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# *The Biology of Pleurotus Corticatus Fries*

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## CONTENTS

# THE BIOLOGY OF PLEUROTUS CORTICATUS FRIES[1]

FRANK H. KAUFERT[2]

## INTRODUCTION

*Pleurotus corticatus,* like other species of the genus *Pleurotus,* is considered a saprophyte or weak wound parasite of several hardwood species in the eastern United States. The biology of *P. corticatus* has been considered similar to that of other species of *Pleurotus* and related agarics and has therefore never been carefully investigated.

During a study of fire and decay damage in the Mississippi Delta hardwoods of Louisiana and Mississippi in 1931, an agaric that formed very striking and unusual asexual spores was isolated from the decay of young fire-scarred hardwoods. The fungus has been tentatively identified as *Pleurotus corticatus* Fries (6). When a fruiting body was slowly dried, large white coremia capped with black drops of conidia formed on the gills and stipe. Similar structures appeared on cultures from sporophores and from the decay of several fire-scarred trees. The coremia were white, tall, capped with dark spore masses, and were formed as outgrowths of the tissue of the sporophore or by compacting of the vegetative mycelium growing on the surface of agar slants (6).

The formation of asexual spores of this type has apparently not been observed for any other species of the *Agaricaceae.* This striking and unusual feature stimulated a more thoro study of the biology of the fungus.

## OBJECTS OF THE STUDY

These investigations consisted of three rather distinct phases:

1. Cultural studies to determine the factors affecting the growth of the vegetative mycelium, the optimum conditions for sporophore production, and the conditions governing the formation of coremia on sporophores and in cultures.

2. A study of the sexuality of the fungus to determine its polarity and the number of sex groups present.

[1] Presented also to the faculty of the Graduate School of the University of Minnesota in partial fullfillment of the requirements for the degree of Doctor of Philosophy. Degree granted June, 1935.

[2] The writer gratefully acknowledges his indebtedness to Drs. E. C. Stakman, Louise T. Dosdall, H. Schmitz, and A. H. R. Buller for their suggestions and criticisms during the course of these investigations and to Dr. Stakman and Dr. Dosdall for aid in the preparation of the manuscript.

3. Wood decay tests to ascertain the optimum conditions for decay and to determine the relative rates of decay caused by dicaryotic, haploid, and various combinations of haploid cultures.

## SOURCE OF MATERIAL

All of the material was obtained from the Mississippi Delta. In addition to a culture obtained from a sporophore collected by the writer, five cultures[3] were obtained from the decayed wood of five fire-scarred hardwoods. The sporophore was found on a fire-scarred and decayed red gum *(Liquidambar styraciflua)* near Greenville, Mississippi. The tissue culture made from it is designated as culture 2B. The remaining cultures were obtained by placing small bits of decayed wood from near the top of decay columns on malt agar. The data concerning the source and designation of these cultures are given below.

| Source of culture isolation | Location | Culture |
|---|---|---|
| *Liquidambar styraciflua* | Oak Grove, La. | 2A |
| do | Parish, Miss. | 2C |
| *Quercus nuttallii* | Waxia, La. | 2D |
| do | Vance, Miss. | 2E |
| *Liquidambar styraciflua* | Louise Rd., Miss. | 2F |

The fire-scarred trees attacked by *Pleurotus corticatus* were young and had very little heartwood. The decay had spread a considerable distance up the bole, as high as 14 feet in one of the trees. Termites and ants were present in every tree from which the fungus was isolated. In two cases the termites and ants had not progressed far, but in the remaining trees their galleries extended almost to the top of the decayed wood. The presence of termites in trees attacked by *P. corticatus* suggests a possible insect relationship. The six cultures used as the basis for this study are shown in Figure 1.

## CULTURAL STUDY

### Life History of the Fungus

The life history of *Pleurotus corticatus* has been given in an earlier paper (6); consequently only a brief résumé of some of the most important points will be given here. The cultures isolated from fire-scarred and decayed trees and made from the single sporophore are all dicaryotic, and prominent clamp connections occur at the cross walls. Large white

[3] Cultures 2A and 2B were isolated by the writer and cultures 2C, 2D, 2E, and 2F by George Hepting while in the employ of the Division of Forest Pathology, Bureau of Plant Industry, Washington, D. C.

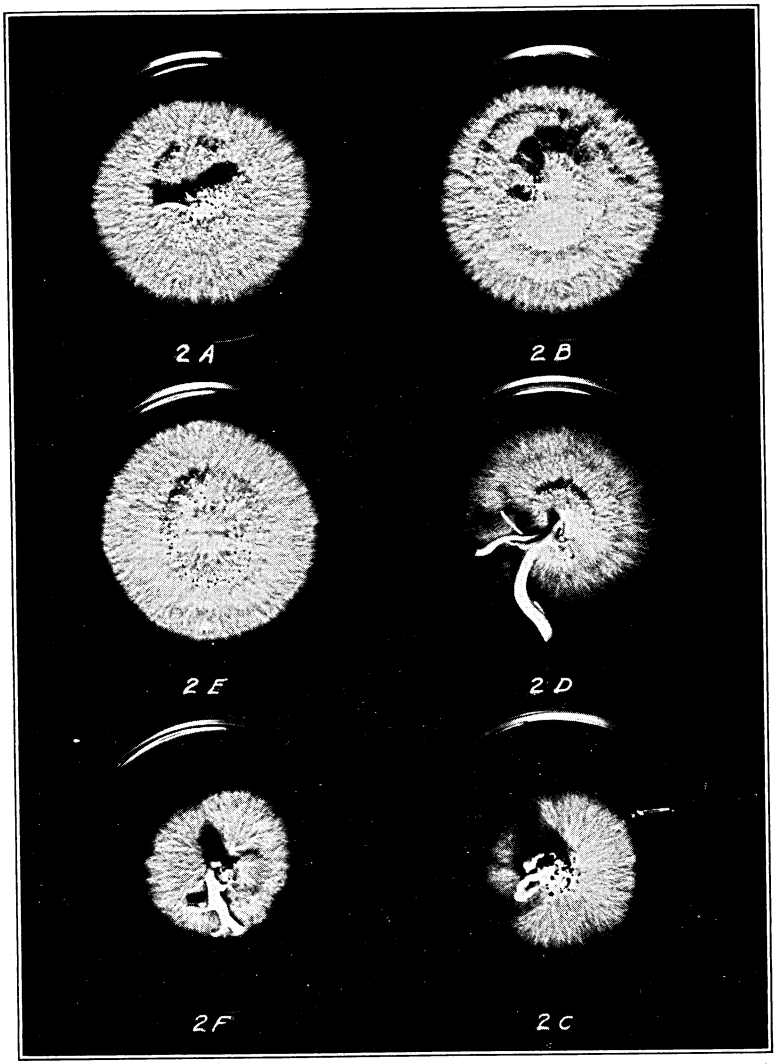



FIG. 1. THE SIX DICARYOTIC CULTURES OF *Pleurotus Corticatus* USED IN THIS STUDY, ON 4.0 PER CENT MALT EXTRACT AGAR

coremia, capped with glistening black masses of conidia, are formed abundantly in all cultures. When these cultures are grown on the proper media under conditions favorable to fruiting, sporophores are formed which produce an abundance of basidiospores. After basidiospore formation has ceased, coremia bearing numerous conidia are formed on the stipe, gills, and pileus of the sporophores (Figs. 3 and 5). The sporophores are very resistant to decomposition by molds and bacteria. Germinating basidiospores give rise to haploid mycelia. The haploid mycelium lacks clamp connections, the cells are uninucleate, and it grows only about three-fourths as rapidly as the dicaryotic mycelium. Coremia and conidia are formed on the haploid mycelium as well as on the dicaryotic, but they are smaller and the spores are formed somewhat differently. In addition to the conidia formed in compound fruiting bodies or coremia, a second kind of asexual spore is formed on both the haploid and dicaryotic mycelium. These spores are very small and are abstricted singly at the tips of hyphal branches. Asexual spores of this type have been described for many other agarics, and they appear to be formed only when the mycelium is growing under unfavorable conditions.

**Table 1. The Size of the Five Types of Spores of *Pleurotus Corticatus***

| Type of spore | Length (microns) | | Width (microns) | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| 1. Binucleate conidia (on coremia) | | | | |
| Isolate 2A | 14.9 ± 1.658 | 7.8 — 22.6 | 6.61 ± .150 | 4.7 — 8.6 |
| 2B | 16.2 ± 1.267 | 11.7 — 19.5 | 7.11 ± .131 | 5.8 — 7.8 |
| 2C | 16.2 ± 1.412 | 11.7 — 19.9 | 6.44 ± .080 | 5.1 — 7.4 |
| 2D | 14.5 ± 1.561 | 9.4 — 19.1 | 6.86 ± .180 | 5.1 — 8.2 |
| 2E | 16.2 ± 2.259 | 11.7 — 27.3 | 6.56 ± .187 | 5.1 — 7.8 |
| 2F | 15.8 ± 1.321 | 11.1 — 23.1 | 6.56 ± .121 | 5.3 — 7.1 |
| 2. Uninucleate conidia (on coremia) from haploid mycelium of | | | | |
| Isolate 2A | 7.94 ± 1.851 | 4.3 — 15.6 | 4.94 ± .146 | 3.9 — 7.0 |
| 2B | 8.29 ± 1.793 | 3.9 — 15.6 | 5.05 ± .098 | 3.9 — 7.8 |
| 2D | 9.54 ± 1.654 | 4.7 — 14.8 | 4.88 ± .190 | 3.9 — 5.9 |
| 2F | 8.81 ± 1.601 | 4.3 — 14.9 | 4.93 ± .112 | 3.9 — 6.1 |
| 3. Binucleate conidia (formed singly) | | | | |
| Isolate 2A | 3.52 ± .118 | 2.3 — 4.3 | 3.51 ± .099 | 2.3 — 4.3 |
| 4. Uninucleate conidia (formed singly) on haploid mycelium | 2.33 ± .084 | 1.7 — 3.5 | 2.31 ± .087 | 1.7 — 3.5 |
| 5. Basidiospores from sporophore of | | | | |
| Isolate 2A | 16.05 ± 1.070 | 4.83 — 19.5 | 4.8 ± .222 | 4.3 — 5.8 |

*Pleurotus corticatus* thus is highly pleomorphic, five distinct types of spores being formed: basidiospores, binucleate conidia formed on coremia, binucleate conidia formed singly on simple conidiophores, uninu-

cleate conidia formed on coremia, and uninucleate conidia formed singly on conidiophores. The spore sizes are given in Table 1.

## Growth on Artificial Media

*Pleurotus corticatus,* like the majority of wood-rotting fungi, grows luxuriantly on malt extract agar. On nutrient agars, such as Richard's, Leonian's, or Czapek, the growth is slower, fewer asexual spores are produced, and sporophores are rarely formed. Potato dextrose agar likewise is not a favorable medium for this fungus.

