In: Land disposal, remedial action, incineration and treatment of hazardous waste: Proceedings of the 13th annual research symposium; 1987 May 6-8; Cincinnati, OH. Cincinnati, OH: U. S. Environmental Protection Agency; 1987: 419–424.

GROWTH OF THE WHITE-ROT FUNGUS *PHANEROCHAETE CHRYSOSPORIUM* IN SOIL

Richard T. Lamar[1], Michael J. Larsen[1], T. Kent Kirk[1] and John A. Glaser[2]
[1]USDA Forest Products Laboratory, Madison, Wisconsin 53705
[2]Hazardous Waste and Engineering Research Laboratory, Cincinnati, Ohio 45268

ABSTRACT

Phanerochaete chrysosporium is a white-rot fungus with a demonstrated ability to degrade chlorinated organics in pure liquid culture to carbon dioxide. This ability suggests that the fungus may have potential as an *in situ* hazardous waste degrader. However, no data exist regarding the ability of *P. chrysosporium* to survive and grow in soil. That information is required for an effective evaluation of the ability of the fungus to degrade organo-pollutants *in situ*. The objective of this study was to investigate the influence of soil biotic and abiotic factors on survival and growth of the organism. This paper will summarize our research results to date on the effects of soil type, temperature, water potential and acidity on growth of the fungus in sterile soils.

INTRODUCTION

The fungus *Phanerochaete chrysosporium* Burds. (Burdsall and Eslyn 1974) (Basidiomycotina, Corticiaceae), has been shown to oxidize several halogenated aromatic pollutants to carbon dioxide and has been proposed as an agent for biological treatment of recalcitrant organo-halides (Eaton 1985; Bumpus *et al.* 1985; Huynh *et al.* 1985). On-site processing or *in situ* hazardous waste treatment methods that employ *P. chrysosporium* will require application of the fungus to soil for treatment of contaminants. *Phanerochaete chrysosporium* is a white-rot wood decay fungus that is often found in wood chip storage piles (Burdsall and Eslyn 1974). Its growth in soils has not been reported. Therefore, the ability of the fungus to grow in soils, as well as abiotic and biotic soil factors affecting fungal survival, growth and degradative ability must be determined before the possibility of developing a biological treatment method can be evaluated.

The investigations reported in this paper were designed to assess the effects of various soil types, water potentials and temperatures on growth of *P. chrysosporium* in sterile soils. Hazardous waste sites occur on a great variety of soil types. Soil water potential and temperature are major factors influencing both fungal growth and decomposition processes (Cooke and Rayner 1984). These parameters were chosen for the initial studies described here, because an assessment of their influence should yield information critical to future investigations into the degradative ability of *P. chrysosporium* in soil, and ultimately to the development of a biological treatment method.

Materials and Methods

Inoculum Preparation

Phanerochaete chrysosporium Burds. (BKM F-1767; ATCC no. 24725) was maintained at room temperature (25°C) on 2% malt agar slants (Kirk *et al.* 1978). Soil inoculum consisted of aspen (*Populus tremuloides* Michx.) pulpwood chips (1.5 x 0.5 x 0.25 cm^3) thoroughly grown through with *P. chrysosporium*. Chips were inoculated with mycelial suspensions of the fungus as follows: The fungus was cultured in 125-ml Erlenmeyer flasks containing 10 ml of basal medium (Kirk *et al.* 1978). Inoculum for these starter cultures consisted of conidial suspensions taken from two-week-old slants (Kirk *et al.* 1978). After incubating at 39°C for 1.5 d in Erlenmeyer flasks, mycelial mats were collected, washed with sterile deionized distilled water and fragmented in a

blender. The resulting slurry was resuspended in the volume of sterile deionized distilled water needed to adjust the moisture content of the aspen chips to 60% (dry weight basis). Aspen chips had been sterilized by autoclaving in aluminum foil-covered 2-L Erlenmeyer flasks at 121°C and 103.5 kPa., for 1 h. Mycelia were added to sterile chips at a rate of 0.02% mycelia:chips (w/w, dry weight basis). Inoculated chips were incubated at 39°C for approximately 3 wk.

