Conditions for soft rot of wood

J. J. WORRALL,[1] S. E. ANAGNOST, AND C. J. K. WANG

College of Environmental Science and Forestry, State University of New York, Syracuse, NY 13210, U.S.A.

Received April 16, 1991

Accepted June 14, 1991

WORRALL, J. J., ANAGNOST, S. E., and WANG, C. J. K. 1991. Conditions for soft rot of wood. Can. J. Microbiol. **37**: 869–874.

Conditions leading to optimal development of soft rot of wood were studied *in vitro*. Rates of weight loss generally remained more or less constant for 12 weeks, after which they decreased. Use of Petri dishes as decay chambers, saturation of blocks with a reduced nutrient solution containing micronutrients and vitamins, and use of a thick nylon mesh as a support between the agar and blocks generally gave better results than alternative conditions. Depending on experimental conditions, decay was either superficial or more or less uniform throughout the block. The uniform pattern was associated with higher weight losses than the superficial pattern. These and other results suggest that, although soft rot is most readily apparent as a surface decay of near-saturated wood in service, moisture conditions for optimal development may be no different than for decays caused by basidiomycetes.

Key words: wood decay, moisture, *Chaetomium globosum*.

WORRALL, J. J., ANAGNOST, S. E., et WANG, C. J. K. 1991. Conditions for soft rot of wood. Can. J. Microbiol. **37** : 869–874.

Les conditions qui favorisent l'atteinte d'un développement optimal de la pourriture molle du bois ont été étudiées *in vitro*. Les taux de pertes de poids sont généralement plus ou moins constants au cours de 12 semaines, période après laquelle ils décroîssent. L'emploi de boîtes de Petri comme chambres d'incubation, la saturation des blocs de bois avec une solution réduite en nutriments contenant des micronutriments et des vitamines et l'emploi d'un support en nylon épais entre la gélose et les blocs donnent généralement de meilleurs résultats que certaines conditions alternatives. Dépendant des conditions expérimentales, la pourriture se manifestera soit superficiellement, soit de façon plus ou moins uniforme à travers les blocs. Le mode uniforme a été associé à des pertes plus élevées en poids que le mode superficiel. Malgré le fait que la pourriture molle soit très apparente comme carie de surface chez les bois presque saturés utilisés, les présents résultats et d'autres suggèrent que les conditions d'humidité requises pour l'obtention d'un développement optimal peuvent ne pas différer de celles qui sont nécessaires pour les caries causées par les basidiomycètes.

Mots clés : carie du bois, humidité, *Chaetomium globosum*.

[Traduit par la rédaction]

Introduction

Since soft rot was recognized as a new and important form of wood decay (Savory 1954), there has been an effort to determine optimal conditions for soft rot *in vitro*. Such information not only facilitates research on mechanisms, ecology, and prevention of soft rot, it also provides insight into features of soft rot that differ from those of other kinds of decay.

Duncan (1960) found that the standard soil-block test used for basidiomycetes was not appropriate for soft-rot fungi. After extensive studies, she recommended an agar-block and a soil-burial technique, which yielded similar results (Duncan 1965). Key features were high nutrient concentrations and maintenance of high moisture content. Bravery (1968) compared seven different techniques available at the time and concluded that agar tests were more severe than soil tests for evaluation of preservatives. A vermiculite-burial technique has also been developed and optimized (Gersonde and Kerner-Gang 1976).

One environmental factor that has been of special interest with regard to soft rot is moisture. Moisture contents are often high where soft rot is economically damaging (Findlay 1984), and Duncan (1965) called attention to the need for maintenance of high moisture content for optimal soft rot *in vitro*. Kerner-Gang (1974), on the other hand, found that intermediate moisture contents were associated with maximum weight loss, but the interpretation is limited by the accompanying variation in available nutrients.

Earlier, we reexamined the need for and mobilization of nutrients in soft rot (Worrall and Wang 1991). In the studies described here, we used those results as a starting point to optimize conditions for soft rot *in vitro*.

