Amer. J. Bot. 62(8): 838–841. 1975.

# GROWTH OF PHELLINUS (PORIA) WEIRII ON DIFFERENT VITAMINS AND CARBON AND NITROGEN SOURCES[1]

C. Y. Li and W. B. Bollen

Forestry Sciences Laboratory, Pacific Northwest Forest and Range Experiment Station, USDA Forest Service, Corvallis, Oregon 97331

ABSTRACT

Mycelial growth of *Phellinus weirii* (Murr.) Gilb., a root pathogen of conifers in the Pacific Northwest, was studied in defined liquid media with different carbon and nitrogen sources and vitamins. The fungus grew significantly more on glucose, xylose, maltose, or fructose than on other carbon sources. Starch did not support growth. Maximum growth occurred in 4 weeks on all carbon sources except dextrin and sucrose, with which maximum growth occurred in 3 weeks. Of the nitrogen sources, peptone supported the best growth of the fungus; glutamic acid, serine, aspartic acid, alanine, leucine, ammonium sulphate, and urea supported significantly better growth than tyrosine, arginine, methionine, threonine, and glycine. Potassium nitrate, phenylalanine, sodium nitrate, lysine, proline, and cysteine inhibited growth. Thiamine hydrochloride was not absolutely required for growth of *P. weirii*, but better growth was obtained with its addition. The fungus showed no significant responses to a range of other vitamins.

Phellinus weirii (Murr.) Gilb. [*Poria weirii* (Murr.) Murr.] is recognized as a widespread and highly destructive pathogen on conifer roots and is responsible for the extensive killing of immature Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] in the Pacific northwestern United States. Although the disease has been studied by several investigators (Buckland, Molnar, and Wallis, 1954; Mounce, Bier, and Nobles, 1940; Childs and Nelson, 1971; Wallis and Reynolds, 1962, 1965) little has been reported on its nutritional requirements. Therefore, we initiated studies on its carbon, nitrogen, and vitamin requirements in chemically defined media; such information is essential for interpreting the complex interactions between *P. weirii*, its substrate, and antagonists in relation to biological control of the disease.

Materials and methods—The experiments were conducted with 250-ml erlenmeyer flasks containing 50 ml of Trione's (1964) synthetic medium, modified by substitution with compounds to be tested. Culture T-124 of *P. weirii*, used for these studies, had been isolated from an infected Douglas fir near Quilcene, Washington, more than 5 years earlier. It has been maintained as a stock culture on malt agar.

For the experiment with carbon sources, the sucrose of the original medium formulation was replaced with a 0.4% carbon equivalent of D-arabinose, D-xylose, D-glucose, D-fructose, maltose, sucrose, lactose, dextrin, starch, or cellulose, respectively. Total carbon in the dextrin, starch, and cellulose had been previously determined by means of dry combustion. Dextrin, starch, and cellulose were sterilized by ether (Shirling and Gottlieb, 1966) and other carbon sources by filtration through ultrafine porosity fritted discs.

In experiments with nitrogen sources, the sucrose of the original formulation was replaced by 10 g glucose, and asparagine was replaced with 0.06% nitrogen equivalent of ammonium sulphate, potassium nitrate, sodium nitrate, urea, peptone, or L-form amino acids, respectively. The L-form amino acids included alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, and tyrosine. The nitrogen sources were sterilized by Millipore filter discs of 0.22 μm pore size.

Experiments with vitamins were preceded by precautions to eliminate traces of vitamins. Flasks were washed in 3.0-N HCl and rinsed with double distilled water. The inoculum was prepared by subculturing in vitamin-free medium for 20 days. The thiamine HCl of the original medium formulation was replaced respectively with biotin, 5 μg; calcium pantothenate, 150 μg; folic acid, 20 μg; *i*-inositol, 5 mg; niacin, 150 μg; pyridoxine HCl, 100 μg; riboflavin, 200 μg, or thiamine HCl, 1.0 mg. A medium containing a mixture of vitamins at the same concentrations was also included to determine if multiple vitamin deficiency exists for *P. weirii*. All vitamins were sterilized by

[1] Received for publication 13 August 1974.

Dr. Bollen is also Professor of Microbiology, Oregon State University, Corvallis 97331.

This study was supported in part by National Science Foundation Grant GB-18050.

[2] Use of trade names does not imply endorsement to the exclusion of similar products.

TABLE 1. *Growth of Phellinus weirii with different compounds as sole sources of carbon*

