JOURNAL OF THE TENNESSEE ACADEMY OF SCIENCE
Volume 42, Number 3, July 1967

# CERTAIN CULTURAL AND PHYSIOLOGICAL VARIATIONS AMONG SOME RESUPINATE POLYPORES

S. K. BALLAL
*Department of Biology,*
*Tennessee Technological University,*
*Cookeville, Tennessee*

## INTRODUCTION

During the past half century the knowledge of systematics of wood-rotting fungi, especially those of the family Polyporaceae, has increased greatly. Many attempts have been made to study these plants in laboratory cultures, but relationships between physiological processes and morphology of the sporophore are unknown. This work was undertaken to study a phase of these relationships, and to determine the range of variation among various isolates in cultures. To achieve this, commonly occurring polypores were collected and cultured. Resupinate species of polypores were chosen for further study because a large number of cultures were readily obtained. These were used as experimental material to determine temperature requirements, rates of growth, and enzyme reactions.

## MATERIALS AND METHODS

*Establishment of cultures.* Wood-rotting fungi were collected individually and brought to the laboratory in separate plastic bags. To obtain a spore print, a piece of agar was cut out of a slant with a sterile needle and placed on the opposite side of the tube. Then, a small portion (approximately 5 x 5 mm.) of the hymenium was cut and placed, pores down, on the small piece of cut agar. The test tubes were left undisturbed overnight. The majority of the fresh specimens failed to produce spore prints. Under sterile conditions successful spore prints were transferred, along with a little of the medium, to malt agar contained in test tubes. These, and transfers from them, served as stock cultures throughout this work. All the cultures were polysporous. No unisporous or tissue cultures were attempted.

*Morphological growth characteristics.* Each isolate to be studied was grown in a 90 mm. Petri dish containing 20-25 cc. of Difco malt agar for six weeks. From an actively growing culture in the test tube a disc of 5 mm. diameter was transferred to the center of the dish. This transfer was made with the mycelium down and in contact with the medium. The plates containing the inoculum were incubated at room temperature in the dark. These were examined and information was collected at weekly intervals.

*The oxidase reaction.* The medium for the oxidase test was prepared as follows: To make one liter of the medium, 45 gms. of Difco malt agar was dissolved in 850 cc. of distilled water, the remaining 150 cc. of water being placed in a separate flask. These were autoclaved separately for 20 minutes at 15 lbs. pressure. While the sterilized distilled water was still hot, 5 gms. of gallic or tannic acid were dissolved in it and this solution was added to the 850 cc. of slightly cooled malt agar and thoroughly mixed. Petri plates were poured with this medium, each plate containing approximately 20-25 cc. of the medium. These plates were inoculated at room temperature, and examined under the microscope.

*Constant temperature studies.* To determine minimum, optimum, and maximum temperatures for growth, the average mat diameters for the different fungi at standard time intervals were determined at 15°, 20°, 25°, 30°, and 35° C in an incubator with a normal variation of less than ± 0.5°. The value of such studies depended upon maintaining as constant experimental conditions as possible. Therefore, the following procedure was adopted: From actively growing cultures, discs of inoculum 0.5 cm. in diameter were removed by means of a sterilized metal holder at the end of which was attached a cylinder with a cutting edge. These discs were placed, mycelial side down, upon Difco malt agar medium in 90 mm. Petri plates containing approximately 20-25 cc. of the medium. The inoculated cultures were kept at the room temperature for 12 hours to allow the mycelium to establish on the agar before placing them in incubators at other temperatures. The dishes were inverted to prevent moisture condensing on the mycelium. Four replicates were maintained for each isolate. Diameters of mat were measured every day for 15 days or until a mat extended across the agar.

*Effect of light and yeast extract on growth.* The basal medium employed contained mineral salts, dextrose, casein hydrolysate, B vitamins and various purine and pyrimidine bases (Robbins and Hervey 1960a). All the glassware used in the experiment was washed in tap water, then in chromic acid. It was rinsed in distilled water repeatedly to remove all traces of chromic acid. The inoculum was obtained from week-old colonies in test tubes. Sterilized water was poured into the tube with the culture and shaken well to dislodge and suspend the fragmented mycelium. This suspension was transferred into another clean sterilized tube and the optical density adjusted to approximately one with distilled water. Triplicate experimental cultures were prepared by transfer of 5 cc. of adjusted suspension to flasks containing 25 cc. of the basal medium with or without yeast extract. After inoculation, one set was