Materials and methods

Cultures

Arthrographis cuboidea (Sacc. & Ellis) Sigler (isolate P-540), *Chaetomium globosum* Kunze ex Steud. (P-591), *Pachnocybe ferruginea* (Sow: Fr.) Berk. (P-231), *Phialocephala dimorphospora* Kendrick (P-109), *Phialophora heteromorpha* (Nannf.) Wang (P-962), *Scytalidium circinatum* Sigler & Wang (P-1018), and *Scytalidium lignicola* Pesante (P-53) were previously isolated from utility poles (Wang and Zabel 1990). *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoemaker (DAOM 160706) was provided by the National Mycological Herbarium, Agriculture Canada, Ottawa. Cultures were grown for about 2 weeks on 2% malt extract agar before plugs 4 mm in diameter were cut from the margins and placed between and in contact with two blocks.

Time course

Wood blocks were prepared and weight losses determined as described below (see Final method). To determine the time course of weight loss caused by various fungi, 6-oz jars with 20 mL reduced nutrient solution agar (Worrall and Wang 1991) were used. Blocks (four per jar, three jars per treatment) lay on a thin (0.2 mm thick) nylon mesh support and were harvested at 4-week intervals for 24 weeks.

Chambers

Although jars are traditionally used for decay studies, Petri dishes may give similar results and are more convenient. We compared the two systems using the 6-oz jars with 20 mL reduced nutrient agar versus plastic Petri dishes (100 × 15 mm) with 35 mL, using a thin mesh support in both cases.

[1]Author to whom all correspondence should be sent to the following address: 350 Illick Hall, 1 Forestry Drive, Department of Environmental and Forest Biology, State University of New York, Syracuse, NY 13210, U.S.A.

Printed in Canada / Imprimé au Canada

TABLE 1. Concentrations (g/L) of nutrients in the reduced nutrient solution (R), double Abrams' solution (2AS), and several intermediate solutions

Solution	NH_4NO_3	KH_2PO_4	K_2HPO_4	$MgSO_4 \cdot 7H_2O$
R	1.5	2.5	2.0	1.0
1.5R	2.25	3.75	3.0	1.5
2R	3.0	5.0	4.0	2.0
~3R	4.5	5.0	4.0	3.0
2AS	6.0	5.0	4.0	4.0

NOTE: All solutions contained thiamine (0.1 mg/L) and glucose (2.5 g/L).

Moisture

To assess the effect of initial moisture content on subsequent decay, blocks were treated to attain, after autoclaving, approximately 100% (near saturation for these wood samples) and 50% moisture content on a dry-weight basis (MC), both with water and with nutrient solution. In a fifth treatment, blocks were equilibrated over distilled water at 25°C. Blocks were then autoclaved at 121°C for 30 min and placed in jars. Actual moisture contents (mean ± standard error) measured in samples ($n = 5$) of birch blocks after autoclaving were 99 ± 7, 43 ± 10, 110 ± 2, 49 ± 18, and 18 ± 0% for the 100 and 50% nutrient solution, the 100 and 50% water, and the equilibrated treatments, respectively. Because observations suggested that the thin mesh permitted agar contact and excessive moisture levels, a thicker nylon mesh (1.3 mm thick, the type used as a needlepoint canvas) was used as a support.

Nutrients

Because initial comparisons of nutrient levels were done in a vermiculite system and by independently varying nutrient concentrations (Worrall and Wang 1991), we compared double Abrams' solution (2AS) with the reduced nutrient solution and several intermediate solutions (Table 1) in the agar system, using Petri dishes and thick mesh. Finally, we compared the reduced nutrients in the same Petri dish system with a widely used vermiculite system using double Abrams' solution (Zabel et al. 1991).

A vitamin and micronutrient amendment (Duncan 1965) was tested using the reduced nutrient solution as a basal medium, thick mesh supports, and Petri dishes as chambers.