Soils

Soil samples from the A horizons of Marsham sandy loam (Fine-silty, mixed, mesic, Mollic-Hapludalf), Xurich sandy loam (Fine-silty over sandy, mixed, mesic, Typic-Haplaquoll) and the B2t horizon of Batavia silty clay loam (Fine-silty, mixed, mesic, Typic-Hapludalf) were used in the present study. Soils were air-dried, passed through a 2 mm sieve and stored in 4-mil plastic bags at 4°C. Samples of each soil were analyzed for physical and chemical properties by Dr. M. F. Jurgensen of the Michigan Technological University, School of Forestry and Wood Products, Houghton, Michigan. These soils were chosen to represent ranges of texture, acidity and organic matter and nutrient contents. Results of soil chemical analyses are presented in Table 1.

Experimental Design

An experiment designed to test the influence of soil type, water potential and temperature on growth of P. chrysosporium in sterile soils consisted of a 3 x 3 x 3 factorial in a completely randomized design; the experiment was repeated once.

Moisture content of each soil was adjusted to levels corresponding to soil water potentials of -0.03, -0.15 and -1.5 MPa. Petri plates (20 x 90mm diam.) were filled with soil; within a soil type, an equal weight of soil on a dry weight basis was added to each plate. The weight of each filled plate was recorded and soils and plates sterilized by autoclaving for 0.5 h at 121°C and 103.5 kPa. Autoclaving was performed on each of three consecutive days to assure sterility; no contamination problems were encountered.

Soils were inoculated by aseptically placing an inoculum chip into the soil in the center of a plate. Controls were prepared by inoculating plates with inoculum chips which had been re-sterilized by autoclaving for 1 h at 121°C and 103.5 kPa. Plates were then placed in incubators held at 25°, 30° and 39°C. Soil water potentials were maintained daily by weighing each plate and adjusting, if necessary, with the appropriate amount of sterile deionized distilled water. Each run of the experiment was 2 weeks in duration.

In a separate experiment the effect of acidity on growth of *P. chrysosporium* in the Batavia soil was investigated. Appropriate amounts of $CaCO_3$ were added to Batavia soil to raise the pH 1 and 2 pH units above the original (pH 4.8). Studies showed that 1 g of $CaCO_3$ per kg of Batavia soil and a 2-week incubation period were required to raise the pH value of the soil 1 unit. After autoclaving, pH values were 4.8, 5.8 and 6.8. Soil water potential was adjusted to -0.03 MPa. Sterilization and inoculation procedures were as previously described. The experiment was 2 weeks long.

Growth Assessment

At the end of 2 wk, growth of P. chrysosporium was assessed using a qualitative rating system. This system was based on a visual assessment of the amount of growth by the fungus over the soil surface. The rating system was as follows:

Growth rating-description

0-no growth of fungus from chip into soil
l-some hyphae growing from chip into soil
2-some scattered hyphal growth in the vicinity of the chip plus some conidiation
3-medium coverage of soil surface by hyphae plus conidiation
4-medium-dense coverage of soil surface plus dense conidiation
5-dense coverage of soil surface plus dense conidia tion

A rank transformation approach was used to analyze the data. The entire set of observations (i.e. growth ratings) were ranked, with the smallest observation having rank 1, the second smallest rank 2, and so on. Average ranks were assigned ties (Conover and Iman 198 1). The transformed data were subjected to analysis of variance and reported as ranks of the growth assessment data. Soil type means were compared using Tukey's HSD method. All other treatment means were compared using single degree of freedom contrasts (Chew 1977).

Results and Discussion

Soil Type

Soil type greatly influenced growth of *P. chrysosporium* ($p = .0001$). Growth of the fungus was greatest in the Marsham, intermediate in the Xurich and least in the Batavia soil (Table 2). It is interesting to consider which factor(s) were responsible for the growth differences of *P. chrysosporium* among the three soils.

Soil acidity is one possibility. Cooke and Rayner

Table 1. Selected soil chemical properties of the three studied soils.