held as a control, one was wrapped individually in aluminum foil and transferred to a Burrell wristaction shaker. The other set was also transferred to the shaker but without the aluminum wrapping. These flasks were shaken continuously at the rate of approximately 100 times a minute in a room at 25° C ± 3°. The light to which the cultures were exposed was a mixture of diffuse day light and artificial light using 2½ ft. KEN-RAD 15 W. tubes kept about one foot from the flasks. After a week the cultures were filtered using individually weighed Whatman No. 1, 9 cm. filter papers. The mycelia on the filter paper discs were treated with 70 per cent alcohol and dried at 50° C, for two hours. After drying, the discs and mycelia were weighed, and the dry weights of the mycelia were calculated.

*Data classification.* Due to the complexity growing out of the increased knowledge of the morphological and physiological characteristics of the wood decaying polypores, the Forest Pathology Division of the United States Department of Agriculture has developed a card index system which is being used by many workers to record the cultural characteristics of different fungi (Davidson, *et al* 1942, Nobles 1948, Davidson, *et al* 1960, Lombard, *et al* 1960). This system was used in this investigation to classify the data.

TABLE I

SUMMARY OF OBSERVED CULTURAL CHARACTERS

| Culture No. | Name | Color of Mat | Oxidase† Test | Septation | Chlamydospores | Conidia | Oidia | Rate of Growth | Effect on Agar | Mat Appearance | Margin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 129 | *Poria ambigua* | White | —ve | Simple | - | - | - | D° | Unchanged | Raised-Downy - | Smooth |
| 177 | *Poria candidissima* | Dull White | —ve | Simple | ° | - | - | B | Yellowish | Irregularly Zonate | Smooth |
| 188 | *Poria candidissima* | Dull White | —ve | Simple | - | - | - | B | Yellowish | Appressed or Raised | Smooth |
| 161 | *Poria cocos* | Yellowish Buff | —ve | Simple or Solid | - | ° | - | A | Unchanged | Appressed - | Regular |
| 134 | *Poria eupora* | Tawny Buff | +ve | Non-Septate Clamped or Solid | - | ° | - | C | Unchanged | Raised with Strands | Irregular |
| 168 | *Poria eupora* | Whitish-Colorless | +ve | Nodose | - | - | - | C | Unchanged | Woolly and Submerged | Smooth |
| 144 | *Poria ferrea* | Olive Brown | +ve | Simple | ° | - | - | C | Unchanged | Intermediate - | Smooth |
| 175 | *Poria incrassata* | White | —ve | Nodose | - | - | - | C | Unchanged | Indefinitely Zonate and Flecked | Even |
| PI 203 | *Poria incrassata* | White | —ve | Simple and Nodose | - | - | - | C | Unchanged | Flecks and Narrow Zones | Even |
| PM 204 | *Poria monticola* | White | —ve | Nodose | ° | - | - | B | Unchanged | Raised-Cottony | Smooth |
| PN 205 | *Poria nigrescens* | Brownish | —ve | Simple and Nodose | - | - | - | C | Slight | Woolly | Undulating |
| PV 201 | *Poria vailantii* | White | —ve | Noose | - | - | - | B | Unchanged | Radiating Cottony-Woolly | Even |
| 173 | *Poria versipora* | Brownish | +ve | Non-Septate | - | ° | ° | D | Unchanged | Flocose Submerged - | Radially Flexuous |
| PX 202 | *Poria xantha* | Dull White | —ve | Non-Septate and Nodose | ° | - | - | A | Unchanged | Raised-Woolly | Smooth |
| 101 | *Trametes americana* | White | —ve | Non-Septate and Nodose | ° | - | ° | B | Unchanged | Appressed - | Irregular |

° Rate of growth
A. Rapid, plates covered in 1-2 weeks.
B. Moderately rapid, plates covered in 3-4 weeks.
C. Slow, plates covered in 5-6 weeks.
D. Very slow, plates not covered in 6 weeks.

° and - Chlamydospores, oidia, conidia and aerial mycelium
° above structures observed.
- above structures not observed.

† Oxidase test
+ve diffusion zone present in the medium.
—ve diffusion zone absent in the medium.