| | Mycelium dry weight[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2 weeks | | 3 weeks | | 4 weeks | | 5 weeks | | 6 weeks | |
| Carbon source | mg | Final pH | mg | Final pH | mg | Final pH | mg | Final pH | mg | Final pH |
| D-glucose | 12.2 | 4.5 | 128.3 | 5.9 | 155.5[b] | 5.1 | 81.3 | 7.3 | 67.5 | 7.2 |
| D-xylose | 15.0 | 4.7 | 116.4 | 5.8 | 155.0 | 5.3 | 77.6 | 7.4 | 72.9 | 7.4 |
| Maltose | 44.4 | 5.8 | 73.7 | 4.5 | 125.5 | 5.2 | 123.1 | 5.2 | 98.4 | 6.6 |
| D-fructose | 11.6 | 4.6 | 80.0 | 6.0 | 124.6 | 5.1 | 104.6 | 5.6 | 66.0 | 7.3 |
| Cellulose | 47.4 | 5.0 | 60.0 | 5.9 | 87.8 | 6.6 | 58.3 | 7.4 | 40.7 | 7.2 |
| Dextrin | 39.0 | 4.8 | 162.8 | 5.5 | 85.1 | 7.4 | 56.8 | 7.1 | 51.0 | 7.2 |
| Sucrose | 3.8 | 4.8 | 56.5 | 6.4 | 56.1 | 6.9 | 54.3 | 7.4 | 16.3 | 7.0 |
| D-arabinose | 3.9 | 4.8 | 5.5 | 6.3 | 35.9 | 7.1 | 25.6 | 6.5 | 17.7 | 6.7 |
| Lactose | 2.5 | 4.9 | 3.2 | 6.0 | 26.0 | 6.8 | 17.1 | 6.5 | 2.7 | 6.3 |
| Starch | 0.7 | 5.1 | 2.6 | 5.4 | 6.7 | 5.8 | 4.1 | 5.5 | 4.2 | 5.8 |
| Control | 0.7 | 5.6 | 2.7 | 5.9 | 6.5 | 6.7 | 4.1 | 6.2 | 4.3 | 6.3 |

[a] Average weight of three replicates; initial *p*H 5.3.
[b] Values along the same line in the 4-weeks column do not differ significantly at the 0.01 confidence level.

means of Millipore[2] filtration. Flasks were covered with sterile aluminum foil.

For inoculation, mycelium was washed three times in double distilled water, fragmented in a Waring blender, and suspended in double distilled water. Each flask was inoculated with 0.1 ml of suspension. After still incubation at room temperature, 23 ± 1 C, mycelia in the carbon source experiment were harvested at the end of the first through the sixth weeks. On the basis of the maximum weekly growth rates shown by that experiment, all mycelia from the nitrogen source and vitamin experiments were harvested after 25 days. Mycelia were harvested on previously weighed No. 42 Whatman filter paper and oven-dried at 85 C for 2 days. The *p*H of all media was measured before inoculation and at the end of the experiment.

Data were submitted to analysis of variance with Scheffé's test on comparisons among compounds at the 99 % confidence level.

RESULTS AND DISCUSSION—*Phellinus weirii* utilized all carbon sources except starch (Table 1). At 4 weeks the mycelial yields were highest in glucose, xylose, maltose, and fructose, and were significantly less in cellulose, dextrin, and sucrose. The fungus grew least in arabinose and lactose. Maximum growth occurred in 3 weeks with dextrin and sucrose and in 4 weeks with all other carbon sources; after this, growth declined. The cessation of growth and loss of mycelial weight following the maximum growth period were probably due to the depletion of carbon sources and autolysis of mycelium. The nonutilization of starch by *P. weirii* may be due in part to the molecular configuration of the starch used and to the type of glycosidic linkage between the units of the molecules (Cantino, 1949). *Coprinus comatus* and *Pestalotia gracilis* are other higher basidiomycetes reported unable to utilize starch as a carbon source (Fries, 1955; Yuself and Allan, 1967).

Of the nitrogen sources, peptone gave by far the highest mycelial yield for *P. weirii* (Table 2). Glutamic acid, serine, and aspartic acid supported significantly better growth than the remaining nitrogen sources. Histidine did not differ from controls. Potassium nitrate, phenylalanine, sodium nitrate, lysine, proline, and cysteine were not utilized and, in fact, produced significantly less growth than controls. The findings confirm previous results (Li et al., 1967) that *P. weirii* does not use nitrates but does use ammonium and certain amino nitrogens.

Thiamine hydrochloride was not absolutely required for growth of *Phellinus*, but 1 mg per liter increased fungal growth (Table 3); the other vitamins in the media did not significantly affect *Phellinus*.

The ability of *P. weirii* to establish on healthy roots in situ is dependent on the availability of a food base (Wallis and Reynolds, 1962). No attempts were made to characterize chemical substances in conifer roots. Holmes and Kurth (1961) revealed that inner bark of Douglas fir

TABLE 2. *Growth of Phellinus weirii with different compounds as sources of nitrogen*

| Nitrogen source | Mycelium dry weight (mg) [a] | Final pH |
|---|---|---|
| Peptone | 261.7 [b] | 3.7 |
| Glutamic acid | 98.4 | 5.5 |
| Serine | 96.4 | 3.6 |
| Aspartic acid | 88.0 | 4.4 |
| Alanine | 83.3 | 3.7 |
| Leucine | 77.0 | 3.7 |
| Ammonium sulphate | 75.5 | 2.8 |
| Urea | 70.0 | 3.5 |
| Tyrosine | 25.7 | 3.9 |
| Arginine | 22.5 | 3.7 |
| Methionine | 17.9 | 4.3 |
| Threonine | 15.4 | 4.4 |
| Glycine | 13.4 | 4.1 |
| Control | 4.8 | 4.0 |
| Histidine | 3.1 | 5.3 |
| Potassium nitrate | 2.6 | 4.2 |
| Phenylalanine | 2.2 | 5.8 |
| Sodium nitrate | 1.7 | 5.8 |
| Lysine | 1.2 | 5.7 |
| Proline | 1.2 | 5.8 |
| Cysteine | 0 | 4.2 |

[a] Average weight of four replicates; initial *p*H 5.3.
[b] Values along the same line do not differ significantly at the 0.01 confidence level.

