PACIFIC NORTHWEST

U. S. FOREST SERVICE

Research Note

FOREST AND RANGE EXPERIMENT STATION · U.S. DEPARTMENT OF AGRICULTURE · PORTLAND, OREGON

PNW-66

October 1967

EFFECT OF pH AND TEMPERATURE ON GROWTH OF *PORIA WEIRII* IN VITRO[1/]

by

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Our studies on the possible role of *Alnus rubra* Bong. in biological control of *Poria weirii* Murr., a destructive pathogen of conifer roots in western North America, have occasionally been thwarted by physiological inactivity of the fungus under certain laboratory conditions. Suspecting either temperature or pH of the medium to be limiting and not finding literature an optima for *P. weirii*, we conducted the studies reported here using the single isolate with which we were concerned.

1/ Research was supported in part by National Science Foundation Grant No. GB-3214.

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The liquid medium developed by Trione (*6*)[3/] was used, except that 10 g. of glucose per liter was substituted for 20 g. of sucrose. The chemical compositions of the medium were as follows:

KH_2PO_4	613	mg.
$MgSO_4 \cdot 7\ H_2O$	246	mg.
$K_2HPO_4 \cdot 3\ H_2O$	114	mg.
$CaCl_2$	55.5	mg.
Sodium ferric diethylene-triaminepentaacetate	20	mg.
$ZnSO_4 \cdot 7\ H_2O$	3.52	mg.
$CuSO_4 \cdot 5\ H_2O$	0.38	mg.
$Na_2MoO_4 \cdot 2\ H_2O$	0.025	mg.
Thiamine · HCl	5	mg.
L-asparagine	2	g.
Glucose	10	g.
Distilled water	1000	ml.

The medium was divided into twelve 200-ml. portions and the pH of each was adjusted with HCl or NaOH to provide series ranging from pH 3.0 to 7.5 at 0.5 intervals. After sterilization by filtration through an ultrafine porosity fritted disk, 50 ml. of medium at each pH were added aseptically to four replicate, sterile, 150-ml. Erlenmeyer flasks. A 4-mm. plug of inoculum, cut from a 7-day-old malt-agar culture of *P. weirii*, then was added. After incubation at 25° C. for 30 days, mycelia were harvested, freed of the agar inoculum plug, dried overnight at 110° C. and weighed.

For study of temperature effects, similar procedures were followed, except that the medium was adjusted to pH 6 and sterilized by autoclaving; five replicates were used at each temperature in the range of 5 to

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[3/] Italic numbers in parentheses refer to Literature Cited, p. 6.

35° C. at 5° intervals. As a quick check on temperature effects, five malt-agar plates inoculated with mycelial plugs were placed in each incubator along with the flasks of liquid medium. Colony diameters of these were measured 12 days after inoculation.

Statistical significance of relationships between final weights or diameters of colonies and treatments was tested by regression analysis. Though the need to use different incubators for temperature treatments precluded randomization, the possibility of extraneous factors affecting fungus growth in this kind of experiment is remote, so regression analysis can be assumed to be valid.

Mean dry weight of *P. weirii* increased with pH at an accelerating rate between pH 3.0 and 6.0 as determined by regression analysis of the data. The fall from maximum growth at pH 6.0 to no growth at pH 6.5 and above was abrupt (fig. 1). At time of harvest, the pH of each medium generally was higher than at the outset except for those starting at pH 5.5 or higher (table 1).

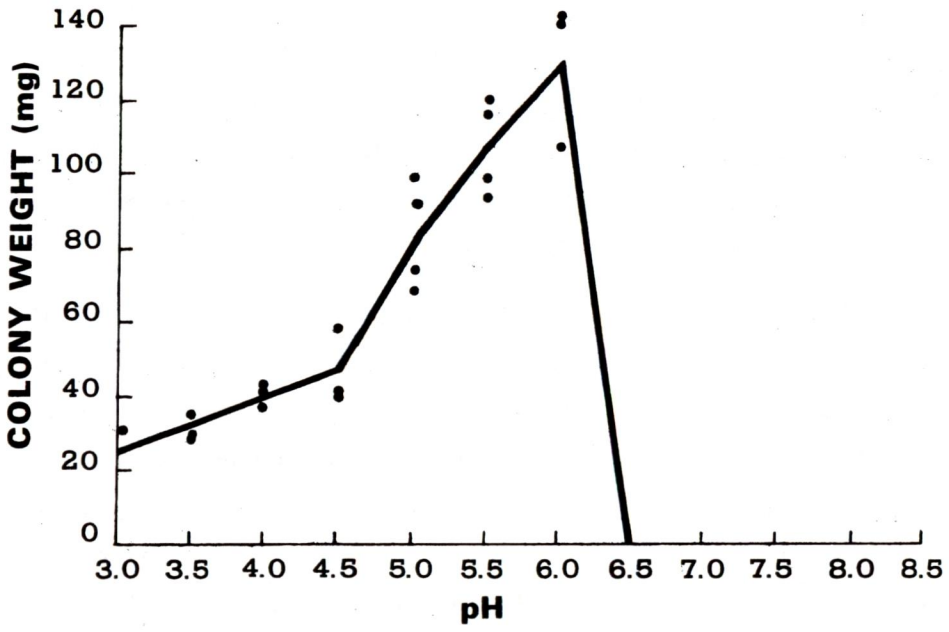

Figure 1.--Weights of colonies of *Poria weirii* grown for 30 days in media with different beginning pH values (four replicates at each pH).