## RESULTS

The fungi which gave + ve oxidase test were: *Poria eupora, P. ferrea* and *P. versipora;* and those which gave — ve oxidase test were: *Poria ambigua, P. candidissima, P. cocos, P. incrassata, P. monticola, P. nigrescens, P. vailantii, P. xantha* and *Trametes americana* (Table I). Different isolates of the same species gave essentially the same response except for a little difference in the concentration of the diffusion zone.

TABLE II

CONSTANT TEMPERATURE STUDIES SHOWING AREA (MM²) BY THE TENTH DAY †

| No. | Name | MM² of Mat Y | Upper Confidence Limit | Lower Confidence Limit |
|---|---|---|---|---|
| 129 | *Poria ambigua* | | | |
| | Temp. °C. | | | |
| | 15 | 163 | 232 | 111 |
| | 20 | 191 | 255 | 143 |
| | 25 | 62.5 | 87.6 | 43.9 |
| | 30 | 6,350* | — | — |
| | 35 | 6,350* | — | — |
| 177 | *Poria candidissima* | | | |
| | Temp. °C. | | | |
| | 15 | 2,300 | 2,400 | 2,200 |
| | 20 | 4,460 | 5,070 | 3,920 |
| | 25 | 3,320 | 4,040 | 2,720 |
| | 30 | 153 | 173 | 135 |
| | 35 | 159 | 185 | 138 |
| 188 | *Poria candidissima* | | | |
| | Temp. °C. | | | |
| | 15 | 4,626 | 5,406 | 3,958 |
| | 20 | 5,408 | 6,486 | 4,508 |
| | 25 | 3,816 | 4,587 | 3,174 |
| | 30 | 6,350* | — | — |
| | 35 | 6,350* | — | — |
| 161 | *Poria cocos* | | | |
| | Temp. °C. | | | |
| | 15 | 1,620 | 1,750 | 1,490 |
| | 20 | 6,060 | 8,060 | 4,560 |
| | 25 | 5,250 | 5,810 | 4,730 |
| | 30 | 6,350* | — | — |
| | 35 | 6,350* | — | — |
| 134 | *Poria eupora* | | | |
| | Temp. °C. | | | |
| | 15 | 58.9 | 142 | 24.4 |
| | 20 | 722 | 812 | 642 |
| | 25 | 1,800 | 1,870 | 1,730 |
| | 30 | 760 | 1,140 | 508 |
| | 35 | 17.7 | 103 | 30 |
| 168 | *Poria eupora* | | | |
| | Temp. °C. | | | |
| | 15 | 2.1 | 12.7 | 0 |
| | 20 | 1,020 | 1,260 | 823 |
| | 25 | 2,000 | 2,220 | 1,806 |
| | 30 | 160 | 595 | 43 |
| | 35 | 13.7 | 52 | 0 |
| 144 | *Poria ferrea* | | | |
| | Temp. °C. | | | |
| | 15 | 37.4 | 87 | 16.9 |
| | 20 | 167 | 214 | 130 |
| | 25 | 202 | 311 | 132 |
| | 30 | 37.8 | 46 | 30.9 |
| | 35 | 8.6 | — | — |
| 175 | *Poria incrassata* | | | |
| | Temp. °C. | | | |
| | 15 | 17.5 | 21.5 | 13.4 |
| | 20 | 250 | 339 | 184 |
| | 25 | 820 | 1,150 | 588 |
| | 30 | 30.6 | — | — |
| | 35 | 8.6 | — | — |
| PI 203 | *Poria incrassata* | | | |
| | Temp. °C. | | | |
| | 15 | 209 | 236 | 182 |
| | 20 | 147 | 199 | 109 |
| | 25 | 1,290 | 1,500 | 1,110 |
| | 30 | 28.5 | 52.5 | 15.5 |
| | 35 | 16.3 | 24.1 | 11 |
| PM 204 | *Poria monticola* | | | |
| | Temp. °C. | | | |
| | 15 | 1,090 | 1,270 | 935 |
| | 20 | 1,120 | 1,305 | 965 |
| | 25 | 1,760 | 2,080 | 1,490 |
| | 30 | 467 | 857 | 255 |
| | 35 | 8.6 | — | — |
| PN 205 | *Poria nigrescens* | | | |
| | Temp. °C. | | | |
| | 15 | 0 | — | — |
| | 20 | 66 | — | — |
| | 25 | 600 | 841 | 426 |
| | 30 | 13 | 26.2 | 8.4 |
| | 35 | 86 | — | — |
| PV 201 | *Poria vailantii* | | | |
| | Temp. °C. | | | |
| | 15 | 514 | 607 | 435 |
| | 20 | 880 | 1,010 | 764 |
| | 25 | 2,110 | 2,380 | 1,880 |
| | 30 | 181 | — | — |
| | 35 | 8.6 | — | — |
| 173 | *Poria versipora* | | | |
| | Temp. °C. | | | |
| | 15 | 835 | 966 | 716 |
| | 20 | 346 | 448 | 261 |
| | 25 | 1,130 | 1,660 | 753 |
| | 30 | 32.1 | 57.2 | 18 |
| | 35 | 8.6 | — | — |
| PX 202 | *Poria xantha* | | | |
| | Temp. °C. | | | |
| | 15 | 650 | 714 | 583 |
| | 20 | 734 | 935 | 594 |
| | 25 | 3,300 | 3,690 | 2,960 |
| | 30 | 2,520 | 3,130 | 2,190 |
| | 35 | 76 | 166 | 34.8 |
| 101 | *Trametes americana* | | | |
| | Temp. °C. | | | |
| | 15 | 481 | 620 | 374 |
| | 20 | 519 | 674 | 400 |
| | 25 | 1,285 | 1,960 | 843 |
| | 30 | 180 | — | — |
| | 35 | 8.6 | — | — |