TABLE 3. *Growth of Phellinus weirii with various vitamins*

| Vitamin | Mycelium dry weight (mg) [a] | Final pH |
|---|---|---|
| Thiamine HCl (1 mg/1) | 58.3 [b] | 5.7 |
| Mixture of vitamins | 54.9 | 5.4 |
| Riboflavin (200 μg/1) | 36.2 | 5.0 |
| Calcium pantothenate (150 μg/1) | 35.1 | 5.0 |
| Niacin (150 μg/1) | 34.7 | 4.9 |
| Control | 34.6 | 5.0 |
| Inositol (5 mg/1) | 34.4 | 4.7 |
| Biotin (5 μg/1) | 33.0 | 4.7 |
| Folic acid (20 μg/1) | 32.2 | 4.7 |
| Pyridoxine HCl (100 μg/1) | 30.0 | 5.0 |

[a] Average weight of four replicates; initial *p*H 5.3.
[b] Values along the same line do not differ significantly at the 0.01 confidence level.

contained isoleucine, valine, *Phellinus*-stimulating glutamic acid, serine, aspartic acid, alanine, leucine, threonine and glycine. It also contained proline that *P. weirii* cannot utilize. Glucose, fructose, and sucrose, good carbon sources for *Phellinus*, were also present. Variation in the chemical contents of conifer roots is suspected of influencing the susceptibility of trees to attack by this pathogen; tree species differ markedly in carbon and nitrogen constituents in tissues (Barnes, 1963; Smith and Zavarin, 1960).

*Phellinus weirii* colonizes root and stem wood of conifers extensively, but no conclusive evidence yet exists on the degree to which it can grow freely through soil. Its isolation from soil is difficult because of competition from other faster-growing organisms when standard media are used. The results reported here suggest ways for improving media used for ecological studies of *P. weirii* with various combinations of carbon or nitrogen sources and phenolic compounds that favor *P. weirii* but suppress other organisms (Li et al., 1969). Moreover, the role of this fungus in carbon and nitrogen cycling in coniferous ecosystems can now be inferred from data rather than speculation only.

## LITERATURE CITED


BARNES, R. 1963. Organic nitrogen compounds in tree xylem sap. For. Sci. 9: 98–102.

BUCKLAND, D. C., A. C. MOLNAR, AND G. W. WALLIS. 1954. Yellow laminated root rot of Douglas fir. Can. J. Bot. 32: 69–81.

CANTINO, E. C. 1949. The physiology of the aquatic phycomycete *Blastocladia pringsheimii* with emphasis on its nutrition and metabolism. Amer. J. Bot. 36: 95–112.

CHILDS, T. W., AND E. E. NELSON. 1971. Laminated root rot of Douglas fir. U.S. Dep. Agric. For. Pestlet 48.

FRIES, L. 1955. Studies in the physiology of *Coprinus*. I. Growth substance, nitrogen and carbon requirements. Sven. Bot. Tidskr. 49: 475–535.

HOLMES, G. W., AND E. F. KURTH. 1961. The chemical composition of the newly formed inner bark of Douglas-fir. Tappi 44: 893–898.

LI, C. Y., K. C. LU, J. M. TRAPPE, AND W. B. BOLLEN. 1967. Selective nitrogen assimilation by *Poria weirii*. Nature 213: 814.

LI, C. Y., K. C. LU, E. E. NELSON, W. B. BOLLEN, AND J. M. TRAPPE. 1969. Effect of phenolic and other compounds on growth of *Poria weirii* in vitro. Microbios 3: 305–311.

MOUNCE, I., J. E. BIER, AND M. K. NOBLES. 1940. A root rot of Douglas fir caused by *Poria weirii*. Can. J. Res. 18: 522–533.

SHIRLING, E. B., AND D. G. GOTTLIEB. 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313–340.

SMITH, L. V., AND E. ZAVARIN. 1960. Free mono- and oligosaccharides of some California conifers. Tappi 43: 218–221.

TRIONE, E. J. 1964. Isolation and in vitro culture of the wheat bunt fungi *Tilletia caries* and *T. controversa*. Phytopathology 54: 592–596.

WALLIS, G. W., AND G. REYNOLDS. 1962. Inoculation of Douglas fir roots with *Poria weirii*. Can. J. Bot. 40: 637–645.

———, AND ———. 1965. The initiation and spread of *Poria weirii* root rot of Douglas fir. Can. J. Bot. 43: 1–9.

YUSELF, H. M., AND M. E. ALLAN. 1967. The carbon and nutrition of certain fungi. Can. J. Microbiol. 13: 1097–1106.