United States
Department of
Agriculture

Forest Service

Pacific Northwest
Forest and Range
Experiment Station

Research Note
PNW-428
October 1985

Maintaining Cultures of Wood-Rotting Fungi

E.E. Nelson and H.A. Fay

Abstract

Phellinus weirii cultures were stored successfully for 10 years in small alder (*Alnus rubra* Bong.) disks at 2 °C. The six isolates tested appeared morphologically identical and after 10 years varied little in growth rate from those stored on malt agar slants. Long-term storage on alder disks reduces the time required for maintaining cultures and the risk of contamination inherent in frequent transfers necessary in storage on agar slants.

Keywords: Root rot, *Phellinus weirii,* cultures, laboratory methods, storage methods, cold storage.

Mycologists, plant pathologists, and others have often wished that cultures of fungi could be kept indefinitely in the laboratory ready to use, with little or no maintenance, and without altering their physiology or structure. Until recently, our culture collection, mainly isolates of *Phellinus weirii* (Murr.) Gilbertson, was kept at 2-5 °C on malt agar slants and transferred to fresh media annually. Isolates appeared normal 15 or more years, but the procedure was time consuming and risk of contamination of the cultures was greater than seemed necessary.

We propose an alternative that requires infrequent attention. Five isolates of *P. weirii* from roots of mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.) growing at high elevations in the Cascade Range in Oregon, and five isolates from roots of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) growing at low elevations in western Oregon and Washington were compared for their abilities to survive over a range of temperatures (Nelson and Fay 1975). With the same procedures used in that study, we have maintained cultures of *P. weirii* for more than 10 years at 2 °C. Thin sections of red alder (*Alnus rubra* Bong.) stemwood were placed over gravel and water in loose-capped, 60-ml-capacity French Square bottles; they were autoclaved and seeded with a small agar plug of the desired fungal isolate. Once the fungus had colonized the disks, bottles were tightly capped and maintained at a constant temperature. We successfully isolated *P. weirii* from all bottles of 6 of the original 10 isolates maintained at 2 °C for 10 years. We used malt agar containing 1 p/m benomyl as our isolation medium.

E.E. NELSON is a research plant pathologist and H.A. FAY is a biological laboratory technician at the Pacific Northwest Forest and Range Experiment Station, Forestry Sciences Laboratory, 3200 Jefferson Way, Corvallis, Oregon 97331.

After 10 years storage, we compared the growth rate and general appearance of the colonies of the six isolates maintained on alder disks with the growth rate and general appearance of the colonies of the same isolates maintained over the same period on malt agar slants. Subcultures of each isolate from both methods of storage were grown in 1.5 percent malt agar in individual petri plates incubated in the dark at 5, 10, 15, 20, 25, and 30 °C. Four replicates of the six isolates from both storage methods were measured periodically, daily in most cases. Colony diameter was plotted over time for each temperature, and slope of maximum growth was determined. Isolates stored in alder disks generally had slightly slower rates of growth than those stored on malt agar slants (fig. 1). Analysis of variance determined that these differences, though small, were significant ($P = 0.05$), as were differences in growth among isolates. We noted no differences in morphology of an isolate stored on disks vs. those stored on slants. No pathogenicity tests were made.

We have not used this means of storing cultures of other fungi; however, there is reason to expect the technique to be applicable to cultures of other species of wood-rotting fungi. We suggest that others explore this alternative to frequent periodic maintenance of their culture collections of wood-rotting fungi.

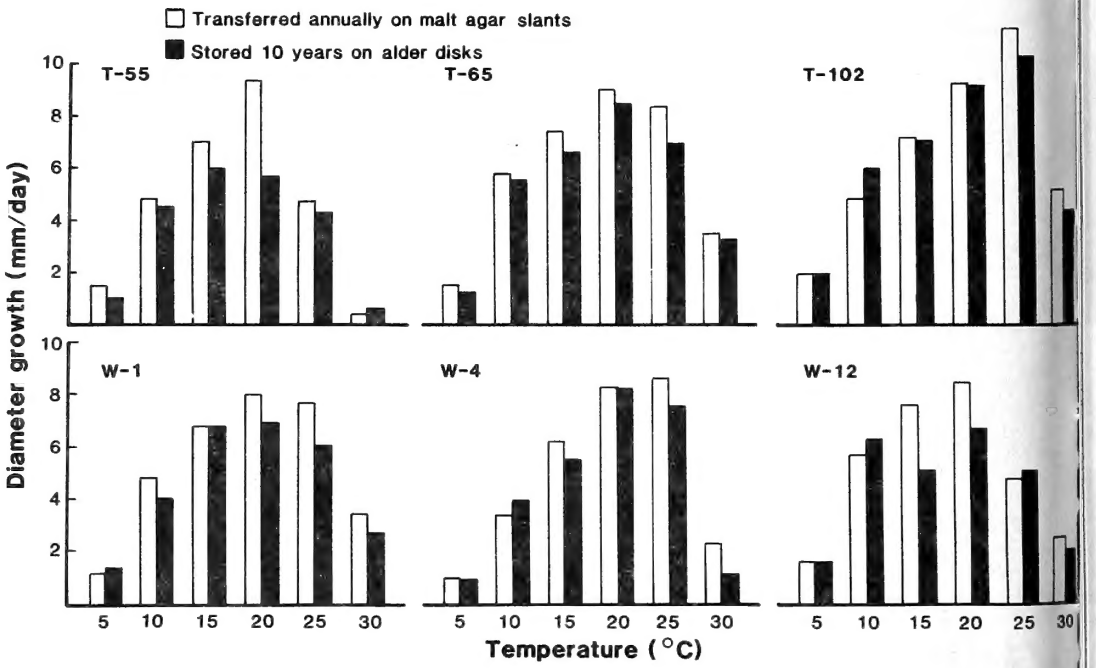

Figure 1.—Maximum rates of growth of *Phellinus weirii* isolates by two storage methods; three isolates are from a high elevation mixed conifer forest (W), and three are from a lower elevation Douglas-fir forest (T).

English Equivalents

1 milliliter (ml) = 0.001056 quart
1 millimeter (mm) = 0.0394 inch
°C = 5/9 (°F - 32)

Literature Cited

Nelson, E.E.; Fay, H.A. Effect of temperature on growth and survival of high- and low-elevation isolates of *Phellinus (Poria) weirii.* Northwest Science. 49: 119-121; **1975.**

The **Forest Service** of the U.S. Department of Agriculture is dedicated to the principle of multiple use management of the Nation's forest resources for sustained yields of wood, water, forage, wildlife, and recreation. Through forestry research, cooperation with the States and private forest owners, and management of the National Forests and National Grasslands, it strives — as directed by Congress — to provide increasingly greater service to a growing Nation.

Pacific Northwest Forest and Range
Experiment Station
319 S.W. Pine St.
P.O. Box 3890
Portland, Oregon 97208