† 95 per cent confidence limit on the. (Y-estimate).
* Increase in area limited by the size of the plate before 10th day.

In constant temperature studies of the fifteen different isolates of polypores growth is expressed in increase in area against time (Table II). The computational procedure followed in the regression analysis is as follows: The measurement of the diameter of each colony was transformed into the log of the change in area (mm.²) versus the log of the elapsed day. This gives

the closest fit to a straight line which is a prerequisite for this type of statistical analysis. A "least squares" regression equation was computed for the linear portion of the curve for each temperature and culture. A 95 per cent confidence interval for the estimated mean value was calculated from the regression equation, and the confidence limits are shown (Table II). In some cases the confidence bands could not be computed because of the artificial limitation of the growth by the size of the plate before the tenth day or because of the completion of only one replicate.

Each isolate had a slightly different rate of growth at the various temperature levels and each species had an optimum temperature of its own (Table II). Even though this difference between species was quite noticeable the response of isolates to optimum temperature of the same species was nearly the same except for *Poria candidissima*, in regard to the optimum temperature. For *Poria candidissima, P. ambigua, P. cocos,* 20° C apparently is the optimum growth temperature as expressed by the area of the colony by the tenth day. For the rest, the optimum temperature is 25° C. Some isolates have a fairly wide range of optimum temperature (*Poria candidissima* and *P. monticola* from 15° to 25° C). It was also noted that different species did not necessarily grow at the same rate even though the optimum temperature for them are the same (*Pora monticola* and *P. nigrescens*).

Light and yeast extract have definite effects on the growth and development of the polypores studied. Growth, as measured by the dry weight of mycelium, was greater in the dark in the absence of yeast extract except in the case of *Poria ferrea, P. incrassata, P. nigrescens* and *P. vailantii.* Dry weights of mycelium grown with low yeast extract levels were lower than the dry weights of mycelium without yeast extract, both in light and dark. At 500 mg. per liter level of yeast extract the dry weights of mycelium were significantly higher in the light than in the dark except for *Poria ambigua* and *P. versipora.*

## Discussion

Probably the most comprehensive investigation of the cultural characters of polypores is by Nobles (1948), who studied 126 species of wood rotting fungi. She observed certain inconsistencies in the cultural characters among different isolates of the same species. Inconsistencies among the isolates of the same species have also been noticed by Davidson, *et al* (1942). In the present investigation, two cultured isolates of *Poria eupora* were slightly different. In one, the hyphae were exclusively nodose septate, while in the other a few nonseptate and solid hyphae were observed along with nodose septate hyphae. This kind of difference has also been noticed between the two isolates of *Poria incrassata.* One isolate of *Poria candidissima* produced chlamydospores in culture while the others did not. Even in the morphological studies, various types of inconsistencies are found among collections of the same species from nature. As an illustration *Poria candidissima* has forms, one with smooth and the other with roughened spores (Lowe 1946). In this study one collection of *Poria eupora* had prominent hyphal pegs; the other collection lacked them.