Soil type	CEC[1]	BS%	Acidity[2] B (pH)	Acidity[2] A (pH)	OM%	N%[3]	Ca (ppm)	Mg (ppm)	K (ppm)	P (ppm)	B (ppm)	Mn (ppm)	Zn (ppm)	S (ppm)
Batavia	22.9	29.5	5.06	4.65	0.5	0.05	1950	850	145	75	0.6	12.5	1.9	9.7
Marsham	50.7	66.4	7.13	6.59	12.0	0.46	4900	1650	90	17	1.3	9.0	12.2	11.3
Xurich	16.5	24.0	7.74	7.12	39.0	0.18	1675	640	80	17	0.8	77.5	6.4	3.9

[1]Determined using the Ammonium Acetate Method (Thomas 1982)
[2]B= before autoclaving; A= after autoclaving
[3]Determined using the Kjeldahl prodedure for Total N (Bremmer and Mulvaney 1982)

Table 2. Mean growth ranking of *Phanerochaete chrysosporium* in three soils.

Soil Type	Growth Ranking
Batavia	27c*
Marsham	80a
Xurich	57b

*Means followed by a different letter are significantly different (Tukey's HSD, $\alpha = .05$)

(1984) observed, however, that the influence of soil acidity on fungal growth is difficult to assess because of the ability of fungi to radically alter the pH value of their environment, and because the effects of acidity are modified by so many other environmental factors. It was not surprising, therefore, that growth of *P. chrysosporium*, which has an optimum of pH 5.5 in liquid culture (Kirk *et al.* 1978), was greater in the neutral to slightly acid Marsham and Xurich soils than in the strongly acid Batavia soil. If acidity were responsible for the poor growth in the Batavia soil, reducing soil acidity should have been beneficial. However, this was not the case ($p = .4523$). The failure of decreasing soil acidity in the Batavia soil to benefit fungal growth, and the observation that optimum growth was found in the slightly acid (*i.e.* pH 6.59) Marsham soil when the optimum for *P. chrysosporium* in liquid culture is pH 5.5, suggests that soil acidity does not play a significant role in mediating the growth of this fungus in soil. Evidently this organism, too, alters the pH value in the vicinity of its hyphae to create a favorable environment.

A second factor likely to influence growth is soil organic matter. The three studied soils differed greatly in this respect (Table 1). The ability of *P. chrysosporium* to assimilate the organic matter in the three soils is unknown. Given the magnitude of the differences, if organic matter content were the primary factor in controlling growth of *P. chrysosporium*, greatest growth would have been expected in the Xurich soil. Because this was not the case (Table 2), some factor other than organic matter content was controlling growth.

A third factor likely to influence growth is soil nitrogen content. In natural environments, this is the nutrient which is most likely to be in such a limited supply as to affect mycelial growth (Cooke and Rayner 1984). Nitrogen content varied greatly between the three soils studied. Regression of fungal growth on soil nitrogen content revealed a strong positive relationship ($R^2 = .924$) (Figure 1). The strength of this relationship suggests that soil nitrogen content played a significant role in mediating growth of *P. chrysosporium*.

Growth habit of the fungus was also affected by soil type. In the Marsham and Xurich soils, hyphae were observed to be growing primarily across the soil surface, although at the highest levels of growth it was evident from observing the bottom of the plates that the fungus had penetrated through the soil. In the Ba-

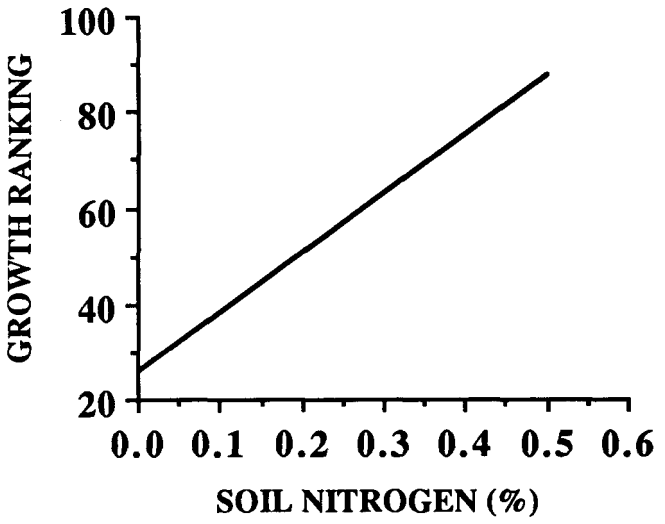

Figure **1.** Regression of growth ranking on soil nitrogen content for *Phanerochaete chrysosporium* grown in three soils ($y = 123.6x + 26.1$, $R^2 = .924$).

tavia soil, hyphae were not observed growing across the soil surface, but appeared to grow through the soil, surfacing at various locations.