To determine the sugars most favorable for its growth a number of studies were made, using maltose, lactose, sucrose, and dextrose in concentrations of from 1 to 10 per cent in agar. In every test the best growth was on agar containing maltose, 4 per cent being the optimum for growth, fructification, and asexual sporulation, altho there is still slight growth on 10 per cent concentration of maltose.

Cultures of the six dicaryotic isolates used in this study are shown in Figure 1. There is some difference in these isolates, both in rate and character of growth. Cultures 2A, 2B, and 2E grow luxuriantly and produce numerous coremia and conidia over the entire surface of the cultures. Cutures 2C, 2D, and 2F, on the other hand, grow more slowly, produce coremia and conidia principally near the center of the culture, and form abortive sporophores earlier than the first group.

## Temperature Relations

The fungus requires a rather high temperature for its optimum vegetative development and for the production of sporophores. The temperature relations of the dicaryotic and haploid mycelia are shown in Figure 2. At 10 degrees C. there is practically no growth, and even at 20 degrees C. growth is not very rapid. The optimum temperature for vegetative growth is 27 to 30 degrees centigrade. At 32 degrees C. growth is still quite rapid but is less than at 27 degrees. The haploid lines have the same optimum as the dicaryotic but grow only about 70 per cent as fast. This difference in rate of growth between the haploid and dicaryotic cultures is illustrated in Figures 8, 9, and 10.

Considerable difficulty was experienced early in this study in inducing basidiospores to germinate. When they were picked directly from the basidia or were placed on agar or water they did not germinate. A number of stimulatory substances were tried, but, even then, only an occasional spore germinated. The author's results were similar to those of Cool (4) and Vandendries (13), who failed to get basidiospores of *Pleurotus corticatus* to germinate. Finally a very simple method was tried with success. Petri dishes of malt agar were exposed for a few

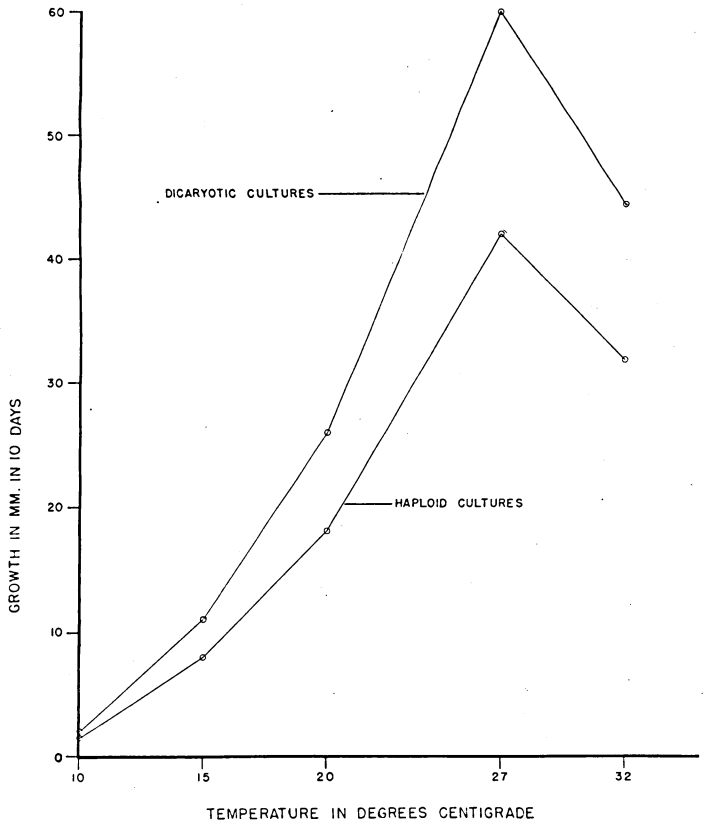



FIG. 2. GROWTH OF DICARYOTIC AND HAPLOID CULTURES OF *Pleurotus Corticatus* AT DIFFERENT TEMPERATURES

seconds directly beneath a sporophore shedding spores and were then incubated at 27° C. By this method it was possible to obtain hundreds of single basidiospore cultures on agar plates. Apparently the primary requisite for germination is the maturity of the spores. Spores picked from the basidium usually are not mature enough to germinate, whereas those abjected are fully mature. Furthermore, the spores apparently require a humid atmosphere for germination, such as is obtained in a closed petri dish filled with agar; but they will not germinate readily when immersed in a liquid. Third, they germinate best at the optimum temperature for vegetative growth, 27° C., which is much higher than the ordinary room temperature at which most germination tests are made.

## Development of Sporophores

As mentioned above, the fungus grows well on a number of media, producing a luxuriant mycelium, abundant coremia, abortive sporophores, and occasionally a sporophore with gills and pileus. But nutrient agar, even in large quantities, does not induce the degree of vegetative development prerequisite to sporophore production by the larger stipitate agarics. To obtain sufficient vegetative growth it is necessary to have a medium that is porous and contains an abundance of food material. Etter (5) used a mixture of sawdust, cornmeal, cornstarch, and malt extract with considerable success with stipitate agarics, such as *Lentinus lepideus*.

Some of the media used in attempts to induce formation of normal sporophores of *Pleurotus corticatus* are listed below.

1. A mixture of basswood sawdust, cornmeal, cornstarch, and malt extract liquid.
2. Basswood sawdust and malt extract liquid.
3. Malt extract agar and basswood sawdust.
4. Basswood sawdust and Richard's agar, Leonian's agar, and Sach's Modified Nutrient Solution.
5. Partially germinated seeds of wheat and barley.[4]
6. A mixture of basswood sawdust and soaked seeds of wheat and barley.

These substances were placed in pint or quart jars, inoculated, and kept in a dark room at 28° C. for three to four weeks, or until the mycelium had grown throughout the medium. The covers of the jars were then removed and the jars placed under large bell jars in which the air was kept humid by means of strips of wet blotting paper. No precautions to prevent contamination had to be taken once the mycelium had covered the surface of the medium, because molds or bacteria do not seem to be able to compete with the luxuriant mycelium of the fungus, once it is established.

Altho sporophores capable of producing basidiospores have been produced on all of the above media, the mixture of barley, wheat, and basswood sawdust has proven most satisfactory. To date, sporophores have never failed to form on this mixture when the above procedure was followed.

Sporophore primordia start to form very soon after the covers are removed, if a luxuriant mycelial development has taken place previously. The structure of these primordia is similar to that of the coremia, the mycelium being tightly compacted into white stalks. These grow rapidly in height and soon protrude from the open jars (Fig. 3). Up to this

[4] This mixture was suggested by Dr. L. O. Overholts, who has used it with success for several stipitate agarics.

point in sporophore development, temperature and nutrients play the most important rôle, light is not essential. At this point, however, unless diffused sunlight or the light from several 100-watt lights is provided, the sporophores will not develop normally, gill formation may be entirely inhibited, and only abortive sporophores are formed. If light conditions are favorable, however, gills are formed in four or five days after the primordia protrude from the jars (Fig. 3). Normally, several sporophores develop from each jar, but rarely more than two or three of these form gills and basidiospores.

Some rather large sporophores have been produced in this manner (Figs. 4 and 5). Basidiospore production begins soon after the gills are

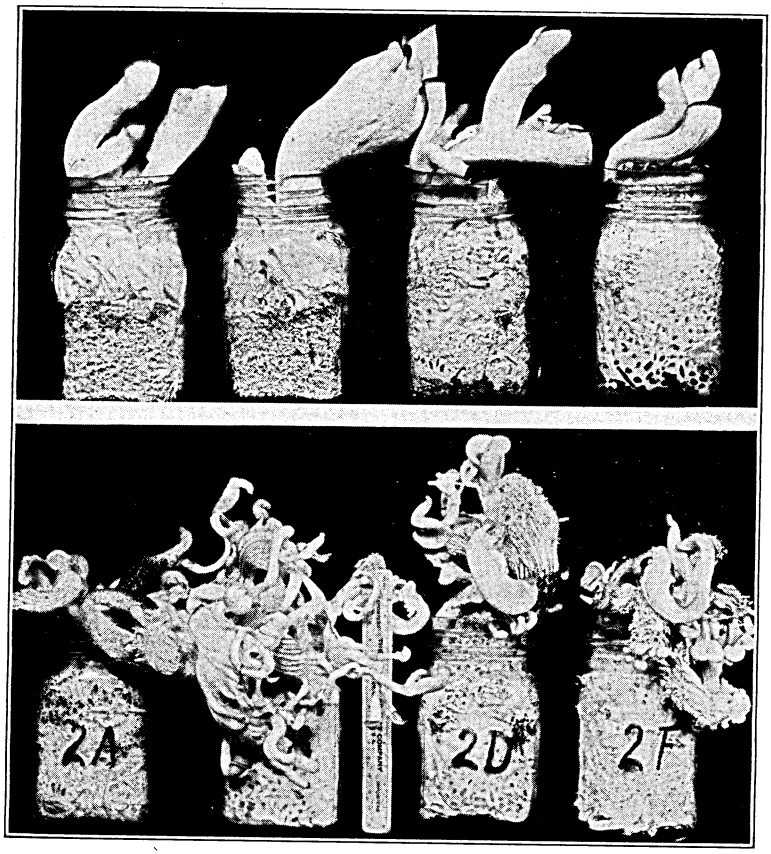



FIG. 3. DEVELOPMENT OF *Pleurotus Corticatus* SPOROPHORES

Upper picture. Sporophores four days after the formation of primordia, on a mixture of grain and basswood sawdust.

Lower picture. The same sporophores two weeks later. Basidiospore production has ceased and coremia have formed on the stipe and gills. Note the branching and formation of secondary sporophores.