Final method

We have adopted the following procedure for experiments on soft rot of wood. Blocks 1 cm (tangential) × 2 cm (radial) × 0.5 cm (longitudinal) are cut from kiln-dried southern yellow pine (*Pinus taeda* L.) and yellow birch (*Betula alleghaniensis* Britton), oven-dried, weighed, vacuum infiltrated, and autoclaved for 30 min. Petri dishes are prepared with 30 mL nutrient agar. The reduced nutrient solution with 0.25% glucose and 10 mL Duncan's (1965) micronutrient–vitamin solution per litre, adjusted to pH 6, is used for infiltration and agar preparation. Nylon needlepoint canvas (1.3 mm thick) is cut into pieces about 5.5 × 4 cm, autoclaved, and laid on the agar. Four blocks are placed on the nylon in each dish (three dishes per treatment for a total of 12 replicate blocks), and two inoculum plugs are placed such that each touches two blocks. Dishes are wrapped with strips of Parafilm to seal the lid, and several needle punctures are made for aeration. Twelve weeks later, blocks are cleaned, oven-dried, and weighed. Weight losses are calculated as $[(O - P)/O] \times 100$ (O, original dry weight; P, postdecay dry weight) and corrected for any weight change in uninoculated controls. Although we have not compared incubation temperatures, others have found that, just as with other factors, the response varies among fungi (Morrell and Zabel 1985). We use 28°C because it is near but not above the optimum of most fungi.

Results

Weight losses of birch blocks were generally much higher than those of pine. Results are presented only for birch, except where results with pine differ substantially and affect the conclusions.

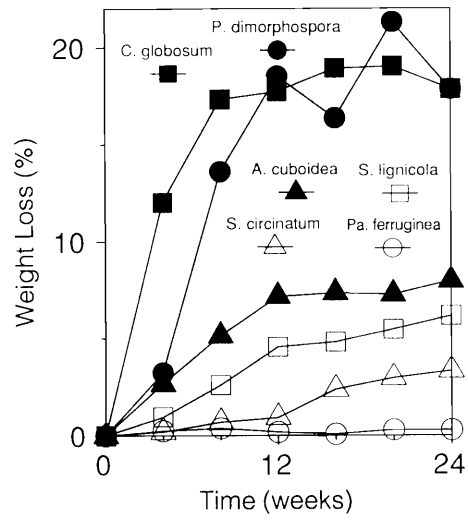

FIG. 1. Changes in weight loss of birch blocks during exposure to six soft-rot fungi. Blocks were incubated on a thin mesh over 20 mL reduced nutrient agar. Each point is the mean of 11 replicates.

For most fungi, the rate of weight loss of birch blocks decreased considerably after 12 weeks (Fig. 1). However, with *S. circinatum* the rate actually increased for a period after 12 weeks. *Pachnocybe ferruginea* caused no meaningful weight loss. On pine, the trends were the same (data not shown). With *S. lignicola* and *Phialocephala dimorphospora*, the rates increased after 12 weeks, but the maximum weight losses of pine caused by these fungi were around 2%.

Petri dish chambers resulted in significantly greater weight losses than did jars with four of five fungi; there was very little weight loss at all with the fifth fungus (Fig. 2). With pine, some of these differences were reversed, but no fungus caused mean weight loss of pine blocks greater than 2% in this experiment (data not shown).

Initial moisture content of birch blocks (using water to establish moisture content) had a statistically significant effect on weight loss with only two of four fungi, and the effect (Fig. 3) was only large with *Phialocephala dimorphospora*. Where there was a difference, initially wetter blocks had more weight loss. When nutrient solution was used to control moisture content, blocks at 100% MC experienced significantly greater weight loss than those at 50% MC with three of the four fungi. Moisture contents after decay were less extreme than the target moisture contents.

For most wood–fungus combinations, the reduced nutrient solution resulted in significantly higher weight losses than 2AS (Fig. 4). Although there is no reduced treatment for *Phialocephala dimorphospora* on pine, the same trend is evident from the nutrient solutions that were represented. However, *C. globosum* on birch was unique in showing the reverse: double Abrams' resulted in significantly greater weight losses than less concentrated solutions.

