In Situ Observations on the Influence of Wood Moisture Content and Temperature on Spore Germination and Wood Colonization by *Poria carbonica*

P. R. Przybylowicz and M. E. Corden

Former graduate research assistant and professor, respectively, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331. Present address of first author: Northwest Mycological Consultants, 702 NW 4th St., Corvallis, OR 97330. Present address of second author: Forest Products Department, Oregon State University, Corvallis 97331.

Research funded in part through the Cooperative Pole Research Program by: U.S. Forest Products Research Laboratory, Madison, WI; Bonneville Power Administration; Portland General Electric Co., and Empire State Electric Energy Research Corp.
Technical Paper 7526, Oregon Agricultural Experiment Station.
Accepted for publication 10 September 1985 (submitted for electronic processing).

ABSTRACT

Przybylowicz, P. R., and M. E. Corden. 1986. In situ observations on the influence of wood moisture content and temperature on spore germination and wood colonization by *Poria carbonica*. Phytopathology 76:212-214.

A method for observing germinating fungal spores on wood was developed in which temperature and wood moisture content could be easily controlled and subsequent wood colonization could be determined. Thin radial sections of Douglas-fir (*Pseudotsuga menziesii*) heartwood (8 mm × 8 mm × 60 μm) were inoculated with a fungal spore suspension and a similar wood section was placed over the inoculated section forming a "spore sandwich." The "spore sandwiches" were incubated between larger blocks of Douglas-fir heartwood to maintain control of the wood moisture content during incubation in controlled temperature-humidity chambers. Spore germination was observed by opening the "spore sandwiches" and staining the spores in situ for microscopic observation. Wood colonization was determined by isolations from the surrounding wood blocks. The "spore sandwich" method was used to study the influences of temperature and wood moisture content on spore germination of *Poria carbonica*. Basidiospores and asexual spores germinated and colonized wood at and above the fiber saturation point (about 30% moisture content), but not below. Both spore types germinated and colonized wood at 22 and 30 C, but basidiospores failed to germinate at 5 and 35 C, whereas asexual spores germinated at 5 and 35 C, but were unable to colonize the wood. The "spore sandwich" method provides a means for assessing spore germination and wood colonization by wood-decaying fungi under conditions simulating those occurring naturally in wood in service.

Additional key words: decay fungi, wood decay.

Most studies of spore germination by wood-decaying basidiomycetes have been on nutrient agar media (3,7,9,11,12, 17,18), and while this allowed easy observation, the results must be viewed in light of the physical and nutritional differences between the agar media and wood. Others have studied spore germination on water agar blocks fused to wood (13, 14) or on dialysis membranes placed on the wood (8). While these methods allowed soluble substances from the wood to diffuse to the spores, the artificial barrier between the spores and wood prevented evaluation of subsequent colonization. Direct observation of spore germination on thin wood-sections has been accomplished (10,14,16,21), but wood moisture content could not be accurately controlled in the thin sections and successful colonization of the wood by mycelium could not be determined.

The purpose of this research was to develop a method for observing germinating fungal spores on wood which would allow colonization to occur while controlling the wood moisture content. This method was used to investigate temperature and wood moisture influences on spore germination and wood colonization by *Poria carbonica* Overh. in situ. *P. carbonica* causes most of the decay in Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] utility poles in service (2,20) but relatively little is known about its establishment in wood.

MATERIALS AND METHODS

Isolation of asexual spores. In culture, *P. carbonica* commonly produces chlamydospores and conidia which appear similar and are extremely difficult to separate. Consequently, the term "asexual spores" is used here for both of these spore types.

Asexual spores were isolated from mycelial mats of *P. carbonica* (O.S.U. No. 265) grown on malt extract medium (25 g of malt extract and 8 g of agar per liter). A steam-sterilized disk of water-permeable cellophane was placed over the culture medium surface before inoculation with mycelium of *P. carbonica*. This permitted rapid removal of the mycelium and spores from the culture medium. The cultures were incubated in the dark at 30 C until the cellophane was covered with mycelium, which then was aseptically scraped from the cellophane and placed in a screw-top test tube containing 25 ml of sterile distilled water and about 3 cm of glass fragments in the bottom. The tube was shaken rapidly which caused the mycelium to be cut into small fragments, then the suspension was filtered through four layers of cheesecloth and a Whatman #4 filter paper. The filtrate was centrifuged at 3,000 *g* and 5 C for 15 min and the supernatant was discarded. The pellet was resuspended in 2 ml of sterile distilled water to produce a spore suspension relatively free of hyphal fragments. The spore concentration was adjusted to about 10^6 spores per milliliter by using a haemacytometer, and the spores were immediately applied to wood.