It often becomes necessary to identify members of the family Polyporaceae lacking fructifications. Where morphological identification is difficult, it is sometimes possible to identify the organism by cultural methods. An example was observed in the present investigation. Young resupinate specimens of *Trametes* resemble *Poria,* the latter being always resupinate. When grown in culture and the medium started drying, the fruiting bodies of *Trametes* formed. The sporophore was clearly sessile and attached to the medium.

Two distinct types of decay, consistent for species of these fungi, occur in the substratum. In one type the decayed wood is brown due to degradation of cellulose by the fungus enzyme or enzymes. In the other type, the substrate attacked is lignin and the decay is white. Nobles (1958) proposed a scheme of classification based upon physiological characteristics. The brown rot fungi which primarily utilize cellulose were considered a primitive, basic group from which developed a more advanced group with a capacity to decompose lignin as well. Lowe (1963) interpreted Nobles' conclusions based on oxidase reactions to mean that the highest degree of specialization was accorded to those white rot species which have thick-walled hyphae of several types. These conclusions were almost diametrically opposite to those which Lowe (1963) had reached by morphological studies.

It is very logical to agree with Nobles regarding the usefulness of this physiological character in broadly dividing the family, but its usefulness could not be sufficiently confirmed in this investigation. It has been noted in a few cases by Davidson *et al* (1942) that the rot type did not agree with the oxidase type. In this study species—consistent results were obtained in the oxidase test.

Most wood destroying fungi possess characteristic optimum temperatures for growth and characteristic temperatures above or below which growth is inhibited. Falck, as cited by Cartwright and Findlay (1934), used minimum, optimum and maximum temperatures as criteria for distinguishing closely related species. The present investigation has shown the difference in optimum temperatures for different species of polypores. For the fifteen different isolates studied there were two different optima for these fungi, one was around 20° C and the other around 25° C. Davidson, *et al* (1942), working with the cultural identification of fungi causing decay of living oaks in the eastern United States, reported that, despite careful control and repeated trials, isolates of *Corticium lividum* (Thelephoraceae) and *Poria cocos* varied considerably in many growth characteristics. However, even in the cases cited above the optimum temperature remained the same. The same phenomenon, variation among isolates, was noticed in the case of some of the isolates examined. Since temperature affects rate of growth and consequently the

rate of decay in timber, this might determine the predominant species in any locality.

Optimum temperatures for many wood destroying fungi were reported by Humphrey and Siggers (1933) and Cartwright and Findlay (1934). They reported close agreement with some species and considerable difference with others. Of the species investigated here, only *Poria xantha* was cited in the work of Humphrey and Siggers (1933). They reported the optimum temperature for its growth as 28° C. The maximum increase in area for this fungus occurred at 25° C (3300 mm²) and at 30° C the increase in area was only 2520 mm².

The optimum for *Poria cocos* was a wide band of temperatures ranging from 20° C to 35° C with a slight slump in growth at 25° C. According to Davidson, *et al* (1942), the constant temperature studies of *Poria cocos* were unsatisfactory due to extensive variation between growth rates of isolates, and they stated that the optimum for this fungus was approximately 30° C. This temperature was the experimental optimum for *Poria candidissima* (Table II). Lombard, Davidson and Lowe (1960) reported that for *Poria ambigua* the temperature for maximum growth was 30° C and growth was almost as high between 28° C and 36° C. In the present studies almost maximum growth was observed between 30° C and 35° C. Even though these temperature studies are useful to clarify factors in the distribution of these fungi, they may not be very effective taxonomic criteria because of the inconsistency observed and because of the wide range of optimum temperatures for growth in certain species.

In studies on the nutrition of some Basidiomycetes, Robbins and Hervy (1960a) noted that a culture of *Poria ambigua* formed basidiospores in light, but produced few or none in the dark. This prompted the investigation on the effect of light on the growth of polypores. According to the authors mentioned above, light reduced the growth of *Poria ambigua* but was required for the formation of sporophores. They also noticed that the natural materials such as wood, malt, tomato juice, cork, casein, etc., added to the media largely eliminated the inhibitory action of light on growth. In the present study growth as expressed by the dry weight of mycelium was greater in the dark for most of the fungi studied (Tables III, IV and V). Yusef (1953) reported that the growth of *Polyporus schweinitzii* was markedly improved when small amounts of malt extract were added to the basal medium. In one of the experiments with *Poria ambigua* Robbins and Hervey (1960a) observed a noticeable increase in dry weight of mycelium between 5 and 25 mg. of yeast extract per flask in the presence of light. They stated that *Polyporus schweinitzii* on a basal medium suffers from a partial deficiency of some unidentified growth substances (Robbins and Hervy, 1960b), one of which was later identified as ferulic acid (Robbins and Hervey *et al* 1963). Growth of *Polyporus schweinitzii* was enhanced by this acid and by other compounds in natural materials like yeast extract.