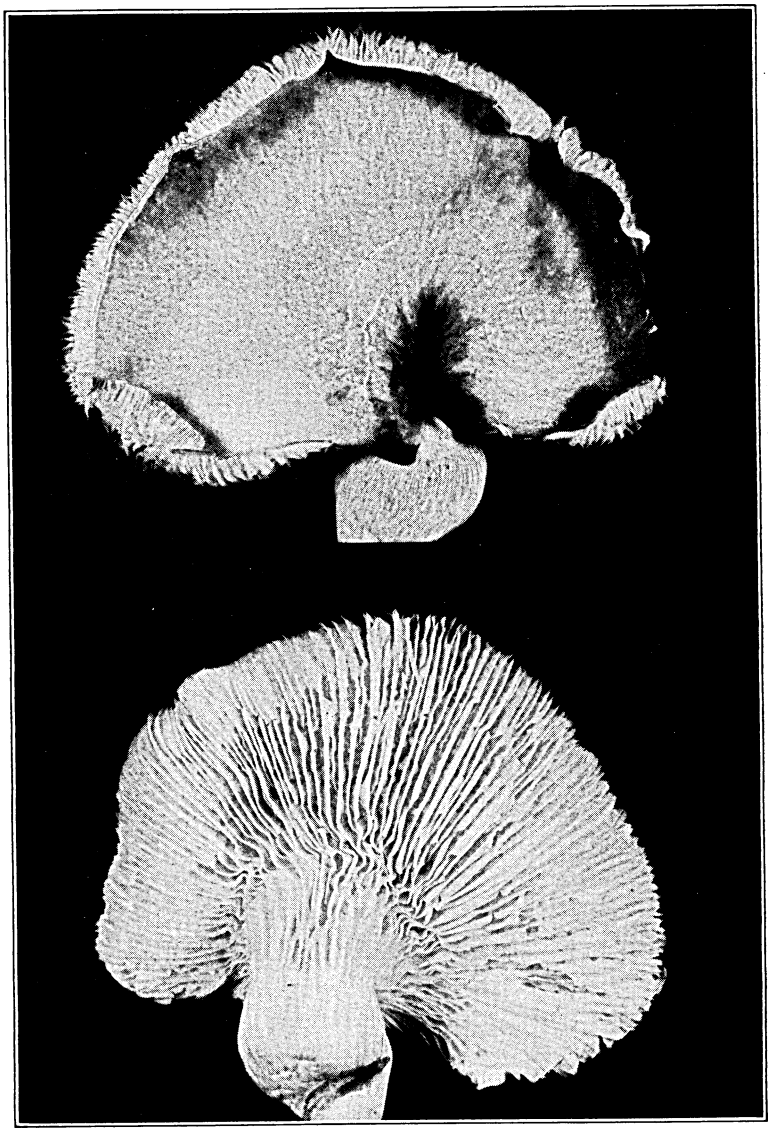


FIG. 4. UPPER AND LOWER VIEWS OF A SPOROPHORE OF *Pleurotus Corticatus* FORMED ON A MIXTURE OF GRAIN AND SAWDUST

formed. The elongated basidiospores are produced in tetrads on the basidia and may be collected in large numbers by placing a slide or petri dish beneath the gills for a second or two when spores are being actively discharged. If moisture and light conditions remain favorable, basidiospores may be shed from some sporophores for a week. The elongated basidiospores are particularly characteristic.

Soon after basidiospore production has ceased, small white protuberances appear on the gills and stipe. These are the primordia of coremia. If the sporophores are kept in a moist chamber, these continue growth and in six or eight days after their inception are capped with black drops of conidia (Fig. 5). The sporophores may be retained in a humid atmosphere for three weeks without material decomposition by molds or bacteria. At the end of this time the sporophores are usually covered with the black-topped coremia.


FIG. 5. SPOROPHORES OF *Pleurotus Corticatus* FORMED IN CULTURE

At the left, sporophores of *Pleurotus corticatus* produced in culture on a mixture of wheat, barley, and basswood sawdust. The largest sporophore was still producing basidiospores when the photo was made.

At the right, the same sporophore 16 days later, 10 days after basidiospore formation had ceased. The sporophore was kept in a humid chamber, as a result of which numerous coremia and black conidial heads have formed on the stipe, gills, and pileus.

This remarkable resistance of the sporophores to molding or bacterial decomposition and the formation of secondary spores, after basidiospore formation has ceased, suggests a unique adaptation of the fungus to its environment. The summer season in the Mississippi Delta is a season of abundant rainfall and high humidity. Air movement during this season is slight, consequently it is possible that the wind-blown basidio-

spores would not be a very effective means of dissemination. Provided with its conidial stage, however, the fungus has provisions for dissemination in damp weather as well as during dry periods. Altho conidia and coremia were not present on the sporophore collected at Greenville, Mississippi, at the time it was found, they did develop when it was slowly dried. This evidence, together with the abundant development of coremia on sporophores formed in culture and then kept in a moist chamber, indicates that the conidial stage is probably formed in nature when the sporophore is formed near the ground or falls to the ground after the production of basidiospores has ceased. Since the conidia are produced in a slightly sticky liquid, they could then be carried to other trees or wood material by insects, birds, or rodents.

So far as the writer is aware, Buller (3) and Brodie (1) are the only investigators who have observed the formation of asexual spores by Hymenomycetes in nature. They observed oidiophores and oidia of *Coprinus lagopus* formed under natural conditions. The oidia of *C. lagopus* are haploid and formed only on the haploid mycelium, whereas the conidia described above are binucleate. It seems likely that the binucleate conidia of *Pleurotus corticatus* are produced and function in nature, altho the evidence is only circumstantial.

## SEXUALITY

The sexuality of the *Agaricaceae* has been studied extensively. Some species have been found to be heterothallic, others homothallic. The heterothallic group consists of two types: bisexual or bipolar species and quadrisexual or tetrapolar species. In the bipolar species the spores borne on a single sporophore may be divided into two groups, according to the reaction of their mycelia with one another. Usually one-half the number belongs to each group. To explain this it has been assumed that a single pair of allelomorphic factors for sex are present in the fusion nucleus of the basidium. Brunswick (2), Kniep (7), Newton (8), and others have found many of the agarics to be of this type.

In the tetrapolar species the mycelia resulting from single spores borne on a sporophore belong to four groups, the members of one group mating and forming clamp connections with the members of one of the other groups and being sterile with the members of the other two groups and with each other. To explain this behavior it has been assumed that two pairs of allelomorphic sex factors, represented by Aa and Bb, are present in the fusion nucleus of the basidium. Four genetically different nuclei, AB, Ab, aB, and ab, result from reduction division of the nucleus. Normally, those haploid mycelia having no factors in common will form hyphal anastomoses when paired and will produce a dicaryotic

mycelium with clamps, ABab and AbaB. Numerous agarics have this type of sexuality, according to Vandendries (13), Buller (3), Smith and Brodie (9), and others.

Since the mycelia resulting from single basidiospores of *Pleurotus corticatus* had no clamp connections and were incapable of forming sporophores when grown alone, the fungus appeared to be heterothallic. The haploid mycelia resulting from the germination of single basidiospores were found to differ from the dicaryotic mycelia in the following respects:

1. The haploid mycelium had no clamp connections, whereas the dicaryotic mycelium had a clamp at every cross wall. The newly formed cells of the haploid mycelium were always uninucleate, those of the dicaryotic were binucleate.

2. Abortive sporophores and sporophores with gills were usually formed in the dicaryotic cultures but were never formed in the haploid cultures.

3. Altho coremia were formed on both types of mycelium, the coremia and conidia of the dicaryotic mycelium were much larger than those on the haploid mycelium. The immature binucleate conidia may be recognized by the remnants of the clamp connections which give these spores a beaked appearance.

4. The haploid mycelium grows only about 70 per cent as fast as the dicaryotic and does not make as luxuriant a growth.

These constant and easily discernible differences between haploid and dicaryotic mycelia of *Pleurotus corticatus* make it easy to distinguish the two. As the fungus appeared to be heterothallic, attempts were made to determine whether it was tetrapolar or bipolar. Matings were made between a large number of single basidiospore cultures from a single sporophore.

## Types of Reactions Between Different Haplonts

Preliminary experiments were first made by mating a single haploid mycelium of isolate 2A with each of 29 other haploid mycelia from the same sporophore. These tests were made in petri dishes of malt agar, the two pieces of inoculum being placed about ½ inch apart and the resulting mycelia allowed to grow together. These preliminary tests indicated that there were four distinct types of reactions between haploid mycelia from the same sporophore: compatible, neutral, antagonistic, and inhibitory (Fig. 6). The distinguishing features of these reactions are given below.

1. *Compatible.*—The haploid mycelia meet, anastomose, and clamp connections are formed all through the cultures. The two mycelia mutually diploidize each other, the resulting mycelium having all the charac-

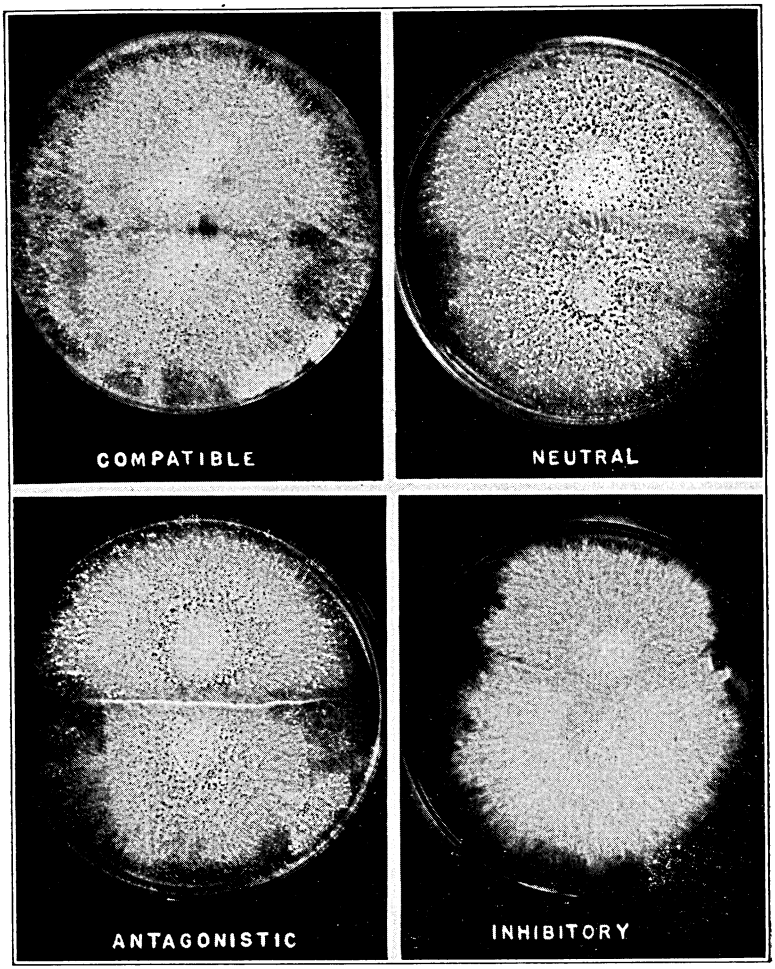



FIG. 6. REACTIONS BETWEEN HAPLOID MYCELIA OF *Pleurotus Corticatus*
Single basidiospore cultures from a single sporophore. Two haploid mycelia mated in each petri dish.

teristics of a dicaryotic culture of the fungus. After diploidization the rate of growth of the mycelium also increases, the identity of the two original haplonts being completely obliterated (Fig. 6).

2. *Neutral.*—The haploid mycelia meet, intermingle, anastomoses occur, but no binucleate mycelium with clamp connections is formed. The hyphal cells remain uninucleate, and the conidia formed in coremia are small and also uninucleate. There is no sharp line of demarcation between the haplonts and no change in their general appearance. They grow together like sub-cultures of the same haplont (Fig. 6).