Isolation of basidiospores. Basidiospores were produced in culture by using a modification of a method described by Morton and French (10). The spores were collected from sporophores produced on malt-agar medium containing two small Douglas-fir heartwood blocks (2 × 1 × 0.5 cm). The blocks were water-saturated under vacuum, autoclaved, and placed in the molten culture medium. The medium was inoculated with mycelium of *P. carbonica*, and the cultures were incubated at 30 C in the dark until colony diameters reached about 2–4 cm. Then the culture plates were inverted under diffuse light at 20–22 C. Basidiospores usually were produced 3–4 wk later and were collected from the petri-dish covers. The covers were changed 24 hr prior to spore use to ensure collection of fresh spores. Spore suspensions were prepared by washing the spores from the covers with sterile distilled water and adjusting their concentration to 1–4 × 10^6 spores per milliliter by using a haemacytometer.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

The "spore sandwich" method. Thin radial sections of Douglas-fir heartwood (8 mm × 8 mm × 60 μm) cut with a sliding microtome were placed between two glass microscope slides in petri dishes and autoclaved (20 min at 103 kPa [15 psi]). The wood sections were removed, placed on sterile filter paper in petri dishes, and inoculated with 25 μl of a fungal spore suspension. Excess water was wicked through the sections by the filter paper, leaving the spores deposited on the upper wood surface. An uninoculated but similarly prepared wood section was then placed on top of the inoculated section, forming a "spore sandwich." The "spore sandwiches" were then placed between sterile Douglas-fir heartwood blocks (1 × 1 × 1 cm) at varying moisture contents and with radial faces in contact. The blocks were secured with a rubber band. The wood cultures were incubated under controlled temperature and humidity conditions for 4 days before spore germination was determined.

Following an incubation period, the "spore sandwiches" were removed from between the blocks and the wood sections were separated. Each section was carefully placed, inner side up, on a small drop of stain (0.05% trypan blue in lactophenol) on a microscope slide; the stain colored the spores without washing them from the wood. Both wood sections were examined microscopically and spores (100 per section) were considered germinated when their germ tube lengths exceeded their diameter.

To determine wood colonization, blocks containing "spore sandwiches" were incubated for 1 mo, then aseptic isolations were made 1 mm into the wood of each block to detect the fungus. Small wood chips removed from each block were cultured on potato-dextrose agar (broth from 200 g of potatoes, plus 10 g of dextrose, 10 μg of benomyl, and 0.075 ml of lactic acid [85%] per liter) at 22 C and observed 1 mo later for growth of *P. carbonica*.

Wood moisture content of the "spore sandwich". For evaluating spore germination at various wood moisture contents, the blocks used to enclose the "spore sandwiches" were first oven dried (24 hr at 110 C), then two blocks were weighed along with a rubber band as a basis for subsequent moisture content determinations. Douglas-fir heartwood below the fiber saturation point (FSP) (about 30% on an oven-dry basis) was obtained by placing blocks on glass supports over saturated solutions of monobasic ammonium phosphate in sealed jars. This resulted in a relative humidity of about 93% at 22 C (19). Wood at the FSP was obtained by incubating the blocks over distilled water, while blocks above the FSP were saturated with water under vacuum for several hours, then placed over distilled water. Jars containing the blocks were autoclaved (45 min at 103 kPa), sealed, and then incubated at 22 C for at least 2 wk before use.

The blocks were weighed before inserting the "spore sandwich" and after incubation to obtain an average wood moisture content for the incubation period. The humidity chambers containing the block cultures were placed inside covered plastic boxes and incubated in a growth chamber at 22 C.