In the comparison of the vermiculite system using double Abrams' solution with the Petri dish system using reduced agar, results varied widely with the fungus and the wood (Table 2). For instance, *C. globosum* caused greater weight loss in the vermiculite system, but *A. cuboidea* tended to cause more in the agar system. *Phialocephala dimorphospora* on birch caused five times greater weight loss in the agar system than in vermiculite, but on pine the vermiculite system gave higher weight losses than agar.

After 6 weeks, birch blocks inoculated with *C. globosum*

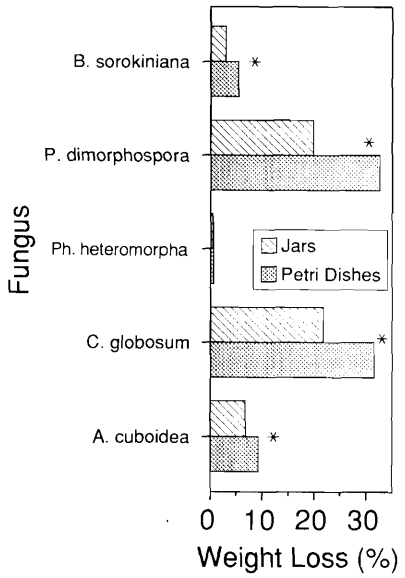

FIG. 2. Comparison of jars (containing 20 mL reduced nutrient agar) with Petri dishes (35 mL) as decay chambers, using thin mesh and birch blocks. Asterisks indicate significant differences according to a t-test ($P = 0.05$, $n = 11$).

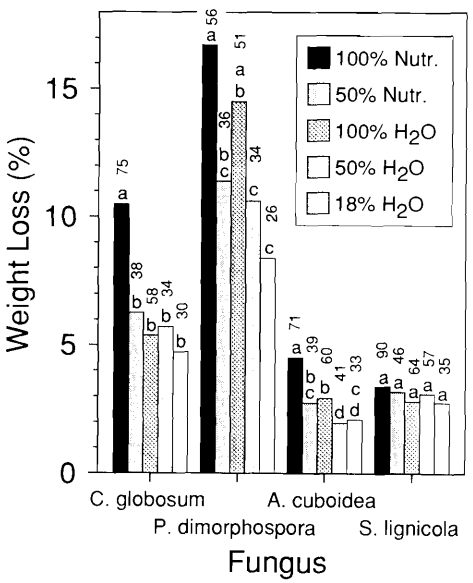

FIG. 3. Comparison of various methods of soaking birch blocks in a reduced nutrient agar system. Bars in a cluster with the same letter are not significantly different according to Scheffé's multiple-range test ($P = 0.05$, $n = 11$). Numbers over the bars are the postdecay moisture contents. Incubation time was 6.5 weeks.

experienced 15.6 and 7.9% weight losses with and without a vitamin and micronutrient amendment, respectively. There was no effect on the weight loss of pine blocks (2.2%).

When cutting tangential sections through the center of decayed birch blocks, we observed two distinct patterns of decay with some intergradation. The uniform pattern was characterized by decay distributed evenly throughout the interior of the blocks (Fig. 5). The superficial pattern was characterized by a distinct, narrow (ca. 1 mm) zone of decay at the surface of the block. In a variety of different experiments and treatments, the uniform pattern was associated with higher weight losses than was the superficial pattern for the same fungus (Table 3). The only

