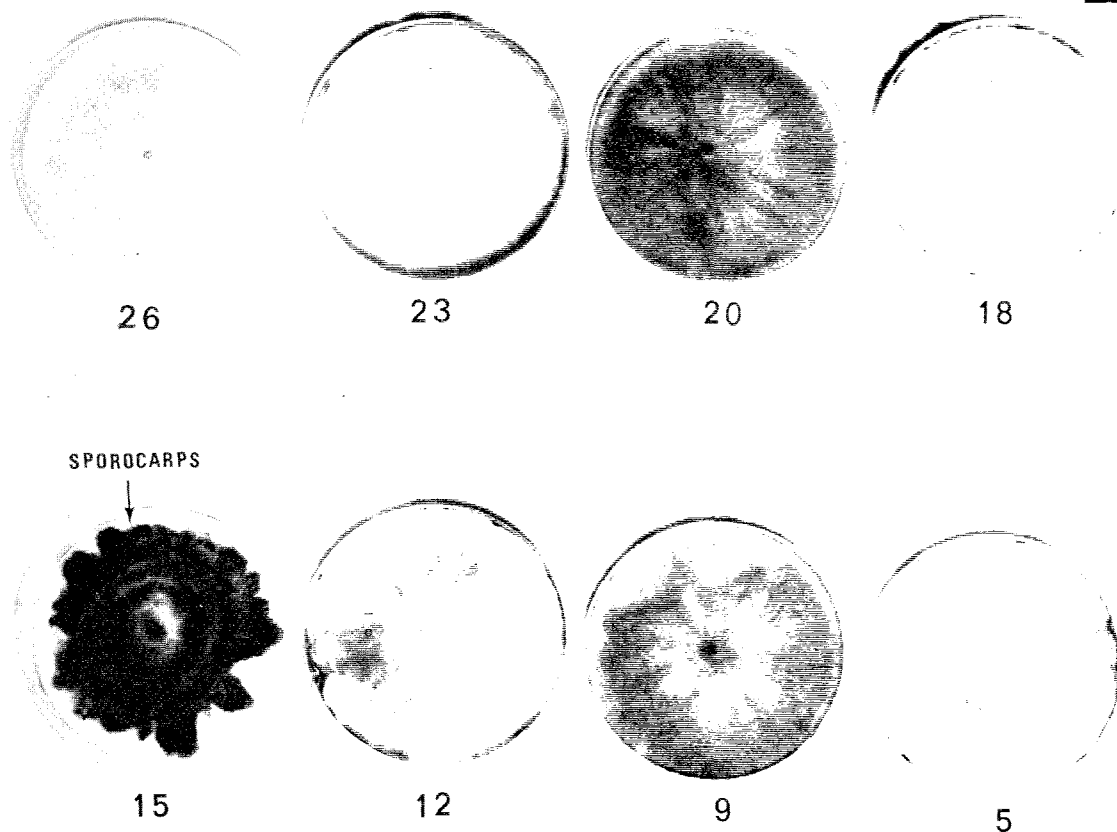


FIG. 1. Sporocarps formed on malt agar after *P. weirii* T-124 was exposed to continuous near-ultraviolet light for 4 days at 15°C; the same exposure at other temperatures induced no sporocarps. The number under each plate corresponds to the temperature (°C) on the temperature-gradient plate during exposure. Scale bar = 1 cm.

caused a maximum difference of 1°C between "night" and "day" temperature at any one point on the plate.

Near-ultraviolet radiation (near-UV) from four General Electric (GE)[®] 40-W, fluorescent black light lamps (BLB), which emit a continuous spectrum of 320–390 nm, and artificial daylight from four GE 40-W white fluorescent lamps with wavelengths from 350 to 700 nm were compared for effectiveness in inducing formation of sporocarps. Lamps were placed 55 cm above the temperature-gradient plate in a reflection chamber lined with aluminum foil to distribute the radiation more uniformly. Values of 516 lx for near-UV and 3229 lx for white light were measured with a Weston model 756 lightmeter at culture level.

Eight isolates of *P. weirii*, maintained as stock cultures on malt agar, were subcultured onto malt agar in petri dishes. G7312, Maryxb-10, and clones designated by the letter T were isolated from Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) at elevations below 600 m in western Oregon and Washington. Those designated by the letter W were isolated from mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.) at elevations above 1500 m in central Oregon.

[®]Trade or proprietary names are included for information purposes only and do not imply any endorsement by the Canadian Journal of Forest Research and the Forest Service of the United States Department of Agriculture.