Temperature of the "spore sandwich". In experiments where temperature was varied, the wood moisture content of the blocks was adjusted to above the FSP to allow optimum spore germination. The "spore sandwiches" were inserted between the sterile blocks which then were suspended in cheesecloth bags over distilled water in tightly stoppered 250 ml Erlenmeyer flasks. The block cultures were incubated at various temperatures for 4 days before the "spore sandwiches" were examined for spore germination. After removing the "spore sandwiches", the blocks were weighed and their moisture contents were calculated.

RESULTS AND DISCUSSION

Moisture content of the "spore sandwich". The moisture content of the wood sections comprising the "spore sandwich" was compared with that of the blocks enclosing them. Pairs of blocks were adjusted to moisture contents from 10 to 86% (oven-dry basis) and "spore sandwiches" were wetted with 25 μl of distilled water to simulate inoculation with a spore suspension. The sandwiches were placed between the blocks and 4 days later they were removed, quickly weighed, oven dried (24 hr at 110 C), then reweighed. The blocks were also weighed and moisture contents were calculated for the blocks and the sections they contained.

Generally the moisture content of the "spore sandwiches" was lower than that of the blocks surrounding them (Fig. 1), and the linear regression line indicated considerable variation ($r^2 = 0.55$). The variation was mostly due to rapid drying of the thin wood sections during weighing, and we presume therefore that the sandwiches were roughly similar in moisture content to the surrounding blocks during the incubation period. This method provides a means of evaluating spore germination on wood by direct observation over a wide range of wood moisture contents but would need refinement for small moisture content increments.

Influence of wood moisture content. Asexual spores and basidiospores of *P. carbonica* failed to germinate or colonize Douglas-fir heartwood below the FSP, but could germinate and colonize wood at or above this moisture level (Table 1). There was no significant difference between basidiospore germination on

TABLE 1. Effect of wood moisture content on spore germination of *Poria carbonica* and colonization of Douglas-fir heartwood

Spore type and wood moisture content[a] (%)	Germination[b] (%)	Colonization (%)
Asexual spores		
17 (15–18)	0	0
29 (25–35)	71 (25.3)	100
60 (38–81)	90 (3.3)	100
Basidiospores		
15 (15–16)	0	0
35 (31–39)	92 (3.6)	100
83 (71–99)	90 (5.1)	100

[a] Each spore type was tested in three replicate experiments with blocks at each wood moisture content. Average percent wood moisture content is given with the range in parentheses.

[b] Average percent germination with standard deviation in parentheses. Asexual spore germination for the two higher moisture contents were significantly different, while basidiospore germination was not (Student's *t*-test, $P = 0.05$, df = 16).

[c] Percent colonization is based on the number of successful isolations of *P. carbonica* from blocks incubated for 1 mo.

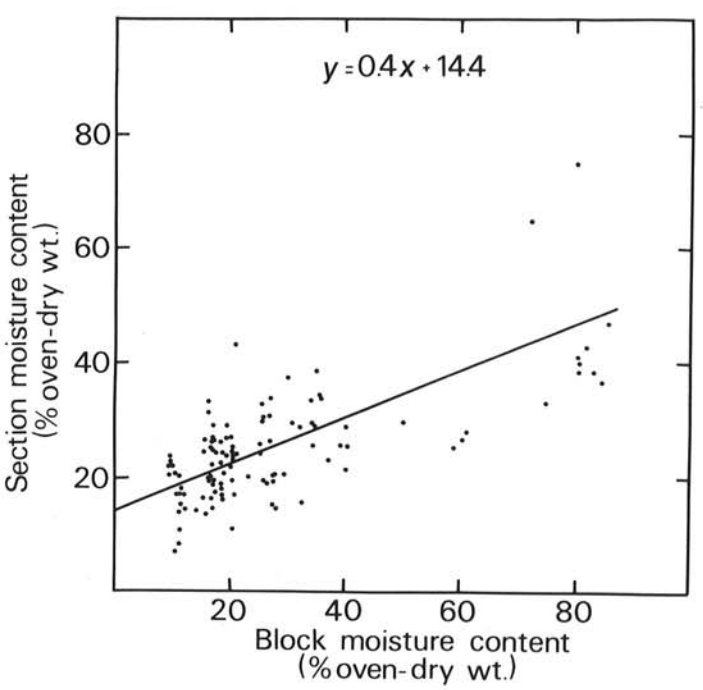

Fig. 1. Moisture content of Douglas-fir heartwood blocks (2 × 1 × 1 cm) used to enclose the "spore sandwiches" compared to the moisture content of the thin wood sections (8 × 8 mm × 60 μm) comprising the "spore sandwiches" after 4 days of incubation at 22 C. Results are based on 112 block-"spore sandwich" cultures.