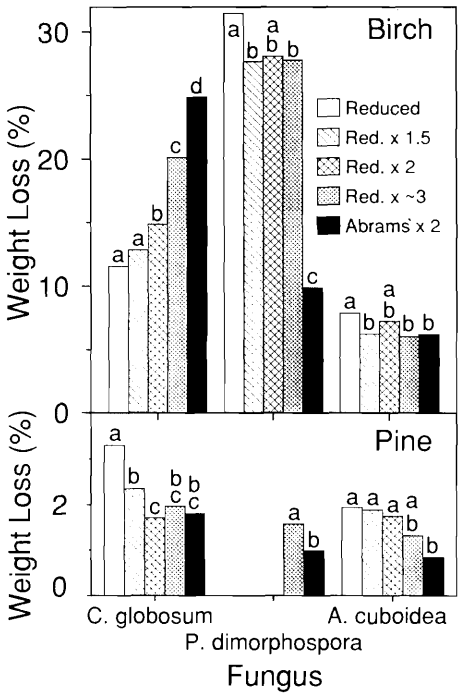

FIG. 4. Effect of various levels of nutrients on weight loss of birch and pine blocks by three fungi. Solutions are those described in Table 1. Bars in a cluster with the same letter are not significantly different according to Scheffé's multiple-range test ($P = 0.05$, $n = 11$).

exceptions, three blocks decayed in a uniform pattern by *Phialocephala dimorphospora*, were unique in that the nutrient solution used for them was 2AS. It was also observed that five of seven blocks exposed to *Phialocephala dimorphospora* on the thin mesh had the superficial pattern, while 10 of 10 on the thick mesh had the uniform pattern.

Discussion

Because weight losses generally tapered off after 12 weeks, we consider this a reasonable time period for experiments. It is also the recommended period in the standard soil-block test used for basidiomycetes (Anonymous 1981, 1986). However, because the rates of weight loss are generally constant up to 12 weeks, shorter periods could be used for comparative studies with little loss of information.

It must be recognized, however, that weight loss as normally measured may not be perfectly correlated with loss of wood mass. Fungal biomass, although often considered insignificant, has been shown to contribute up to 50% of postdecay dry weight (Swift 1973). Thus "weight loss" is always an underestimate of wood decay.

Contrary to expectations, initial moisture content had no strong impact on weight loss. However, the actual moisture contents at the end of the experiment were less extreme than the target moisture contents. Thus, the small overall difference in weight loss may reflect the small difference in moisture content. On the other hand, postdecay moisture contents ranging from 35 to 90% were associated with no significant differences in weight loss by *S. lignicola*.

In general, Petri dishes proved to be a better chamber for the agar system than the jars. This is most likely related to the 50% greater volume of nutrient agar available to the fungi in Petri dishes. Besides the added advantage of convenience, Petri dishes are probably more consistent in aeration: the gap between the lid and the chamber is more precisely controlled. Because the