There was evidence for an interaction between soil type and experimental run ($p = .0003$). This interaction was probably the result of better growth in the Marsham and Xurich soils in Run 2 than in Run 1 (Figure 2). During Run 1, relative humidity in the incubators was maintained at levels which resulted in appreciable daily soil moisture losses, particularly at 39 °C. During the second run, humidity levels in the incubators were increased and soil moisture losses greatly reduced. *Phanerochaete chrysosporium* might have been able to benefit from the increased incubator humidity levels during Run 2 by obtaining moisture directly from the atmosphere, or loss of hyphal vigor due to partial desiccation could have been prevented. Either might explain the significantly greater growth of the fungus in the Marsham and Xurich soils during Run 2. Because *P. chrysosporium* did not grow on the soil surface in the Batavia soil it would not be expected to benefit as much from an increase in atmospheric moisture. Indeed, growth of the fungus in the Batavia soil during Run 2 was not significantly different from that in Run 1 (Figure 2). In light of the above, the data from each run were analyzed separately to assess the effect of soil water potential on growth of *P. chrysosporium.*

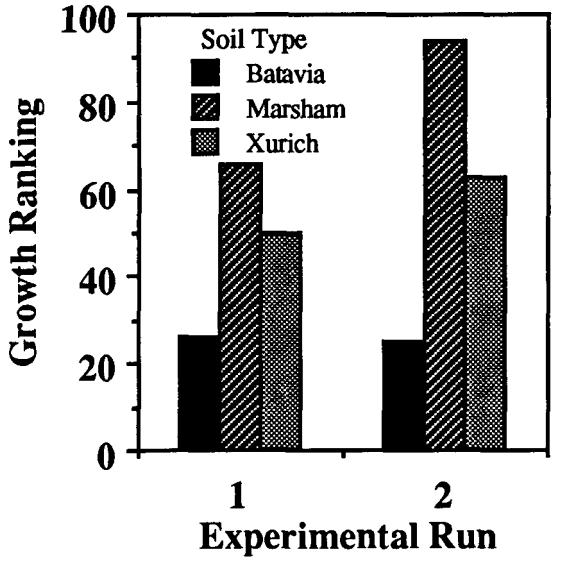

Figure 2. Growth ranking of *Phanerochaete chrysosporium* in three soils by experimental run.

Soil Water Potential

A significant ($p = 0.0002$) linear relationship between growth of *P. chrysosporium* and soil water potential over all soils was observed in Run 1 (Figure 3). In contrast, soil water potential had less influence on growth in Run 2 ($p = .1399$). As indicated above, however, the fungus growing in the Marsham and Xurich soils might have benefited from increased levels of atmospheric moisture which effectively masked the influence of soil water potential on growth. In the Batavia soil where surface growth of the fungus was sparse, there was a significant ($p = 0.0017$) linear relationship between growth of the fungus and soil water potential (Fig. 4).

The linear increase in growth of *P. chrysosporium* in response to increasing water potential over the range