3. *Antagonistic.*—The haploid mycelia meet and the hyphae intermingle in a narrow region between the two. This is evident by the development of a narrow line of aerial mycelium (Fig. 6). Hyphae of both mycelia are present in this white line of aerial mycelium, but their mutual antagonism prevents them from growing further into the region of the other. If transfers are made from the mycelium of the white line separating the two haploid cultures, the two mycelia separate and each grows as in its original condition (Fig. 8).

4. *Inhibitory.*—The most striking feature of this reaction is the almost complete inhibition of coremial and conidial formation (Fig. 6). Either haplont when growing alone is capable of producing abundant coremia and conidia, but when mated they react upon each other in such a way that this capability is suppressed. The hyphae intermingle freely, and hyphal anastomoses occur. A few clamp connections, resembling the pseudoclamps described by Buller (3), occur scattered throughout the culture. If transfers are made from the point of union of two inhibitory haplonts, the resulting cultures are found to grow very slowly, the mycelium is thin and lies flat on the agar, and coremia and conidial production is suppressed (Fig. 9).

The compatible and neutral reactions are common to most agarics whose sex reactions have been determined. The occurrence of antagonism or sexual barrages between haploid mycelia has been described by Vandendries (10, 12) and Vandendries and Brodie (9) for *Pholiota aurivella, Pleurotus ostreatus,* and several other agarics. These authors have also pointed out the usefulness of such reactions in analyses of sex. As far as the writer is aware, no reaction between the haploid mycelia of other agarics has been described that compares with the inhibitory reaction observed between haploid mycelia of *Pleurotus corticatus.*

## Matings of Monosporous Mycelia

In order to determine how the reactions between the haploid mycelia were grouped, a large number of haploid mycelia from basidiospores of a single fruiting body were mated. Small bits of inoculum of two haploid mycelia were placed on opposite sides of agar slants in test tubes. The cultures were allowed to grow about three weeks and were then examined. As shown in Figure 7, the reactions were even more characteristic than in petri dishes. Altho it was possible to classify most of the reactions by a macroscopic examination, the mycelium and spores in each tube were examined microscopically as well. The characteristics used in separating the cultures into groups according to the reaction type were:

1. For the compatible reactions—
   a. The presence of aborted sporophores
   b. The presence of binucleate conidia
   c. The presence of mycelium with clamp connections
2. For the neutral reactions—
   a. The lack of a sharp line between mycelia
   b. The presence of coremia and uninucleate conidia
   c. The absence of clamp connections
3. For the inhibitory reactions—
   a. The absence of coremia and conidia
   b. The lack of a sharp line between haplonts
   c. The slow growth of the mycelium and its white powdery appearance
4. For the antagonistic reactions—
   a. The presence of a sharp white line between haplonts
   b. The presence of coremia and uninucleate conidia
   c. The absence of clamp connections

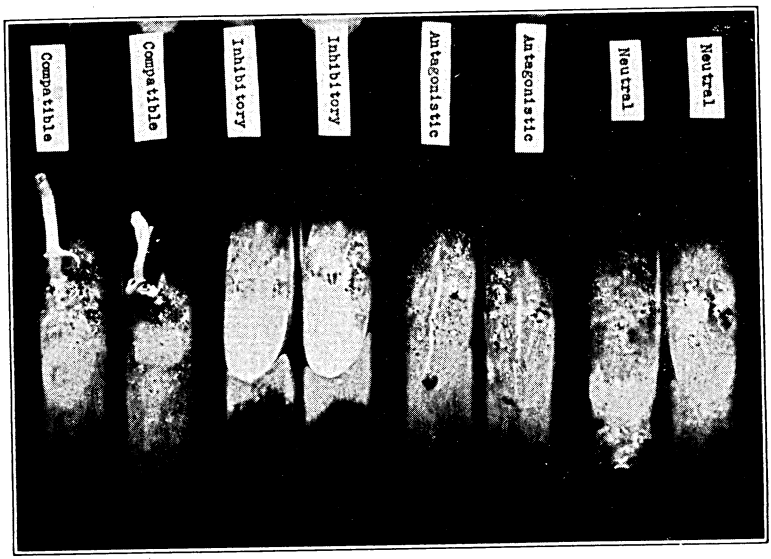



FIG. 7. REACTIONS BETWEEN HAPLOID MYCELIA OF *Pleurotus Corticatus*

The four types of reactions obtained when single basidiospore cultures, all from the same sporophore, are paired. This shows how the matings were made, two haplonts being placed in each tube and allowed to interact.

By means of the above method, 30 haploid or single basidiospore cultures of three isolates of *Pleurotus corticatus,* 2A, 2B, and 2F, were tested for their sex reactions. The cultures of each form were numbered from 1 to 30 and paired in all possible combinations, making 900 crosses for each of the 3 isolates. Three weeks to a month after the pairings were made, the cultures were examined microscopically and macroscop-

TABLE 2

MATINGS OF 29 MONOSPOROUS MYCELIA DERIVED FROM A SINGLE SPOROPHORE OF PLEUROTUS CORTICATUS ISOLATE 2A

| | | AB | | | | | | | | ab | | | | | | | | Ab | | | | | | | | aB | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 7 | 8 | 15 | 21 | 22 | 30 | 4 | 5 | 10 | 12 | 23 | 25 | 3 | 6 | 9 | 11 | 20 | 24 | 27 | 28 | 13 | 14 | 16 | 17 | 18 | 19 | 29 |
| AB | 1 | N | N | N | N | N | N | N | N | + | + | + | + | + | + | A | A | A | A | A | A | A | A | I | I | I | I | I | I | I |
| | 2 | N | N | N | N | N | N | N | N | + | + | + | + | + | + | A | A | A | A | A | A | A | A | I | I | I | I | I | I | I |
| | 7 | N | N | N | N | N | N | N | N | + | + | + | + | + | + | A | A | A | A | A | A | A | A | I | I | I | I | I | I | I |
| | 8 | N | N | N | N | N | N | N | N | + | + | + | + | + | + | A | A | A | A | A | A | A | A | I | I | I | I | I | I | I |
| | 15 | N | N | N | N | N | N | N | N | + | + | + | + | + | + | A | A | A | A | A | A | A | A | I | I | I | I | I | I | I |
| | 21 | N | N | N | N | N | N | N | N | + | + | + | + | + | + | A | A | A | A | A | A | A | A | I | I | I | I | I | I | I |
| | 22 | N | N | N | N | N | N | N | N | + | + | + | + | + | + | A | A | A | A | A | A | A | A | I | I | I | I | I | I | I |
| | 30 | N | N | N | N | N | N | N | N | + | + | + | + | + | + | A | A | A | A | A | A | A | A | I | I | I | I | I | I | I |
| ab | 4 | + | + | + | + | + | + | + | + | N | N | N | N | N | N | I | I | I | I | I | I | I | I | A | A | A | A | A | A | A |
| | 5 | + | + | + | + | + | + | + | + | N | N | N | N | N | N | I | I | I | I | I | I | I | I | A | A | A | A | A | A | A |
| | 10 | + | + | + | + | + | + | + | + | N | N | N | N | N | N | I | I | I | I | I | I | I | I | A | A | A | A | A | A | A |
| | 12 | + | + | + | + | + | + | + | + | N | N | N | N | N | N | I | I | I | I | I | I | I | I | A | A | A | A | A | A | A |
| | 23 | + | + | + | + | + | + | + | + | N | N | N | N | N | N | I | I | I | I | I | I | I | I | A | A | A | A | A | A | A |
| | 25 | + | + | + | + | + | + | + | + | N | N | N | N | N | N | I | I | I | I | I | I | I | I | A | A | A | A | A | A | A |
| Ab | 3 | A | A | A | A | A | A | A | A | I | I | I | I | I | I | N | N | N | N | N | N | N | N | + | + | + | + | + | + | + |
| | 6 | A | A | A | A | A | A | A | A | I | I | I | I | I | I | N | N | N | N | N | N | N | N | + | + | + | + | + | + | + |
| | 9 | A | A | A | A | A | A | A | A | I | I | I | I | I | I | N | N | N | N | N | N | N | N | + | + | + | + | + | + | + |
| | 11 | A | A | A | A | A | A | A | A | I | I | I | I | I | I | N | N | N | N | N | N | N | N | + | + | + | + | + | + | + |
| | 20 | A | A | A | A | A | A | A | A | I | I | I | I | I | I | N | N | N | N | N | N | N | N | + | + | + | + | + | + | + |
| | 24 | A | A | A | A | A | A | A | A | I | I | I | I | I | I | N | N | N | N | N | N | N | N | + | + | + | + | + | + | + |
| | 27 | A | A | A | A | A | A | A | A | I | I | I | I | I | I | N | N | N | N | N | N | N | N | + | + | + | + | + | + | + |
| | 28 | A | A | A | A | A | A | A | A | I | I | I | I | I | I | N | N | N | N | N | N | N | N | + | + | + | + | + | + | + |
| aB | 13 | I | I | I | I | I | I | I | I | A | A | A | A | A | A | + | + | + | + | + | + | + | + | N | N | N | N | N | N | N |
| | 14 | I | I | I | I | I | I | I | I | A | A | A | A | A | A | + | + | + | + | + | + | + | + | N | N | N | N | N | N | N |
| | 16 | I | I | I | I | I | I | I | I | A | A | A | A | A | A | + | + | + | + | + | + | + | + | N | N | N | N | N | N | N |
| | 17 | I | I | I | I | I | I | I | I | A | A | A | A | A | A | + | + | + | + | + | + | + | + | N | N | N | N | N | N | N |
| | 18 | I | I | I | I | I | I | I | I | A | A | A | A | A | A | + | + | + | + | + | + | + | + | N | N | N | N | N | N | N |
| | 19 | I | I | I | I | I | I | I | I | A | A | A | A | A | A | + | + | + | + | + | + | + | + | N | N | N | N | N | N | N |
| | 29 | I | I | I | I | I | I | I | I | A | A | A | A | A | A | + | + | + | + | + | + | + | + | N | N | N | N | N | N | N |

+ = COMPATIBLE  A = ANTAGONISTIC

N = NEUTRAL  I = INHIBITORY

ically and the reactions recorded. Symbols and letters were used to denote the reactions, the compatible by (+), the neutral by (N), the antagonistic by (A), and the inhibitory by (I).

The results of these matings with the haploid mycelia of isolates 2A, 2F, and 2B are given in Tables 2, 3, and 4. The spores of isolates 2A and 2F fall into four groups, which shows that the fungus is tetrapolar. The reactions resulting from these pairings may be explained on a factorial basis if we assign the factors (AB) to the first group of spores; their allelomorphs (ab) to the second group; the first factor and the allelomorph of the second (Ab) to the third group; and the second factor and the allelomorph of the first (aB) to the fourth group.