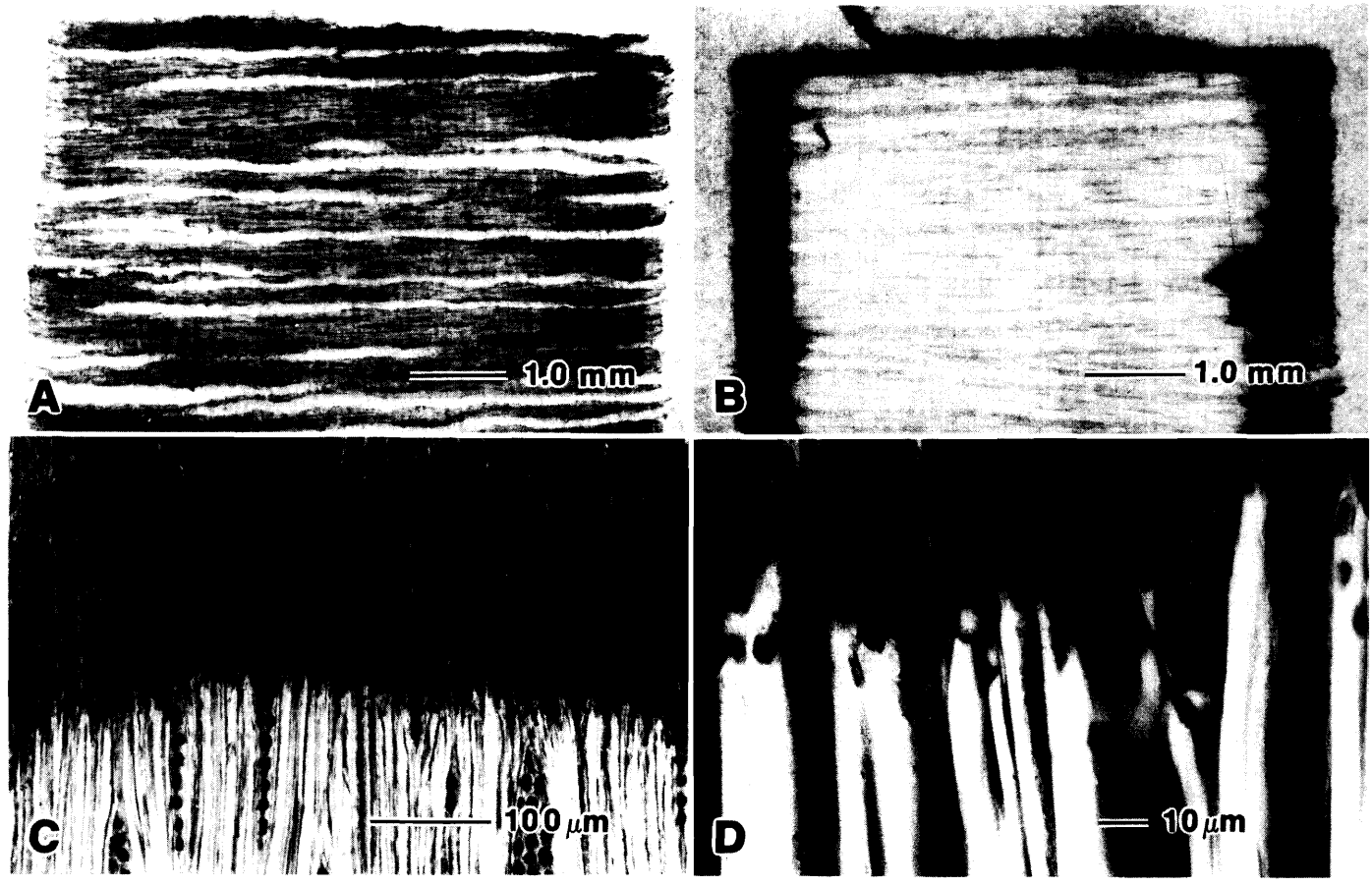

FIG. 5. Uniform and surface patterns of soft rot in tangential sections from the center of birch blocks decayed by *Phialocephala dimorphospora* P-109. (A) The uniform pattern of soft rot in a block supported by thick mesh during testing. Weight loss after 12 weeks was 30%. 13×. (B) Superficial soft rot in a block decayed on thin mesh. The dark soft-rotted zone extends 0.25–1.22 mm from the surface. Weight loss was 7%. 13×. (C) Superficial soft rot extending approximately 1.0 mm in a block decayed on thin mesh. The transition is abrupt between the outer zone, where the S2 cell wall layer has been almost totally eliminated, and the inner zone, where no cell wall damage is apparent. 160×. (D) Diamond-shaped cavities, typical of soft rot at the edge of the transition zone, 0.8 mm from the surface of a block decayed on thin mesh. 640×. (A and B) Epi-illumination of unstained microtome-cut sections, 16 μm thick, Zeiss dissecting microscope. (C and D) Nomarski differential interference contrast microscopy of unstained microtome-cut sections, 16 μm thick, Nikon Optiphot microscope.

TABLE 2. Comparison of weight losses (%) in a vermiculite system using double Abrams' solution with an agar – petri dish system using a reduced nutrient solution

	Birch		Pine	
	Vermiculite	Reduced agar	Vermiculite	Reduced agar
C. globosum	14.1	10.5*	5.1	2.2*
P. dimorphospora	2.9	16.7*	2.9	1.5*
A. cuboidea	2.1	4.5*	2.2	2.3
S. lignicola	nd	3.4	1.9	1.8

NOTE: Incubation period was 6.5 weeks. Each value in body of table is the mean of 11 replicates. nd, not determined.