TABLE III

DRY WEIGHT OF MYCELIUM AT DIFFERENT LEVELS OF YEAST EXTRACT AFTER SEVEN DAYS OF GROWTH IN LIGHT

| | Yeast Extract in mg. per Liter | | | | | Culture No. | Name |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 100 | 250 | 500 | | |
| Dry Weight of Mycelium in mg. (mean of three replicates) | 118.4 | 26.6 | 43.1 | 79.1 | 120.1 | 129 | *Poria ambigua* |
| | 62.4 | 32.2 | 27.6 | 65.6 | 89.9 | 177 | *Poria candidissima* |
| | 71.3 | 26.2 | 51.7 | 81.6 | 119.6 | 188 | *Poria candidissima* |
| | 43.1 | 26.6 | 32.6 | 53.5 | 93.7 | 161 | *Poria cocos* |
| | 103.8 | 20.2 | 32.1 | 101.6 | 124.6 | 134 | *Poria eupora* |
| | 68.6 | 23.2 | 31.2 | 79.6 | 93.6 | 168 | *Poria eupora* |
| | 71.6 | 17.4 | 39.5 | 75.2 | 102.3 | 144 | *Poria ferrea* |
| | 77.9 | 18.7 | 27.6 | 65.7 | 90.4 | 175 | *Poria incrassata* |
| | 56.4 | 18.8 | 24.9 | 66.3 | 110.9 | PI203 | *Poria incrassata* |
| | 40.5 | 19.9 | 44.1 | 78.1 | 106.3 | PM204 | *Poria monticola* |
| | 71.8 | 22.8 | 21.9 | 69.5 | 83.5 | PN205 | *Portia nigrescens* |
| | 81.6 | 21.6 | 22.5 | 68.4 | 98.9 | PV201 | *Poria vailantii* |
| | 73.3 | 33.8 | 38.9 | 70.8 | 94.9 | 173 | *Poria versipora* |
| | 87.2 | 20.4 | 30.2 | 85.4 | 181.1 | PX202 | *Poria xantha* |
| | 63.2 | 20.5 | 22.4 | 63.2 | 81.4 | 101 | *Trametes americana* |

TABLE IV

DRY WEIGHT OF MYCELIUM AT DIFFERENT LEVELS OF YEAST AFTER SEVEN DAYS OF GROWTH IN DARK

| | Yeast Extract in mg. per Liter | | | | | Culture No. | Name |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 100 | 250 | 500 | | |
| Dry Weight of Mycelium in mg. (mean of three replicates) | 173.6 | 23.9 | 45.5 | 95.1 | 160.1 | 129 | *Poria ambigua* |
| | 69.5 | 29.8 | 32.7 | 47.1 | 65.4 | 177 | *Poria candidissima* |
| | 73.3 | 30.4 | 29.6 | 59.1 | 65.2 | 188 | *Poria candidissima* |
| | 87.2 | 26.4 | 39.3 | 60.4 | 81.2 | 161 | *Poria cocos* |
| | 113.2 | 27.3 | 39.9 | 69.7 | 103.4 | 134 | *Poria eupora* |
| | 75.5 | 24.6 | 27.3 | 42.6 | 70.1 | 168 | *Poria eupora* |
| | 50.4 | 47.4 | 35.5 | 41.7 | 47.2 | 144 | *Poria ferrea* |
| | 58.0 | 23.3 | 23.4 | 42.2 | 51.2 | 175 | *Poria incrassata* |
| | 47.5 | 24.1 | 27.2 | 35.3 | 41.0 | PI203 | *Poria incrassata* |
| | 71.9 | 21.2 | 24.9 | 31.3 | 68.6 | PM204 | *Poria monticola* |
| | 62.1 | 27.4 | 28.6 | 39.5 | 59.8 | PN205 | *Poria nigrescens* |
| | 56.3 | 20.9 | 23.2 | 36.5 | 51.5 | PV201 | *Poria vailantii* |
| | 128.6 | 28.4 | 28.3 | 59.6 | 118.9 | 173 | *Poria versipora* |
| | 249.7 | 18.4 | 28.3 | 71.3 | 104.2 | PX202 | *Poria xantha* |
| | 89.2 | 25.4 | 27.5 | 40.8 | 80.8 | 101 | *Trametes americana* |