TABLE 3

MATINGS OF 30 MONOSPOROUS MYCELIA DERIVED FROM A SINGLE SPOROPHORE OF PLEUROTUS CORTICATUS ISOLATE 2F

| | | AB | | | | | | ab | | | | | | | | | | | | | | | Ab | | | | | | | | | | aB | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 8 | 11 | 12 | 13 | 16 | 22 | 3 | 5 | 9 | 10 | 14 | 19 | 21 | 24 | 26 | 27 | 28 | 1 | 2 | 4 | 6 | 7 | 15 | 18 | 20 | 23 | 30 | 17 | 25 | 29 |
| AB | 8 | N | N | N | N | N | N | + | + | + | + | + | + | + | + | + | + | + | A | A | A | A | A | A | A | A | A | A | I | I | I |
| | 11 | N | N | N | N | N | N | + | + | + | + | + | + | + | + | + | + | + | A | A | A | A | A | A | A | A | A | A | I | I | I |
| | 12 | N | N | N | N | N | N | + | + | + | + | + | + | + | + | + | + | + | A | A | A | A | A | A | A | A | A | A | I | I | I |
| | 13 | N | N | N | N | N | N | + | + | + | + | + | + | + | + | + | + | + | A | A | A | A | A | A | A | A | A | A | I | I | I |
| | 16 | N | N | N | N | N | N | + | + | + | + | + | + | + | + | + | + | + | A | A | A | A | A | A | A | A | A | A | I | I | I |
| | 22 | N | N | N | N | N | N | + | + | + | + | + | + | + | + | + | + | + | A | A | A | A | A | A | A | A | A | A | I | I | I |
| ab | 3 | + | + | + | + | + | + | N | N | N | N | N | N | N | N | N | N | N | I | I | I | I | I | I | I | I | I | I | A | A | A |
| | 5 | + | + | + | + | + | + | N | N | N | N | N | N | N | N | N | N | N | I | I | I | I | I | I | I | I | I | I | A | A | A |
| | 9 | + | + | + | + | + | + | N | N | N | N | N | N | N | N | N | N | N | I | I | I | I | I | I | I | I | I | I | A | A | A |
| | 10 | + | + | + | + | + | + | N | N | N | N | N | N | N | N | N | N | N | I | I | I | I | I | I | I | I | I | I | A | A | A |
| | 14 | + | + | + | + | + | + | N | N | N | N | N | N | N | N | N | N | N | I | I | I | I | I | I | I | I | I | I | A | A | A |
| | 19 | + | + | + | + | + | + | N | N | N | N | N | N | N | N | N | N | N | I | I | I | I | I | I | I | I | I | I | A | A | A |
| | 21 | + | + | + | + | + | + | N | N | N | N | N | N | N | N | N | N | N | I | I | I | I | I | I | I | I | I | I | A | A | A |
| | 24 | + | + | + | + | + | + | N | N | N | N | N | N | N | N | N | N | N | I | I | I | I | I | I | I | I | I | I | A | A | A |
| | 26 | + | + | + | + | + | + | N | N | N | N | N | N | N | N | N | N | N | I | I | I | I | I | I | I | I | I | I | A | A | A |
| | 27 | + | + | + | + | + | + | N | N | N | N | N | N | N | N | N | N | N | I | I | I | I | I | I | I | I | I | I | A | A | A |
| | 28 | + | + | + | + | + | + | N | N | N | N | N | N | N | N | N | N | N | I | I | I | I | I | I | I | I | I | I | A | A | A |
| Ab | 1 | A | A | A | A | A | A | I | I | I | I | I | I | I | I | I | I | I | N | N | N | N | N | N | N | N | N | N | + | + | + |
| | 2 | A | A | A | A | A | A | I | I | I | I | I | I | I | I | I | I | I | N | N | N | N | N | N | N | N | N | N | + | + | + |
| | 4 | A | A | A | A | A | A | I | I | I | I | I | I | I | I | I | I | I | N | N | N | N | N | N | N | N | N | N | + | + | + |
| | 6 | A | A | A | A | A | A | I | I | I | I | I | I | I | I | I | I | I | N | N | N | N | N | N | N | N | N | N | + | + | + |
| | 7 | A | A | A | A | A | A | I | I | I | I | I | I | I | I | I | I | I | N | N | N | N | N | N | N | N | N | N | + | + | + |
| | 15 | A | A | A | A | A | A | I | I | I | I | I | I | I | I | I | I | I | N | N | N | N | N | N | N | N | N | N | + | + | + |
| | 18 | A | A | A | A | A | A | I | I | I | I | I | I | I | I | I | I | I | N | N | N | N | N | N | N | N | N | N | + | + | + |
| | 20 | A | A | A | A | A | A | I | I | I | I | I | I | I | I | I | I | I | N | N | N | N | N | N | N | N | N | N | + | + | + |
| | 23 | A | A | A | A | A | A | I | I | I | I | I | I | I | I | I | I | I | N | N | N | N | N | N | N | N | N | N | + | + | + |
| | 30 | A | A | A | A | A | A | I | I | I | I | I | I | I | I | I | I | I | N | N | N | N | N | N | N | N | N | N | + | + | + |
| aB | 17 | I | I | I | I | I | I | A | A | A | A | A | A | A | A | A | A | A | + | + | + | + | + | + | + | + | + | + | N | N | N |
| | 25 | I | I | I | I | I | I | A | A | A | A | A | A | A | A | A | A | A | + | + | + | + | + | + | + | + | + | + | N | N | N |
| | 29 | I | I | I | I | I | I | A | A | A | A | A | A | A | A | A | A | A | + | + | + | + | + | + | + | + | + | + | N | N | N |

+ = COMPATIBLE  A = ANTAGONISTIC

N = NEUTRAL  I = INHIBITORY

Tables 2 and 3 show that a neutral reaction results when mycelia of the same factorial composition (AB x AB), (ab x ab), (Ab x Ab), and (aB x aB) are mated. Mycelia having both sets of factors (AB x ab) or (Ab x aB) are compatible, and the two dicaryotic mycelia (ABab) and (AbaB) are formed. When mycelia having the factor (A) or its allelomorph (a) in common are mated, (AB x Ab) or (ab x aB), the antagonistic reaction occurs. When mycelia having the factor (B) or its allelomorph (b) in common are mated, (AB x aB) or (Ab x ab), an inhibitory reaction results; the mycelia intermingle but coremia and conidia are not produced. As can be seen from Tables 2 and 3, the

spores of these two forms fall into four groups.[5] It is possible to recognize these groups when all four reactions are used or when the spores are arranged according to any one of the four reactions.

TABLE 4

MATINGS OF 30 MONOSPOROUS MYCELIA DERIVED FROM A SINGLE SPOROPHORE OF PLEUROTUS CORTICATUS ISOLATE 2B

| | | AB | | | | | | ab | | | | | aB | | | | | | | | | | | | Ab | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 15 | 16 | 21 | 22 | 28 | 1 | 6 | 10 | 12 | 30 | 3 | 8 | 9 | 11 | 13 | 14 | 17 | 18 | 19 | 20 | 23 | 26 | 29 | 4 | 27 | 5 | 7 | 24 | 25 |
| AB | 2 | N | N | N | N | N | N | + | + | + | + | + | I | I | I | I | I | I | I | I | I | I | I | I | I | I | A | + | + | + | + |
| | 15 | N | N | N | N | N | N | + | + | + | + | + | I | I | I | I | I | I | I | I | I | I | I | I | I | I | A | + | + | + | + |
| | 16 | N | N | N | N | N | N | + | + | + | + | + | I | I | I | I | I | I | I | I | I | I | I | I | I | I | A | + | + | + | + |
| | 21 | N | N | N | N | N | N | + | + | + | + | + | I | I | I | I | I | I | I | I | I | I | I | I | I | I | A | + | + | + | + |
| | 22 | N | N | N | N | N | N | + | + | + | + | + | I | I | I | I | I | I | I | I | I | I | I | I | I | I | A | + | + | + | + |
| | 28 | N | N | N | N | N | N | + | + | + | + | + | I | I | I | I | I | I | I | I | I | I | I | I | I | I | A | + | + | + | + |
| ab | 1 | + | + | + | + | + | + | N | N | N | N | N | A | A | A | A | A | A | A | A | A | A | A | A | A | A | I | + | + | + | + |
| | 6 | + | + | + | + | + | + | N | N | N | N | N | A | A | A | A | A | A | A | A | A | A | A | A | A | A | I | + | + | + | + |
| | 10 | + | + | + | + | + | + | N | N | N | N | N | A | A | A | A | A | A | A | A | A | A | A | A | A | A | I | + | + | + | + |
| | 12 | + | + | + | + | + | + | N | N | N | N | N | A | A | A | A | A | A | A | A | A | A | A | A | A | A | I | + | + | + | + |
| | 30 | + | + | + | + | + | + | N | N | N | N | N | A | A | A | A | A | A | A | A | A | A | A | A | A | A | I | + | + | + | + |
| aB | 3 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| | 8 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| | 9 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| | 11 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| | 13 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| | 14 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| | 17 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| | 18 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| | 19 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| | 20 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| | 23 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| | 26 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| | 29 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| | 4 | I | I | I | I | I | I | A | A | A | A | A | N | N | N | N | N | N | N | N | N | N | N | N | N | N | + | + | + | + | + |
| Ab | 27 | A | A | A | A | A | A | I | I | I | I | I | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | + | + | + | + |
| | 5 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | N | N | N |
| | 7 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | N | N | N |
| | 24 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | N | N | N |
| | 25 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | N | N | N |

+ = COMPATIBLE A = ANTAGONISTIC
N = NEUTRAL I = INHIBITORY

The results given in Table 4 for isolate 2B of *Pleurotus corticatus,* however, are not as clear-cut and easy to explain. The same reactions were obtained with 26 of the 30 haploid mycelia mated, and if only these are considered the spores again fall into four distinct groups. This grouping may be explained on a factorial basis as was done for forms

[5] The results with matings of only 29 spores are shown for isolate 2A. The culture from spore number 26 was lost through contamination.

2A and 2F. As shown by the table, however, there appeared a fifth group, composed of mycelia numbers 5, 7, 24, and 25. These mycelia are capable of forming dicaryotic mycelia with all the other 26 mycelia but when mated among themselves give neutral reactions. This last fact shows that the mycelia were not dicaryotic at the beginning. Careful microscopic examination of these four mycelia also showed that they were typically haploid in every respect.

Vandendries (14) obtained somewhat similar results with the haploid mycelia of *Hypholoma hydrophilum*. He found that one of the 21 mycelia he used in his mating tests could mate with all of the remaining 20 mycelia and formed dicaryotic mycelium with them. He attributes this behavior to a mutation of some type which had changed the factorial composition of the spore from which the mycelium was obtained and had made it possible for this mycelium to mate with all the other mycelia. If only a single mycelium of isolate 2B had shown a similar reaction, such an explanation might be tenable, but when 4 out of 30 haplonts exhibit this characteristic, it seems that there must be some other explanation.