*Significantly different from the corresponding vermiculite treatment according to a *t*-test ($P = 0.05$).

Parafilm that we have wrapped around Petri dishes as protection against desiccation and contamination has a tendency in some cases to crack, leading to potential variation among chambers, we perforate the film in several places on all plates at the outset.

The present results with an improved agar system confirm earlier results that suggested that nutrient concentrations in 2AS may be too high (Worrall and Wang 1991). It is not clear why these results differ from Duncan's (1965). She used different woods, different fungi, and the micronutrient–vitamin solution. Perhaps most importantly, she impregnated blocks with water rather than nutrient solution, so the concentrations of nutrients in the block were probably lower than in the bulk solution. Our results suggest an advantage in impregnating with the solution.

In agreement with Duncan's (1965) findings, the vitamin–micronutrient solution increased weight loss. We have not studied the effect on other fungi, but it is unlikely that the solution would be deleterious.

Two observations frequently made of soft rot are that it is

TABLE 3. Patterns of decay in representative birch blocks from various experiments and treatments

	Surface decay		Uniform decay	
	Conditions[a]	Weight loss (%)	Conditions[a]	Weight loss (%)
C. globosum	Agar, thin, R	18	Agar, thin, R	22
	Agar, thin, R	18	Agar, thin, R	32
			Agar, thick, R	19
			Vermiculite, 2AS	40
			Vermiculite, 2AS	25
P. dimorphospora	Agar, thin, R	12	Agar, thin, R	33
	Agar, thin, R	9	Agar, thin, R	32
	Agar, thin, R	12	Agar, thick, R	30
	Agar, thin, R	7	Agar, thick, R	20
	Agar, thin, R	10	Agar, thick, R	26
			Agar, thick, R	19
			Agar, thick, R	15
			Agar, thick, R	31
			Agar, thick, 1.5R	28
			Agar, thick, 2R	28
			Agar, thick, ~3R	28
			Agar, thick, 2AS	10
			Vermiculite, 2AS	10
			Vermiculite, 2AS	5

[a]Agar, agar support system in decay chamber; vermiculite, vermiculite support system; thin, blocks incubated over thin mesh; thick, blocks incubated over thick mesh. Abbreviations representing nutrient solutions in the support system are defined in Table 1.

generally restricted to the wood surface and it is most severe under very wet conditions (Findlay 1984). Oxygen availability under such conditions would virtually prevent growth and decay by aerobic fungi deep in the wood (Worrall and Parmeter 1983). Soft-rot fungi may have some advantage over basidiomycetes in establishment and colonization under such conditions (Duncan 1961), but those same conditions apparently restrict their activity to a narrow surface zone. Under conditions conducive to basidiomycetes, soft-rot fungi may be excluded by competition. On the other hand, because soft rot is less well known and more difficult to detect than decays caused by basidiomycetes, it may be simply overlooked in their presence.

In our experiments, the association of the uniform decay pattern with higher weight losses than the superficial pattern suggests that whatever conditions lead to the superficial pattern do not represent optimal conditions for development of soft rot. The only exceptions to this association were blocks decayed by *Phialocephala dimorphospora* in a uniform pattern but with relatively low weight losses. These blocks were decayed in the presence of 2AS, which we have found to be poorer than the reduced solution for this fungus.

Clearly, moisture content and related oxygen availability are the most likely factors determining the decay pattern and the associated magnitude of weight loss. Moisture content is notoriously difficult to control during studies of wood decay (Cartwright and Findlay 1958). Although Duncan (1960, 1965) concluded that relatively high moisture conditions are optimal, most of the experiments that apparently led to the conclusion used thin veneer strips rather than blocks. Kerner-Gang (1974) found that an intermediate volume of nutrient solution in her vermiculite system led to greater weight losses than higher or lower volumes. The postdecay moisture content of beech blocks at the optimal level was about 100%; that at the maximum level was 150%.