Table 1.--Dry weight of mycelium of *Poria weirii* grown 30 days in synthetic medium with various H-ion concentrations

Initial pH	Mean colony weight (mg.)	Final pH
3.0	24	4.3
3.5	31	5.0
4.0	40	5.0
4.5	46	4.6
5.0	84	5.7
5.5	107	5.3
6.0	130	5.7
6.5	0	6.5
7.0	0	7.0
7.5	0	7.4

Mean dry weight of colonies of *P. weirii* at pH 6 increased with temperature from 5° C. to a maximum at 20° C., then fell off abruptly at temperatures above 20° C. to no growth at 30° C. or higher (fig. 2). The resulting complex curve proved highly significant as determined by regression analysis of the data. Similar trends in diameter growth on malt agar also were highly significant.

Having determined for this isolate of *P. weirii* that a pH of 6.0 and temperature 20° C. were optimal for the conditions of our research, we plan no further studies of this kind at present. Though the range of variation within this species remains to be defined, we can reasonably anticipate that isolates of *P. weirii* will vary from each other in pH and temperature optima for growth, as strains or geographical isolates of the same species may respond differently (*1, 2, 3, 4, 5, 7*).

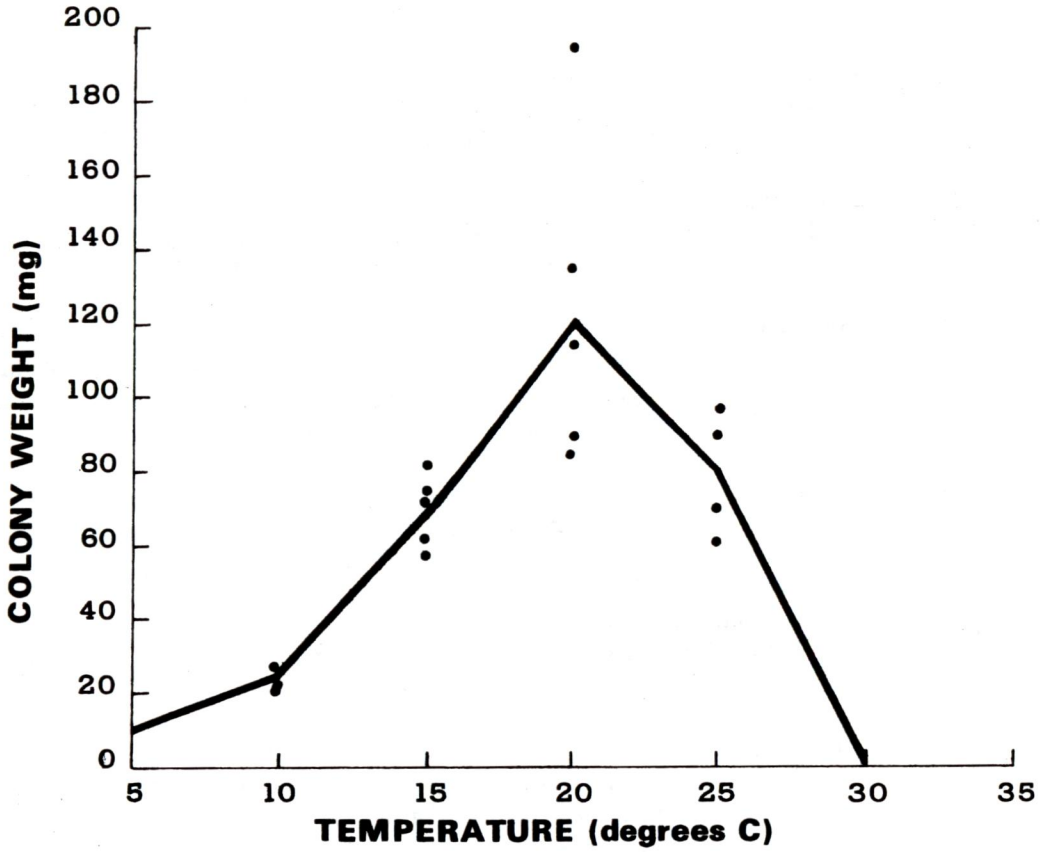

Figure 2.--Weights of colonies of *Poria weirii* grown for 30 days in synthetic medium at different temperatures (five replicates at each temperature).

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