## Growth of the Mycelial Combinations

In connection with the mating tests, several experiments were made to compare the rate and type of growth of dicaryotic mycelia, haploid mycelia, and the mycelia resulting from the crosses of haploid mycelia. The results with the parent dicaryotic culture and haploid mycelia of isolate 2B are illustrated in Figure 8. Four haploid cultures, 2, 11, 25, and 30, were mated with haploid culture 13. The mating (2 x 13) shows an inhibitory reaction, (11 x 13) is neutral, (25 x 13) is compatible, and (30 x 13) is antagonistic. Transfers from the point of union of the mated haploid mycelia are shown in the bottom row of cultures. All the cultures shown in Figure 8 are the same age and are growing on the same type of medium. The sharp line of demarcation between haploid mycelia of the antagonistic reaction and the tendency of the mycelia to grow separately when transfers are made from the mycelium of the white line is clearly shown. Matings of mycelia that are neutral do not exhibit any unusual features; the culture made from mycelium taken at the point of union of the two mycelia grows like either of the haplonts. The suppression of coremia and conidia in the mating of mycelia having an inhibitory effect on each other is clearly illustrated, as is the extremely slow growth rate of transfers from such matings. The similarity between the transfer from the compatible mating and the parent dicaryotic mycelium is strikingly apparent.

The antagonistic and inhibitory matings are particularly interesting. The lack of attraction between the mycelia in the case of the antagonistic

reaction is clearly shown by the separation of the mycelia when a transfer is made from the point of union of the mycelia. Apparently the antagonism is too great to permit the mycelia to intermingle and grow together. The reduced growth of the inhibitory matings must be due to some strange attraction between the two mycelia, which keeps them from separating as in the antagonistic matings and which is detrimental to both mycelia. Transfers of these matings were used in the decay tests described in another part of this bulletin. Transfers of these matings have also been made to jars of sterilized grain and sawdust in attempts to obtain sporophores. To date, however, success has been had only with the compatible matings. None of the mycelia resulting from the neutral, antagonistic, or inhibitory matings appear to be capable of sporophore production on the above medium.

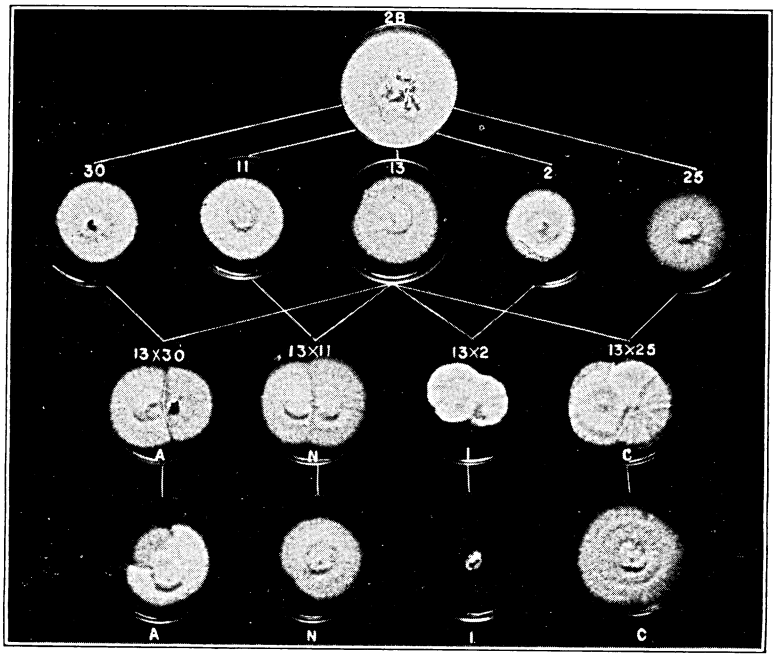



FIG. 8. THE GROWTH OF DICARYOTIC, HAPLOID, AND MATINGS OF HAPLOID MYCELIA OF *Pleurotus Corticatus*, ISOLATE 2B

The parent dicaryotic culture 2B at the top. The five haploid mycelia 2, 11, 13, 25, and 30 in row 2. Matings between haploid mycelium 13 and mycelia 2, 11, 25, and 30 in row 3. Transfers from the point of union of the mycelia mated are shown in row 4. All cultures of the same age.

## Diploidization of Haploid Mycelia by Dicaryotic Mycelia

Buller (3) was the first to observe that certain haploid mycelia could be diploidized by the parent dicaryotic mycelia. He was able to transform haploid mycelia of *Coprinus lagopus,* with a composition of (AB)

or (ab), into dicaryotic mycelia by mating with the dicaryotic mycelium (ABab), and the haplonts (Ab) or (aB) into dicaryotic mycelia by mating with the dicaryotic mycelium (ABaB). He explains this by assuming that when the haploid and dicaryotic hyphae meet, fusions occur; that the nucleus required to change the haploid mycelium into a dicaryotic mycelium divides, one of the resulting nuclei passing into the haploid mycelium, dividing and migrating from cell to cell until the entire haploid mycelium is transformed. He has also found that partial diploidization took place when certain "illegitimate" matings of haploid and dicaryotic mycelia were made.

Studies similar to those made by Buller with *Coprinus lagopus* were made with the haploid and dicaryotic mycelia of *Pleurotus corticatus*. The parent dicaryotic mycelium of the three isolates 2A, 2B, and 2F were mated in petri dishes with each of their 30 haploid derivatives. In each case the parent mycelium diploidized two of the groups of mycelia given in Tables 2, 3, and 4, but not the others. The dicaryotic mycelium of isolate 2A diploidized the mycelia with the factorial composition (AB) and (ab) but not the groups with composition (Ab) and (aB). This parent dicaryotic culture was thus shown to have the composition (ABab). The dicaryotic parent culture 2F diploidized the haplonts with the factorial composition (Ab) and (aB) but not those with a composition of (AB) and (ab). This mycelium therefore must have the composition (AbaB). When the parent dicaryotic culture of isolate 2B was mated with each of its haplonts, it was found to diploidize those mycelia with the factorial composition (Ab) and (aB) but not those with the composition (AB) and (ab). It was thus shown to have the factorial composition (AbaB). When the parent mycelium of isolate 2B was mated with the four aberrant haplonts described above, numbers 5, 7, 24, and 25, however, no diploidization was observed. This fact further illustrates the peculiar nature of these mycelia. They are capable of diploidizing any of the other haploid mycelia but are not diploidized when mated with the parent dicaryotic mycelium.

The diploidization process in the case of *Pleurotus corticatus* is not as rapid or as regular as with *Coprinus lagopus*. It usually requires two weeks or longer for a haploid mycelium to be transformed into a dicaryotic mycelium. The process is rather irregular, diploidization occurring in patches. The diploidization of haploid by dicaryotic mycelia is illustrated in Figure 9. If the haploid mycelium is diploidized, it immediately begins to grow like a dicaryotic mycelium, and the parent dicaryotic mycelium does not continue to grow over it. If, however, diploidization does not take place, the parent dicaryotic mycelium continues growth and, being more vigorous than the haploid mycelium, grows over the latter and covers it with a dense white mycelium (Fig. 9). When the

parent dicaryotic mycelium of isolate 2B was mated with the four aberrant mycelia, numbers 5, 7, 24, and 25, it neither diploidized them nor grew over them. This may be due to the fact that these four haploid mycelia are somewhat more vigorous than the others and so are able to repel the fast-growing dicaryotic mycelium.

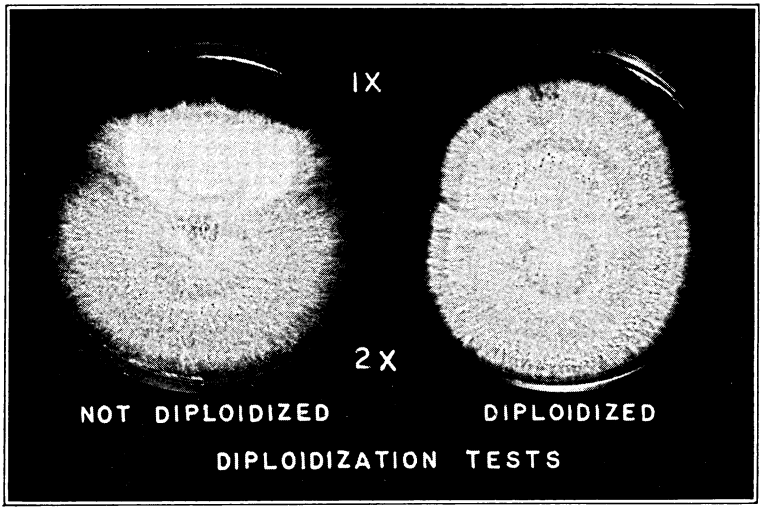



FIG 9. DIPLOIDIZATION TESTS WITH *Pleurotus Corticatus*
Two dicaryotic mycelia (2X) were mated with two haploid mycelia (1X). The haploid culture at the right has been diploidized, the one at the left has not.

## WOOD DECAY STUDIES

In nature, *Pleurotus corticatus* was found attacking young fire-scarred trees of red gum and Nuttall oak. The decay columns in these trees were always limited to the centers of the trees and often extended 10 or 12 feet up the bole. Since most of these trees were only 30 to 40 years old and fast growers, they had very little heartwood, this being particularly true of the red gum. Second-growth red gum trees 18 inches in diameter often have no trace of heartwood. Uninjured trees without heartwood are usually considered resistant to attack by wood-rotting fungi. If injured, decay ordinarily occurs in the dead sapwood around the wound but rarely spreads far. For this reason it is considered safe to prune young trees before heartwood has begun to form. The small amount of rot that gets in through pruning wounds rarely spreads far enough to be of any consequence. For the same reason, fire scars on young trees without heartwood have been considered less dangerous than on more mature trees. This apparent resistance to decay

has always been thought to be due to the following characteristics of sapwood:

1. High moisture content. Sapwood of practically every known tree species has a higher moisture content than the heartwood. In Norway pine the sapwood may have a moisture content of 150 per cent (based on oven-dry weight), whereas the heartwood rarely has a moisture content of more than 50 per cent. Whether the difference in red gum and oak is as striking as this is not definitely known.

2. The living cells of the sapwood, the parenchyma of the medullary rays, may produce substances which prohibit the growth of the fungous mycelium.

3. Lack of oxygen, a recognized corollary of moisture content, may be another factor that accounts for the inability of wood destroyers to penetrate far into living sapwood.

It is interesting, therefore, to find instances where wood destroyers have gained entrance to trees without detectable heartwood formation and then have continued to spread up the bole in a manner characteristic of heart-rotting fungi. Such is the case with *Pleurotus corticatus, Lentinus tigrinus, Polyporus lucidus,* and several other fungi found attacking young fire-scarred trees in the Mississippi Delta.[6] They must have the ability to attack the sapwood despite its high moisture content and the presence of living cells. Once they have gained entrance through basal fire scars they continue to spread up the trunk after the manner of heart-rotters.