TABLE 2. Effect of temperature on spore germination of *Poria carbonica* and colonization of Douglas-fir heartwood

Temp. (C)	Wood moisture content[a] (%)	Germination[b] (%)	Colonization[c] (%)
Asexual spores			
5	106 (95–137)	22 (23.1)	0
22	112 (73–151)	90 (3.8)	100
30	89 (71–113)	71 (27.8)	100
35	116 (94–157)	41 (29.9)	0
Basidiospores			
5	93 (64–117)	0	0
22	93 (67–115)	95 (2.5)	100
30	94 (80–106)	33.5 (10.4)	100
35	91 (72–112)	0	0

[a] Each spore type was tested in three replicate experiments with four blocks at each temperature. Average percent wood moisture content is given with the range in parentheses.

[b] Average percent germination with standard deviation in parentheses. Asexual spore germination at 5 and 35 C was significantly different from that at 22 and 30 C (analysis of variance, $F_{3,42} = 20.8$, LSD = 20.2). Percent basidiospore germination at 22 C was significantly different from that at 30 C (Student's t-test, $P = 0.05$).

[c] Percent colonization is based on the number of successful isolations from each block after 1 mo of incubation.

wood at and above the FSP, but asexual spore germination was significantly lower on wood at the FSP than in the wetter wood. In an additional experiment with wood at about 24% moisture content, asexual spores failed to germinate on the wood.

These results suggest that wood in service can be colonized by *P. carbonica* when it reaches or exceeds the FSP. Moisture contents in this range, for example, commonly occur in freshly cut utility poles, at the soil-pole contact zone in poles in service (6), and in checks and cracks in poles where rainwater may be trapped.

Influence of temperature. The temperature range for basidiospore germination of *P. carbonica* was more limited than that found for asexual spores (Table 2). Basidiospores failed to germinate at 5 and 35 C, although some spore swelling was observed at the higher temperature. Spore germination was significantly lower at 30 C than at 22 C, but wood colonization occurred at both temperatures. Many of the ungerminated basidiospores at 30 C were swollen after 4 days of incubation, which suggested that they might germinate later.

Asexual spores of *P. carbonica* germinated readily and colonized the wood at 22 and 30 C (Table 2), but germination frequencies at 5 or 35 C were significantly lower than those at 22 and 30 C. After 3 days' incubation at 22 or 30 C, the asexual spore germ tube lengths were between 100 and 1,000 μm, while those at 5 and 35 C ranged from 10 to 100 μm, but were usually less than 50 μm. Asexual spores of *P. carbonica* incubated at 5 and 35 C were unable to establish colonies 1 mm into the wood.

Temperatures between 22 and 30 C were favorable for wood colonization by asexual spores and basidiospores of *P. carbonica* and this range is similar to that reported for other wood-decaying basidiomycetes (1,3,4,9,18). Successful colonization probably would decrease during periods when temperatures were outside this range, but on the basis of observations of other basidiomycetes (3,9) these spores probably could survive until more favorable conditions occurred.

Results of these experiments confirm that temperature and wood moisture content are important factors that define favorable periods for wood colonization as is the case with other decay fungi (17,21). Further studies at temperatures between 5 and 22 C, however, are needed to determine the minimum temperature for wood colonization. While temperature seldom would be limiting in the mild maritime climate of the Pacific Northwest, optimum moisture levels favorable for wood colonization are more likely to occur during the wet winter months.

The "spore sandwich" methodology developed in this study places fungal spores on wood where the influence of environmental factors (e.g. temperature and wood moisture content) on spore germination and colonization can be studied. The airflow around the "spore sandwich" was restricted by the surrounding blocks and this reduced the wetting and drying typical of wood in service. Nevertheless, the "spore sandwich" creates an environment roughly similar to that of spores in seasoning checks in wood in service where successful colonization is presumed to occur (1,2,5,20).