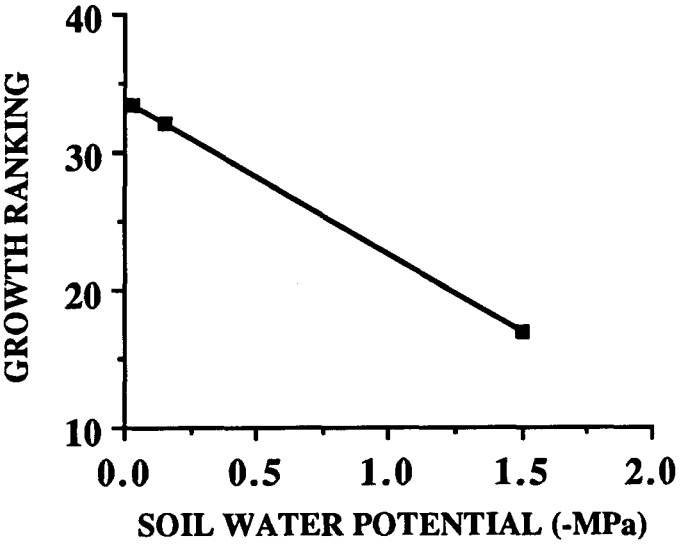

Figure 3. Regression of growth ranking on soil water potential for *Phanerochaete chrysosporium* grown in three soils-Run 1 ($y = 1.119x + 33.767$, $R^2 = .233$).

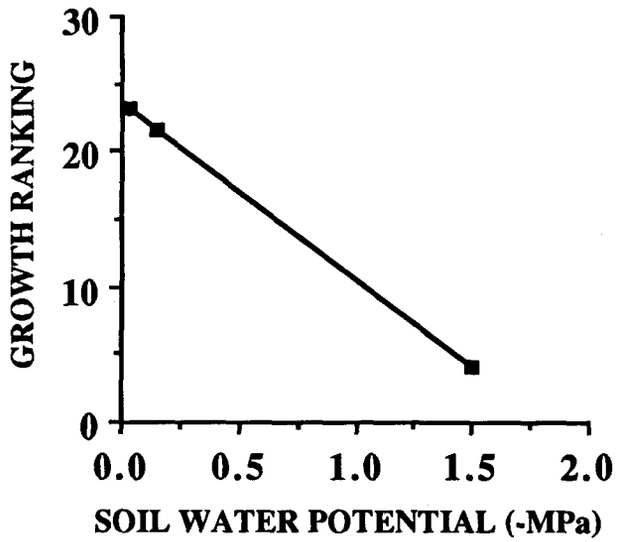

Figure 4. Regression of growth ranking on soil water potential for *Phanerochaete chrysosporium* grown in the Batavia soil-Run 2 ($y = 1.299x + 23.551$, $R^2 = .47$)

-1.5 to -0.03 MPa is consistent with what other workers have observed for both wood decay and soil fungi in general. Griffin (1977) proposed that growth of wood decay fungi occurs at a decreasing rate from 0 to -4.0 MPa, and presented data indicating that maximum growth of five wood decay basidiomycetes occurred at -0.15 MPa osmotic potential. Wood decay fungi have been placed in a group of organisms, which includes some soil basidiomycetes and gram-negative bacteria, that is extremely sensitive to water potential, with an optimum growth response *ca.* -0.1 MPa osmotic potential, and little growth below -2.0 MPa (Griffin 1981). The optimum soil water potential range for microbial growth and metabolic activity is generally considered to be between -0.01 and -0.03 MPa (Sommers *et al.* 1981). The soil water potential growth optimum for *P. chrysosporium* might be greater than -0.03 MPa.

Sommers *et al.* (1981) suggested that water potential influences decomposition of soil organic materials in a 2-phase process whereby an initial rapid decrease in decomposition in the -0.03 to -1.5 MPa matric potential range is followed by a second phase in which decomposition decreases linearly with decreasing water potential. Therefore, soil water potential optima for growth of, and degradative activity by, *P. chrysosporium* may be very similar.

Temperature

Evidence was obtained for better growth of *P. chrysosporium* at 30° and 39°C compared to growth of the fungus at 25°C (Table 3). The magnitude of this difference appeared to be directly related to the relative growth among soil types. Most soil fungi are mesophiles with temperature optima between 25° and 35°C, but with an ability to grow from *ca* 15° to 45°C (Cooke and Rayner 1984). *Phanerochaete chrysosporium* is also mesophilic and has a temperature optimum of *ca.* 39°C on 2% malt agar (Burdsall and Eslyn 1974). Ability of the fungus to grow as well at 30°C as it did at 39°C was not expected. It is possible that at 39°C some other factor (e.g. nutrient availability or water stress) offsets the benefits of the more optimal temperature. However, *P. chrysosporium* did not appear to exploit the total soil volume in any of the plates, indicating that nutrient pools were also not fully exploited. Studies are underway to determine the minimum temperature at which *P. chrysosporium* will grow in the soil.