Preliminary tests made with *Pleurotus corticatus* on blocks of red gum and several of the oaks showed that it produced the same pulpy white rot found in the trees in nature. Since the woods of its natural hosts were not readily available, all of the decay studies were made with basswood *(Tilia americana)*. Several series of decay tests were made to determine:

1. The minimum, optimum, and maximum moisture contents of basswood blocks at which decay would take place.

2. The rate of decay of the dicaryotic mycelium as compared to the haploid mycelium.

3. The rate of decay by various combinations of haploid lines involving the four types of reactions described above, namely, compatible, inhibitory, neutral, and antagonistic.

4. Whether coremia and conidia could be produced on and in wood blocks.

[6] Hepting, G. H. Decay Following Fire in Young Mississippi Delta Hardwoods. U.S.D.A. Tech. Bull. 494, 32 pp., 6 figs. 1935.

## The Effect of Moisture Content on Rate of Decay

As this fungus was one of several found rotting sapwood of living trees, it must be able to decay wood with a high moisture content. Wood-rotting fungi vary greatly in their ability to attack wood at different moisture contents. Some, such as the so-called dry rot fungi, *Merulius lachrymans, Coniophora cerebella,* and *Poria incrassata,* are able to attack wood at fiber saturation or below. This ability to attack dry wood has been ascribed to a number of factors: conduction of moisture by strands of mycelium from more moist places; absorption and storage of moisture when this is available; and recently a third explanation, the ability of certain fungi to obtain water by decomposition of the wood substance itself. As a rule, however, fungi can not attack wood until a moisture content well above fiber saturation is reached. The optimum moisture content for decay varies greatly between different fungi and different woods. Optimum moisture contents for decay usually vary from 60 to 100 per cent, based on oven-dry weight. The maximum moisture content at which decay occurs also varies considerably, usually being well over 100 per cent. Moisture content is often a more important factor in wood decay than is temperature and is a far more difficult variable to control and measure.

In these tests to determine the minimum, optimum, and maximum moisture content for decay by *Pleurotus corticatus,* basswood blocks were used. Blocks 1.5 x 2.5 x 5.0 cm. in size were cut from young trees of uniform growth. The test pieces were matched longitudinally so as to eliminate as much variability as possible. They were first oven-dried at 60° C. for 24 hours, and moisture was then added by immersing the blocks and drawing a partial vacuum on them. To obtain the lower moisture contents the blocks were removed, wiped dry, and weighed soon after they were immersed. Those having the desired moisture content were then removed and placed in tightly capped pint jars. Simple immersion was sufficient to obtain moisture contents of 30 and 60 per cent, but for higher moisture contents a vacuum was drawn to hasten the penetration of water. In this way about 65 blocks were obtained for each of 5 moisture contents, 30, 60, 90, 130, and 170 per cent.

The blocks were then steamed for 30 minutes without pressure and inoculated with two dicaryotic cultures of *Pleurotus corticatus,* isolates 2A and 2B. These jars were then placed in a room in which the humidity was kept between 90 and 95 per cent and the temperature varied between 24° and 26° C. In about two weeks the blocks with the higher moisture contents were covered with a dense white mycelium. The covers of the jars were then loosened to prevent accumulation of carbon dioxide. After 150 days the blocks were removed from the jars and the

mycelium removed from the surface. They were then dried at 60° C. for 24 hours. The difference between the original oven-dry weight and final oven-dry weight was taken as the measure of decay.

It is realized that this method is subject to a large number of uncontrolable variables. The most serious is perhaps the distribution of the moisture in the wood. At the lower moisture contents the moisture is concentrated near the surface of the block, giving the surface layers a high initial moisture content, whereas the inner layers remain dry. However, it is believed that this moisture gradient is reduced soon after the wood is placed in the humid chamber, the moisture being gradually distributed and coming to an equilibrium. Another objection is the loss in moisture during the test period; this is particularly true of the blocks at the higher moisture contents. Since it is impossible to keep the air completely saturated, some evaporation takes place through the loosened covers.

Even after deducting for all possible variables, however, the results of the tests do show certain facts concerning the moisture relations of this fungus. The results of these tests are presented in Table 5.

**Table 5. The Relation of Moisture Content to Decay of Basswood Blocks by *Pleurotus Corticatus***

| Form 2A | | | Form 2B | | |
|---|---|---|---|---|---|
| Moisture content in per cent | Number of blocks | Loss in weight in per cent of oven-dry weight | Moisture content in per cent | Number of blocks | Loss in weight in per cent of oven-dry weight |
| 30 | 24 | 0 | 30 | 23 | 0 |
| 55 to 60 | 28 | 1.4 | 55 to 60 | 23 | 1.1 |
| 80 to 90 | 26 | 7.2 | 80 to 90 | 29 | 9.6 |
| 110 to 130 | 25 | 14.1 | 110 to 130 | 22 | 15.4 |
| 150 to 170 | 21 | 1.3 | 150 to 170 | 24 | 2.4 |

These data show that no decay occurred at fiber saturation. As a matter of fact, none of the cultures placed on these blocks developed more than a small patch of mycelium around the piece of inoculum. Since at the higher moisture contents some loss in moisture occurred during the test period, a range of moistures, such as 55 to 60 per cent, is given. This indicates that some of the blocks, which had an original moisture content of 60 per cent, lost 5 per cent during the test period. At 55 to 60 per cent moisture the blocks were covered with a thin hyphal weft and some decay occurred. At 80 to 90 per cent moisture decay increased appreciably, being 7.2 per cent in the case of isolate 2A and 9.6 per cent in the case of isolate 2B. The optimum moisture content for decay lies somewhere between 110 and 130 per cent. The increase

in decay up to this point has been rapid. At 150 to 170 per cent moisture, however, there is a sharp falling off in amount of decay.

These results show that *Pleurotus corticatus* has a rather high optimum moisture content for decay, higher than the majority of wood-rotting fungi. This explains in part at least why this fungus is able to attack young trees without apparent heartwood formation, in which the moisture content of the wood is high.

### The Rate of Decay by Dicaryotic and Haploid Cultures

Practically all decay tests that have been made have been made with the dicaryotic mycelium of wood-destroying fungi. This is probably due to the fact that the haploid phase does not seem to be abundant in nature and is probably rarely obtained when isolations are made from decayed wood. It is interesting, therefore, to compare the wood-destroying abilities of haploid and dicaryotic mycelia in pure culture. Verrall (15) found that the haploid mycelia of *Fomes igniarius* caused only about one-half as much decay in a given period of time as the dicaryotic.

To determine whether haploid and dicaryotic cultures of *Pleurotus corticatus* differed in ability to cause decay, tests were made with basswood sawdust as the decay medium. The use of sawdust for such comparative tests is considered preferable to the use of blocks, because such variables as moisture and variations in the wood of different blocks can be eliminated.

The sawdust was prepared by running air-dry shavings through a hammer mill. The sawdust was then thoroly mixed, and small weighed samples placed in half-pint jars. The sawdust was not oven-dried before using, because such treatment seems to make it resistant to attack by this fungus. To obtain the oven-dry weight of the samples used in the tests, a number of check jars were placed in an oven at 104° C. and the contents dried for 24 hours. The oven-dry weight of the test samples was then computed from these checks. Enough water was added to the sawdust in the test jars to bring the moisture content up to 300 per cent, which preliminary experiments had shown to be the optimum for decay of sawdust. The jars were then sterilized for 15 minutes at 15 pounds pressure, inoculated, and placed in a humidity room for 90, 150, and 210 days, respectively. At the end of each period one-third of the jars were removed, the contents were oven-dried at 104° C., and the weights were recorded. The difference in original and final oven-dry weight was taken as the criterion of decay.

The results are given in Table 6. It may be well to mention at this point that the variation in loss of weight in the sawdust from jars of the same group was negligible. Altho each figure in the table is an

average of from 15 to 27 jars, it was found that 2 or 3 jars would have given equally consistent results.

**Table 6. The Rate of Decay by Haploid and Dicaryotic Cultures of *Pleurotus Corticatus**

| Strain of the fungus | Nuclear condition | Loss in weight in per cent of the oven-dry weight in | | |
|---|---|---|---|---|
| | | 90 days | 150 days | 210 days |
| 2A | Dicaryotic or binucleate | 9.6 | 17.5 | 26.0 |
| 2B | do | 9.6 | 15.4 | 21.7 |
| 2C | do | 14.5 | 23.8 | ..... |
| 2D | do | 17.8 | 21.6 | ..... |
| 2E | do | 14.5 | 21.6 | 31.6 |
| 2F | do | 15.6 | 20.5 | ..... |
| 1A | Haploid or uninucleate | 3.3 | 12.1 | 24.0 |
| 1B | do | 5.0 | 11.0 | 24.0 |
| 1C | do | 4.1 | 14.5 | 23.0 |
| 1D | do | 5.0 | 14.3 | 31.6 |
| 1E | do | 3.3 | 13.4 | 27.4 |
| 1A x 1D | Dicaryotic or binucleate | 15.6 | 19.5 | 28.2 |
| 1B x 1D | do | 14.5 | 21.6 | 28.2 |
| 1C x 1D | do | 14.9 | 21.6 | 30.4 |
| 1E x 1D | do | 14.4 | 17.4 | 19.0 |

* Each figure is based on an average of from 15 to 27 jars or tests.

The results of these tests are rather interesting. In the tests run for 90 days there are consistent differences between the haploid and dicaryotic cultures. The haploid cultures caused losses in weight varying from 3 to 5 per cent, whereas the dicaryotic lines caused losses of 9 to 17 per cent. Since the haploid lines were single basidiospore cultures from a sporophore of dicaryotic culture 2A, they are directly comparable with this culture and no other. It is evident, therefore, that the dicaryotic cultures cause two to three times as much decay in 90 days as the haploid cultures. Of the haploid mycelia, 1D when mated with either 1A, 1B, 1C, or 1E produced a dicaryotic mycelium (Fig. 10). Table 6 shows furthermore that in 90 days dicaryotic mycelia resulting from these crosses produced three times as much decay as the haploid and considerably more than the parent culture, 2A, with which they are directly comparable.

The difference in amount of decay caused by the haploid and dicaryotic lines is still considerable after a decay period of 150 days but is not as striking as it is at 90 days. After 210 days, however, it is interesting to note that the difference between the haploid and dicaryotic mycelia has been practically eliminated, the haploid lines having caused as much decay as the dicaryotic.