After several days of growth, a 3-mm disc from the periphery of a uniformly growing culture was transferred to the center of a plastic petri dish (100 mm × 15 mm), containing 25 mL of 3% malt agar (DIFCO). After 5 days of incubation in the dark at 25°C, the petri dishes were placed on a temperature-gradient plate for 4 days under one of five light treatments: (i) continuous fluorescent white light, (ii) continuous near-UV, (iii) 12 h of fluorescent white light alternated with 12 h of darkness, (iv) 12 h of near-UV alternated with 12 h of darkness, and (v) continuous darkness. The experiment was run one isolate at a time. Eight petri dishes were placed at the same temperature on the temperature-gradient plate for each light treatment. A total of 64 petri dishes for each isolate, therefore, were placed on the temperature-gradient plate with eight various temperatures under one of the light treatments.

After the light-temperature treatments, the petri dishes were removed and incubated on a laboratory bench at normal laboratory temperature (22–24°C) and lighting conditions (118 lx for 8–9 h), with four of the eight petri dishes receiving the same treatment in an inverted position, the other four in an upward position. For control, petri dishes in either position were kept continuously in the dark at 25°C or exposed only to normal laboratory lighting at normal laboratory temperature.

Sporocarp formation was assessed every week for 5 weeks. Degree of development was recorded as follows: —, no

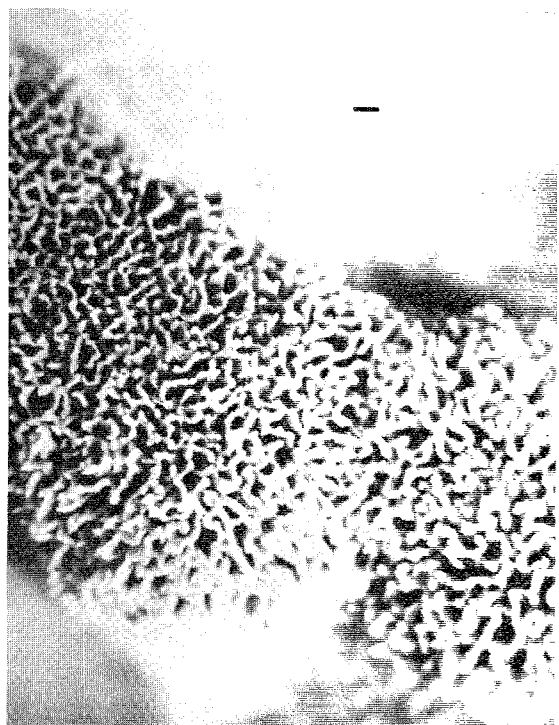


FIG. 2. Sporocarps of *P. weirii* T-124 on malt agar. Scale bar = 0.5 mm.

sporocarps; +, sparse development; ++, light development; +++, moderate development; and +++, abundant development.

Results and Discussion

One or 2 weeks after cessation of irradiation, tan crater-like depressions, representing the earliest stage of sporocarp development, appeared in some isolates in the portion of the mycelium that was peripheral at the time of irradiation. When sporocarps were initiated, placing the cultures in the dark stopped their development. Sporocarp initials continued to develop into flat, thin, and brownish-gray

pore layers, with 3–5 pores/mm (Figs. 1, 2). Examination of sporocarps under microscope invariably revealed the presence of basidiospores, which germinated readily under moist conditions. Macroscopically, the sporocarps closely resemble those formed in nature as described by Gilbertson (1956). Anatomical structures such as setal hyphae exactly fit Gilbertson's description.

Isolates T-55 and G7312 produced sporocarps either when cultures were in normal position or inverted; the other isolates produced sporocarps only in inverted culture. Only T-55 and G7312 produced sporocarps at all temperatures and light combinations tested. In uninterrupted darkness, all eight isolates remained as white vegetative mycelia.

All eight isolates produced sporocarps after exposure to continuous near-UV at certain temperatures, which varied among isolates (Table 1). Near-UV alternated with darkness failed to induce sporocarp formation for Maryxb-10 and T-125 at any temperature tested, but induced sparse to abundant sporocarp formation at some temperatures for other isolates (Table 2). Of all the isolates, only G7312 and T55 were induced to form sporocarps by fluorescent white light.