Table 3. Effect of soil temperature on growth ranking of *Phanerochaete chrysosporium* in three soils.

Soil	Soil Temperature (°C) 39°	30°	25°	P*
Batavia	28	31	21	.1198
Marsham	86	89	66	.0170
Xurich	64	59	47	.0531

*** Probability of a larger F value for the single degree of freedom contrast of growth at 30° and 39°C versus growth at 25°C.**

Conclusions

All parameters investigated, except acidity, influenced growth of *P. chrysosporium* in the three soil types. Soil type had a significant effect on growth and growth habit. Nitrogen content appeared to play a major role in mediating growth of the fungus in the soils, and could well have been responsible for the differences in fungal growth among the soil types. Future studies to elucidate the extent to which available nitrogen influences growth of *P. chrysosporium* in soil are warranted. Increasing soil water potential from -1.5 MPa to -0.03 MPa resulted in greatly increased growth of P. *chrysosporium.* The data indicate that growth of the fungus might benefit from soil water potentials above-0.03 MPa. Growth of the fungus was significantly greater at 30° and 39°C than at 25°C.

Acknowledgments

The authors wish to thank C. B. Davey for critical review of the manuscript and D. M. Dietrich for technical assistance.

Literature Cited

1. Bremmer, J.M. and C.S. Mulvaney. 1982. Nitrogen-Total. In: *Methods and Principles of Soil Analysis, Part 2. Chemical and Microbiological Properties-Agronomy Monograph* no. 9 (2nd Edition).

2. Bumpus, J.A., M. Tien, D. Wright and S.D. Aust. 1985. Oxidation of persistent environmental pollutants by a white rot fungus. *Science* 228:1434-1436.

3. Burdsall, H.H., Jr. and W.E. Eslyn. 1974. A new *Phanerochaete* with a *Chrysosporium* imperfect state. *Mycotaxon* 1:123-133.

4. Chew, V. 1977. Comparisons among treatment means in analysis of variance. *Agric. Res. Ser. H-6*. 60 pp.

5. Conover, W.J. and R. L. Iman. 1981. Rank transformations as a bridge between parametric and non-parametric statistics. *American Statistician* 35(3):124-129.

6. Cooke, R.C. and A.D.M. Rayner. 1984. *Ecology of saprophytic fungi*. New York:Longman 415 pp.

7. Eaton, D.C. 1985. Mineralization of polychlorinated biphenyls by *Phanerochaete chrysosporium* : a ligninolytic fungus. *Enzyme Microb. Technol.* 7:194-196.

8. Griffin, D.M. 1981. Water and microbial stress. *Adv. Microbial Ecol.* 5:91-136.

9. Griffin, D.M. 1977. Water potential and wood decay fungi. *Ann. Rev. Phytopathology* 15:319-329.

10. Huynh, V.-B., H.-M. Chang, T.W. Joyce and T.K. Kirk. 1985. Dechlorination of chloro-organics by a white-rot fungus. *Tappi J.* 68:98-102.

11. Kirk, T.K., E. Schultz, W.J. Connors, L.F. Lorenz and J.G. Zeikus. 1978. Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. *Arch. Microbiol.* 117:277-285.

12. Seitz, L. M., D. B. Sauer, R. Burroughs, H. E. Mohr and J. D. Hubbard. 1979. Ergosterol as a measure of fungal growth. *Phytopathology* 69:1202-1203.

13. Sommers, L.E., C.M. Gilmour, R.E. Wilding and S.M. Beck. 1981. The effect of water potential on decomposition processes in soils. pp. 97-118. In: *Water potential relations in soil microbiology.* Soil Sci. Soc. Am. Spec. Pub. No. 9.

14. Thomas, G.W. 1982. Exchangeable cations. In: Methods of Soil Analysis, Part 2. *Chemical and Microbiological Properties-Agronomy Monograph* no. 9 (2nd Edition).