Spore germination is the stage when decay fungi are most susceptible to inhibitory chemicals; at this time the fungus is poorly established in the wood and has few food reserves (15). Preservatives and wood fumigants that remain effective for many years probably prevent spore germination by decay fungi. Using the "spore sandwich" method it would be possible to evaluate new fungicides against decay fungi in a test that simulates natural colonization of wood in service where inoculum levels seldom reach the high levels used in the standard soil-block test and related assays.

LITERATURE CITED

1. Cartwright, K. St. G., and Findlay, W. P. K. 1958. Decay of timber and its prevention. Her Majesty's Stationery Office, London. 332 pp.
2. Eslyn, W. E. 1970. Utility pole decay. Part II. Basidiomycetes associated with decay in poles. Wood Sci. Technol. 4:97-103.
3. Good, H. M., and Spanis, W. 1958. Some factors affecting the germination of spores of *Fomes igniarius* var. *populinus* (Neuman) Campbell, and the significance of these factors in infection. Can. J. Bot. 36:421-437.
4. Gottlieb, D. 1950. The physiology of spore germination in fungi. Bot. Rev. 16:229-257.
5. Graham, R. D. 1980. Converting 1979 wood preservation problems into opportunities for 1999. For. Prod. J. 30(2):17-20.
6. Graham, R. D., and Helsing, G. G. 1979. Wood pole maintenance manual: Inspection and supplemental treatment of Douglas-fir and western red cedar poles. Forest Research Laboratory, Oregon State University, Corvallis. 62 pp.
7. Hintikka, V. 1970. Stimulation of spore germination of wood-decomposing hymenomycetes by carbon dioxide. Karstenia 11:23-27.
8. Manion, P. D., and French, D. W. 1969. The role of glucose in stimulating germination of *Fomes igniarius* var. *populinus* basidiospores. Phytopathology 59:293-296.
9. McCracken, F. I. 1974. Effects of some factors on *Pleurotus ostreatus* spore germination. (Abstr.) Proc. Am. Phytopathol. Soc. 1:63.
10. Morton, M. L., and French, D. W. 1966. Factors affecting germination of spores of wood-rotting fungi on wood. For. Prod. J. 16(3):25-30.
11. Morton, H. L., and French, D. W. 1974. Stimulation of germination of *Polyporous dryophilus* basidiospores by carbon dioxide. Phytopathology 64:153-154.
12. Scheld, H. W., and Perry, J. J. 1970. Basidiospore germination in the wood-destroying fungus *Lenzites saepiaria*. J. Gen. Microbiol. 60:9-21.
13. Schmidt, E. L., and French, D. W. 1979. Sterilization method effects on germination of wood decay fungus spores observed by the contact agar method. Phytopathology 69:688-689.
14. Schmidt, E. L., and French, D. W. 1979. Spore germination of *Gloeophyllum trabeum* on wood in relation to mass of the sample. Plant Dis. Rep. 63:30-31.
15. Schmidt, E. L., and French, D. W. 1979. CCA and sodium pentachlorophenate inhibition of basidiospore germination of decay fungi: Contact agar method. For. Prod. J. 29(5):53-54.
16. Toole, E. R. 1971. Germination of spores of wood decay fungi on wood. Phytopathology 61:88-90.
17. Tsuneda, I.1978. Spore germination of *Fomes* species. M.S. thesis. University of Alberta. Edmonton, Alberta, Canada. 132 pp.
18. Whitney, R. D. 1966. Germination and inoculation tests with basidiospores of *Polyporus tomentosus*. Can. J. Bot. 44:1333-1343.
19. Winston, P. W., and Bates, D. H. 1960. Saturated solutions for the control of humidity in biological research. Ecology 41:232-237.
20. Zabel, R. A., Lombard, F. F., and Kenderes, A. M. 1980. Fungi associated with decay in treated Douglas-fir transmission poles in the northeastern United States. For. Prod. J. 3(4):51-56.
21. Zeller, S. M. 1920. Humidity in relation to moisture imbibition by wood and to spore germination on wood. Ann. Mo. Bot. Garden 7:51-74.