A visual indication that photochemical responses were occurring in *P. weirii* was evidenced by color changes in the vegetative mycelium. When the culture was in continuous darkness, the vegetative mycelium was white; when the culture was exposed to light, the vegetative mycelium became brown. Sporocarps formed only from the pigmented mycelium at certain temperatures, an indication of a physiological mechanism apparently triggered by specific light-temperature combinations.

The mechanism of fruiting in Basidiomycetes is incompletely known, although factors affecting fruiting have been studied for several years. Light is one of the factors shown to be involved with sporophore

TABLE 1. Sporocarp formation by *Phellinus weirii* after exposure to 4 days of continuous near-ultraviolet light on malt agar at various temperatures*

Isolate	Temperature, °C							
	26	23	20	18	15	12	9	5
G7312	++	++	+++	++++	++++	++++	++++	++++
Maryxb-10	-	-	-	-	-	-	-	-
T-55	++	+++	+++	++++	++++	++++	++++	++++
T-115	+	+	+	+	+	+	+	+
T-124	-	-	-	-	+	+	+	+
T-125	-	-	+	+	+	+	+	+
W-2E	-	+	++	+++	+++	+++	+++	+++
W-4W	-	-	-	+++	+++	+++	+++	+++

*Sporocarps: +, sparse; ++, light; +++, moderate; +++, abundant; -, no sporocarps. Based on eight cultures for each isolate.

TABLE 2. Sporocarp formation by *Phellinus weirii* after exposure to 4 days of an alternating 12-h cycle of near-ultraviolet light and darkness on malt agar at various temperatures*

Isolate	Temperature, °C							
	26	23	20	18	15	12	9	5
G7312	++	++	++	+++	+++	+++	+++	+++
Maryxb-10	-	-	-	-	-	-	-	-
T-55	++	+++	+++	+++	+++	+++	+++	+++
T-115	+	+	+	-	-	-	-	-
T-124	-	-	+	++	+++	+	-	-
T-125	-	-	-	-	-	-	-	-
W-2E	-	+	+	+	+	+	+	+
W-4W	-	-	-	-	-	++++	++++	++++

*Sporocarps: +, sparse; ++, light; +++, moderate; +++++, abundant; -, no sporocarps. Based on eight cultures for each isolate.

formation in other fungi. Leonard and Dick (1968) found that a brown pigment appeared in the region of fruiting in *Schizophyllum commune* and suggested that deposition of melanin-like pigments was an early stage of morphogenesis. Faro (1972) demonstrated that the brown pigment formed before fruiting in *Panus tigrinus* was melanin. The significance of pigments in the fruiting phenomena of *P. weirii* is not understood. Further studies will relate to the nature and mode of action of the pigments in the mycelium.

This study does not establish optima for length, periodicity, intensity, or quality of irradiation. It does, however, provide a more dependable method than has been available for inducing spore production for most isolates.

For better sporocarp formation on malt agar, *P. weirii* should be preincubated at 25°C in the dark for 5 days followed by exposure to continuous near-UV with 516 lx intensity at 5°C. Some isolates may require a warmer temperature. Afterward incubate the culture in an inverted position at normal tempera-

tures (22–24°C) and lighting conditions (118 lx for 8–9 h).

- CARLILE, J. M. 1970. The photoresponses of fungi. In *Photobiology of microorganism*. Edited by P. Halldal. Wiley Interscience, London and New York. pp. 309–344.
- FARO, S. 1972. Physiological aspects of pigment production in relation to morphogenesis in *Panus tigrinus*. *Mycologia*, **64**: 374–387.
- GILBERTSON, R. L. 1956. The genus *Poria* in the central Rocky Mountains and Pacific Northwest. *Lloydia*, **19**: 65–85.
- GILLETTE, W. D., and C. H. DRIVER. 1974. Sporophore formation by *Poria weirii* in culture. *Proc. Am. Phytopathol. Soc.* **1**: 32.
- LEACH, C. M. 1967. Interaction of near-ultraviolet light and temperature on sporulation of the fungi *Alternaria*, *Cercospora*, *Fusarium*, *Helminthosporium*, and *Stemphylium*. *Can. J. Bot.* **45**: 1999–2016.
- . 1971. A practical guide to the effects of visible and ultraviolet light on fungi. In *Methods in microbiology*. Vol. IV. Edited by C. Booth. Academic Press, London and New York. pp. 609–664.
- LEONARD, T. J., and S. DICK. 1968. Chemical induction of haploid fruiting bodies in *Schizophyllum commune*. *Proc. Natl. Acad. Sci. U.S.A.* **59**: 745–751.