Our results suggest that an initial MC near saturation may be somewhat better than the lower levels, but under our conditions those moisture contents may moderate during the experiments. This is especially likely on a thick mesh support. For *Phialocephala dimorphospora* on birch, use of the thick mesh was generally associated with the uniform decay pattern and higher weight losses than the thin mesh. The thick mesh prevents direct contact of the block with the agar and reduces capillary uptake of water. Better aeration associated with the thick mesh apparently promotes a uniform decay pattern and higher weight losses.

These findings suggest that soft-rot fungi may not differ from basidiomycetes in their optimal decay under moderate moisture conditions. However, they may be unique in their ability to colonize and decay in a very narrow surface zone of near-saturated wood.

Acknowledgement

This work was supported in part by a research contract from the Electric Power Research Institute.

ANONYMOUS. 1981. Standard method of accelerated laboratory test of natural decay resistance of woods. Designation D 2017-81. American Society for Testing and Materials, Philadelphia, PA.

——— 1986. Standard method of testing wood preservatives by laboratory soil-block cultures. Method M10-77. *In* American Wood-Preservers' Association Standards. American Wood-Preservers' Association, Stevensville, MD.

BRAVERY, A. F. 1968. Determining the tolerance of soft-rot fungi to wood preservatives: a comparison of test methods. Mater. Org. **3**: 213–227.

CARTWRIGHT, K. ST. G., and FINDLAY, W. P. K. 1958. Decay of timber and its prevention. Her Majesty's Stationery Office, London.

DUNCAN, C. G. 1960. Wood-attacking capacities and physiology of soft-rot fungi. U.S. For. Prod. Lab. Rep. No. 2173.

——— 1961. Relative aeration requirements by soft rot and basidiomycete wood-destroying fungi. U.S. For. Prod. Lab. Rep. No. 2218.

——— 1965. Determining resistance to soft-rot fungi. U.S. For. Prod. Lab. Res. Pap. No. FPL-48.

FINDLAY, W. P. K. 1984. Soft rot of timber—a review. J. Indian Acad. Wood Sci. **15**: 1–11.

GERSONDE, M., and KERNER-GANG, W. 1976. A review of information available for development of a method for testing wood preservatives with soft-rot fungi. Int. Biodeterior. Bull. **12**: 5–13.

KERNER-GANG, W. 1974. Beitrag zur Moderfäuleprüfung in Vermiculit-Eingrabe-Verfahren. Mater. Org. **9**: 269–282.

MORRELL, J. J., and ZABEL, R. A. 1985. Wood strength and weight losses caused by soft-rot fungi isolated from treated southern pine utility poles. Wood Fiber Sci. **17**: 132–143.

SAVORY, J. 1954. Breakdown of timber by Ascomycetes and Fungi Imperfecti. Ann. Appl. Biol. **41**: 336–347.

SWIFT, M. J. 1973. The estimation of mycelial biomass by determination of the hexosamine content of wood tissue decayed by fungi. Soil Biol. Biochem. **5**: 321–332.

WANG, C. J. K., and ZABEL, R. A. (*Editors*). 1990. Identification manual for fungi from utility poles in the eastern United States. American Type Culture Collection, Publisher, Rockville, MD.

WORRALL, J. J., and PARMETER, J. R., JR. 1983. Inhibition of wood-decay fungi by wetwood of white fir. Phytopathology, **73**: 1140–1145.

WORRALL, J. J., and WANG, C. J. K. 1991. Importance and mobilization of nutrients in soft rot of wood. Can. J. Microbiol. **37**. This issue.

ZABEL, R. A., WANG, C. J. K., and ANAGNOST, S. E. 1991. Soft-rot capabilities of the major microfungi isolated from Douglas-fir poles in the Northeast. Wood Fiber Sci. **23**: 220–237.