In the present investigation the analysis of variance showed significantly greater growth (dry weight of mycelium) of mycelium grown in light with 500 mg. of yeast extract per liter as compared with growth in dark, except in the case of *Poria ambigua, P. versipora* and *Trametes americana.* Another observation made in the present study is that the growth was much less in both light and dark when very little yeast extract was added to the basal medium. In *Poria ambigua,* even though light reduced growth, it was necessary for fruiting (Robbins and Hervey 1960a). The effect of light and yeast extract on sporulation of polypores could not be ascertained because isolates were grown only for seven days.

Bessey (1950) recognized forty-eight genera in Polyporaceae, while Overholts (1953) recognized only thirteen. The latter's generic concepts are accepted by contemporary students of the family. Bessey's bases for division of *Poria* and its related genera were the difference in color of sporophores, presence or absence of volva-like structures, true cystidia, and the size of pores. The present study indicated differences of this nature among the isolates of the same species. For example,

TABLE V

EFFECT OF LIGHT AND DARK ON DRY WEIGHT IN mg. OF MYCELIUM AFTER SEVEN DAYS AT DIFFERENT LEVELS OF YEAST EXTRACT *

| Culture No. | Name | Levels of Yeast Extract mg./l. 0 | 10 | 100 | 250 | 500 |
|---|---|---|---|---|---|---|
| 129 | *Poria ambigua* | D[a] > L[b] ± 5.09 | D = L ± 5.25 | D = L ± 8.09 | D > L ± 8.56 | D > L ± 2.53 |
| 177 | *Poria candidissima* | D > L ± 6.58 | D = L ± 5.25 | D = L ± 1.11 | L > D ± 8.00 | L > D ± 13.12 |
| 188 | *Poria candidissima* | D = L ± 8.30 | D = L ± 9.94 | L > D ± 8.10 | L > D ± 16.13 | L > D ± 20.61 |
| 161 | *Poria cocos* | D > L ± 12.18 | D = L ± 4.95 | D > L ± 0.13 | D > L ± 6.76 | L > D ± 8.65 |
| 134 | *Poria eupora* | D = L ± 24.81 | D = L ± 17.51 | D > L ± 2.22 | L > D ± 10.45 | L > D ± 19.79 |
| 168 | *Poria eupora* | D > L ± 13.51 | D = L ± 10.50 | D = L ± 10.50 | L > D ± 11.23 | L > D ± 8.73 |
| 144 | *Poria ferrea* | L > D ± 6.04 | D > L ± 4.46 | D = L ± 13.81 | L > D ± 11.31 | L > D ± 10.49 |
| 175 | *Poria incrassata* | L > D ± 5.24 | D = L ± 5.98 | D = L ± 9.68 | L > D ± 8.60 | L > D ± 3.44 |
| PI 203 | *Poria incrassata* | L > D ± 4.94 | D = L ± 6.58 | D = L ± 6.49 | L > D ± 18.29 | L > D ± 4.60 |
| PM 204 | *Poria monticola* | D > L ± 2.92 | D = L ± 8.43 | L > D ± 2.58 | L > D ± 9.59 | L > D ± 6.15 |
| PN 205 | *Poria nigrescens* | L > D ± 6.41 | D = L ± 10.58 | D = L ± 9.78 | L > D ± 10.41 | L > D ± 14.37 |
| PV 201 | *Poria vailantii* | L > D ± 5.72 | D = L ± 8.82 | D = L ± 10.37 | L > D ± 12.22 | L > D ± 24.23 |
| 173 | *Poria versipora* | D > L ± 26.42 | L > D ± 0.98 | L > D ± 6.38 | D = L ± 17.94 | D > L ± 5.20 |
| PX 202 | *Poria xantha* | L > D ± 147.6 | D = L ± 8.99 | D = L ± 9.59 | L > D ± 8.56 | L > D ± 31.41 |
| 101 | *Trametes americana* | D = L ± 7.06 | D = L ± 1.05 | D = L ± 7.48 | L > D ± 11.83 | D > L ± 18.03 |