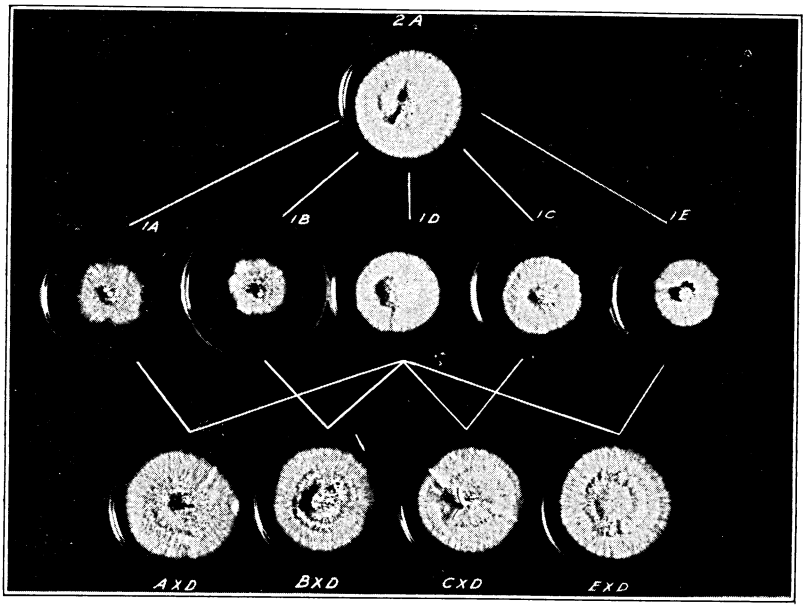



FIG. 10. DICARYOTIC, HAPLOID, AND MATINGS OF HAPLOID CULTURES OF *Pleurotus Corticatus*

Five single basidiospore cultures, 1A, 1B, 1C, 1D, and 1E, were obtained from a sporophore grown from the dicaryotic mycelium 2A. Culture 1D was mated with each of the other four to form the dicaryotic mycelia A x D, B x D, C x D, and E x D. Transfers of these cultures were used in the decay tests of Table 7.

These results can be explained by the differential rate of growth of the haploid and dicaryotic mycelia. As shown in Figures 8, 9, and 10, the dicaryotic mycelium grows much more rapidly than the haploid; consequently it apparently can attack the sawdust vigorously from the very first, producing considerable losses in weight in 90 days. The haploid mycelium, on the other hand, grows more slowly and attacks the sawdust less vigorously at first. After 150 days the difference in amount of decay begins to disappear. In 210 days the haploid mycelia have caused as much decay as the dicaryotic cultures, probably because the rate of decay by the latter is much reduced. Unfortunately, a decay period longer than 210 days was not tried to check on the further behavior of the cultures.

### The Rate of Decay by Matings of Haploid Mycelia

Having found differences in rate of decay caused by haploid and dicaryotic mycelia, similar tests were made with various combinations of

haploid mycelia. Culture 2A and several haplonts obtained from single basidiospores produced on sporophores developed in culture were used. The tests were made as described above except that the jars were incubated in a room kept at 27° C., the optimum temperature for vegetative growth. Before beginning the tests matings were made between chosen haploid lines in order to obtain the various reactions described in a previous section, namely, compatible, neutral, antagonistic, and inhibitory. Mycelium from the line of union of these crosses was used. In this way any tendency of the two haplonts to grow as separate entities before meeting was eliminated. The tests were run for 90 days.

The results of this experiment are given in Table 7. The parent dicaryotic culture caused about twice as much decay as any of the haploid lines alone. The matings which resulted in dicaryotic mycelia produced as much or more decay than the parent culture, two to three times the amount produced by either of the constituent haploid lines acting alone. The neutral and antagonistic matings differed little from the haploid lines acting alone. Apparently the haploid lines grow as separate entities, together producing an amount of decay equal to that of either of the haplonts when grown alone. The inhibitory crosses, however, show the most striking difference. The mycelial growth in these cultures is weak, and the rate of decay is correspondingly slow. These remarkable differences in rate of decay agree closely with the behavior of the mycelia when grown on agar plates (Fig. 8). This reduction in decay when two haploid lines resulting from the same sporophore are mated and placed on sawdust resembles somewhat the antagonism of different fungi when grown together (16). Either haploid line when growing alone is capable of causing a loss in weight of basswood sawdust amounting to 6.7 per cent. When combined, however, the losses are sharply reduced, being only 2.1 per cent. This is a clear illustration of antagonism and reduction in virulence of two cultures of the same fungus and derived furthermore from basidiospores of a single fruiting body.

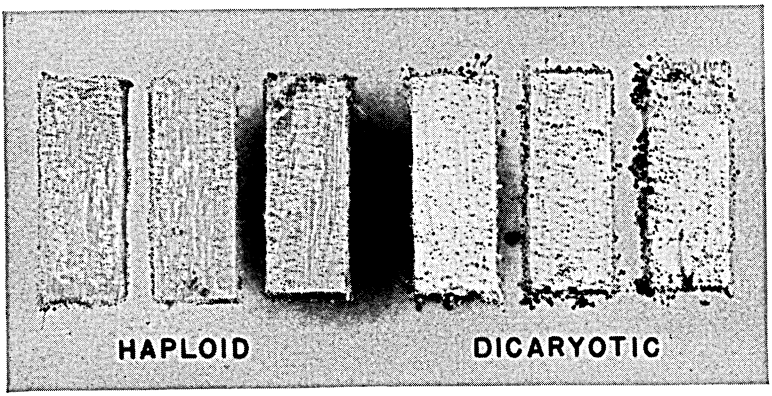
### The Production of Coremia and Conidia on Wood Blocks

When wood blocks are inoculated with haploid or dicaryotic cultures of *Pleurotus corticatus,* the fungus rapidly spreads over the surface of the wood and then penetrates from all sides. Two weeks after inoculation the blocks are usually completely covered with white mycelium, which then begins to form typical coremia and black conidial heads all over the surface of the blocks (Fig. 11).

Table 7. The Rate of Decay by Dicaryotic, Haploid, and Various Combinations of Haploid Lines of *Pleurotus Corticatus*

| Line or cross of form 2A | Nuclear condition of line or of the cross | Number of test jars used for each line or cross | Original weight of sawdust in grams | Oven-dry weight of sawdust after decay in grams | Loss in weight of sawdust during period in grams | Loss in weight of sawdust in per cent of oven-dry weight |
|---|---|---|---|---|---|---|
| 2A | Binucleate or dicaryotic | 42 | 19.1 | 16.5 | 2.6 | 15.8 |
| A-1 to A-15 | Haploid or uninucleate | 54 | 19.1 | 17.9 | 1.2 | 6.7 |
| Compatible crosses | Binucleate or dicaryotic | 11 | 19.1 | 16.1 | 3.0 | 18.6 |
| Neutral crosses | Haploid or uninucleate | 14 | 19.1 | 17.7 | 1.4 | 7.9 |
| Antagonistic crosses | do | 14 | 19.1 | 17.9 | 1.2 | 6.7 |
| Inhibitory crosses | Questionable | 14 | 19.1 | 18.7 | 0.4 | 2.1 |

The difference in size of coremia formed on the haploid and dicaryotic mycelia is shown in Figure 11. Usually the coremia are much larger on the blocks inoculated with the dicaryotic mycelium than those inoculated with the haploid mycelium.



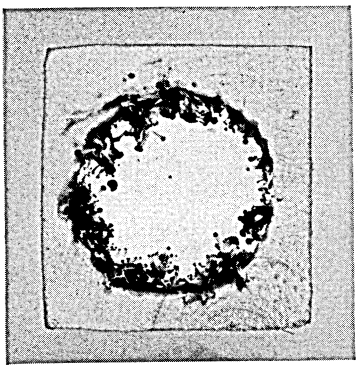



FIG. 11. COREMIA OF *Pleurotus Corticatus* FORMED ON WOOD

Upper picture. Basswood blocks inoculated with haploid and dicaryotic cultures of *Pleurotus corticatus*. The difference in size of coremia on the blocks is very apparent.

Left. A one-inch hole was drilled through a basswood block, the ends coated with paraffin, and the block inoculated with the dicaryotic mycelium of *P. corticatus*. This section through the center of this block shows the coremia and conidial heads of the fungus on the inner face of the hole.

Since the coremia formed so abundantly on the surface of inoculated wood blocks, tests were made to determine whether they would also form on the walls of holes bored through blocks. Holes were bored longitudinally through basswood blocks two to four inches long, the ends of the blocks were then covered with paraffin to prevent the mycelium from entering the open holes, and the blocks were inoculated on the radial and tangential surfaces. After two months the blocks were cut up and examined. In practically every case the mycelium had penetrated through the blocks and had formed coremia and black conidial heads on the walls of the holes (Fig. 11).

Since these holes resemble somewhat the tunnels made in trees by termites and ants, and coremia were formed in them, there is additional circumstantial evidence that the fungus is admirably adapted for insect dissemination.

## SUMMARY AND CONCLUSIONS

1. *Pleurotus corticatus* Fries was one of several fungi isolated from young fire-scarred and decayed trees growing in the Delta hardwood region of Louisiana and Mississippi.

2. The production of a striking asexual spore stage in culture attracted the writer's attention to the fungus and stimulated further investigations of its biology.

3. Binucleate conidia are formed on coremia. These coremia are formed from the dicaryotic vegetative mycelium, abortive sporophores, and fertile sporophores developed in culture. Simple binucleate conidia are also formed singly on the dicaryotic mycelium. Uninucleate conidia are formed on coremia on the haploid mycelia developing from single basidiospores. Simple conidia formed singly are also formed on the haploid mycelium.

4. Numerous large sporophores capable of producing basidiospores have been developed in culture. Sporophores are very resistant to decomposition by molds and bacteria.

5. The optimum temperature for vegetative growth is about 27° C. The most favorable medium for vegetative growth is malt agar.

6. Mating experiments were made between 30 haploid mycelia of three isolates of the fungus. When haploid mycelia from the same sporophore were mated, four types of reactions resulted: compatible, neutral, antagonistic, and inhibitory. These reactions are described, and one, the inhibitory reaction, to the writer's knowledge, has not been observed for other agarics.

7. These mating experiments have shown the fungus to be heterothallic and tetrapolar.

8. The diploidization of haploid by dicaryotic mycelia is described.

9. The dicaryotic mycelium causes the greatest decay of basswood blocks at a moisture content of 110 to 130 per cent.

10. The dicaryotic mycelia decay basswood sawdust more rapidly than haploid mycelia in tests lasting 90 to 150 days. When the decay period was lengthened to 210 days, however, the haploid mycelia caused as much decay as the parent dicaryotic mycelium or compatible matings of haploid mycelia.

11. The antagonistic and neutral matings of haploid mycelia decay basswood sawdust to the same extent as haploid cultures, about one-third to one-half as fast as the dicaryotic mycelia or compatible pairs of haploid mycelia. The inhibitory pairs of haploid mycelia decay sawdust much more slowly, only one-third as fast as the haploid mycelia and one-seventh to one-eighth as fast as the parent dicaryotic cultures.

12. Coremia and conidia are formed on and in basswood blocks.

This supports the supposition that the fungus may be disseminated by termites or other insects in nature.

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