* At 95 per cent significance level.
[a] D—Dark [b] L—Light

the two collections of *Poria eupora* were slightly different in color and in cultural characters. On the other hand, Overholt's delimitations of genera were based on more uniform macroscopic characters like nature of attachment of sporophores to the substratum, shape of pores, and consistency of fructification. The present investigation has proved that the characters used by Bessey are of value only at the species level, which makes the writer prefer to adopt the use of a smaller number of genera. Nobles (1958), investigated the phylogenetic relationships among different genera of the family. She relied somewhat upon cultural characters for the identification of genera and species. The characters used were presence or absence of extracellular oxidase, the type of interfertility, hyphal characters, presence or absence of chlamydospores and oidia, color of mycelial mats, and changes in color of the substrate. Ultimate divisions were based on the shape and color of basidiospores. In her earlier work Nobles (1948) recorded differences among isolates of the same species, for example in *Poria xantha* and *P. monticola.* Inconsistencies mentioned by Nobles regarding cultural characters was observed in this work. Rates of growth of these fungi under identical cultural conditions were different for some isolates of the same species. In nature *Poria ferrea* had prominent setae which were not noticed in cultures. Most resupinate polypores can be identified using morphological characters. When determination based on sporophore morphology becomes difficult or misleading, it is helpful to resort to cultures, as was illustrated in the case of *Trametes americana.*

The present studies have confirmed that, in certain cases, cultural characters may be of taxonomic value.

Since these fungi are of economic importance due to their destructive capacity, physiological studies on the effect of temperature or nutrition may be of great value. Cytological studies, critical studies of pigments, and studies on geographic distribution are also promising approaches for future investigation.

## Literature Cited


Bessey, E. A. 1950. Morphology and taxonomy of fungi. The Blakiston Co., Toronto. i-xiii - 791 pp.

Cartwright, K. St. G. and Findlay, W. P. K. 1934. Studies in the physiology of wood-destroying fungi—II. Temperature and rate of growth. Ann. Botany. 48: 481-495.

Davidson, R. W., Campbell, W. A. and Vaughn, D. B. 1942. Fungi causing decay of living oaks in the eastern United States and their cultural identification. U.S. Dept. Agr. Tech. Bulletin. 785.

Davidson, R. W., Lentz, P. L. and McKay, H. H. 1960. The fungus causing pecky cypress. Mycologia 52(2): 260-279.

Humphrey, C. J. and Siggers, P. V. 1933. Temperature relations of wood-destroying fungi. J. Agr. Research. 47: 997-1008.

Lombard, F. F., Davidson, R. W. and Lowe, J. L. 1960. Cultural characteristics of *Fomes ulmarius* and *Poria ambigua*. Mycologia 52(2). 280-294.

Lowe, J. L. 1946. The Polyporaceae of New York State (The genus Poria). N.Y. State Coll. Forestry. Syracuse University Tech. Pub. 65.

Lowe, J. L. 1963. The polyporaceae of the world. Mycologia 55(1): 1-12.

Nobles, M. K. 1948. Studies in Forest Pathology VI. Identification of Cultures of wood-rotting Fungi. Canad. J. Research. 26: 281-431.

Nobles, M. K. 1958. Cultural characters as a guide to the taxonomy and phylogeny of the Polyporaceae. Canad. J. Botany 36: 883-926.

Overholts, L. O. 1953. The Polyporaceae of the United States, Alaska and Canada. University of Michigan Press. i-xiv - 466 pp.

Robbins, W. J. and Hervey, A. 1960a. Light and the development of *Poria ambigua*. Mycologia. 52(6): 231-247.

Robbins, W. J. and Hervey, A. 1960b. Growth substances for *Polyporus Schweinitzii*. Mycologia. 52(6): 946-957.

Robbins, W. J., Hervey, A., Page Jr., A. C., Gale, P. H., Hoffman, C. H., Moscatelli, E. A., Koniuszv, F. R., Smith, M. C., and Folkers, K. 1963. Growth factors for *Polyporus schweinitzii*. The identification of ferulic acid as a new co-factor. Mycologia. 55(6): 742-757.

Yusef, H. M. 1953. The requirements of some Hymenomycetes for essential metabolites. Bull. Torrey Bot. Club. 80: 43-64.