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Cultural and Microscopical Studies Designed to Differentiate between Similar but Geographically Distinct Forms of Fungi

by
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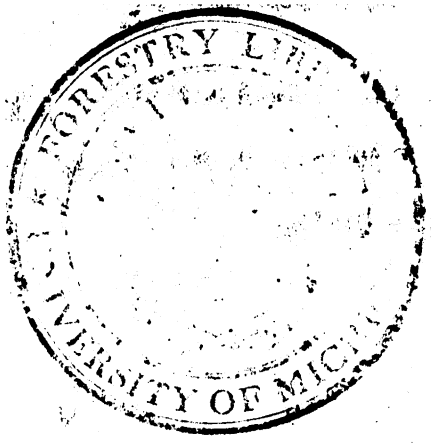
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ACKNOWLEDGMENTS

Grateful acknowledgment is here made to Dr. Dow V. Baxter under whose direction this study was made.

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INTRODUCTION

In making a diagnosis of the causal organism involved in the decay of wood a study of the fruiting body, if present, and of cultural characteristics is necessary. Color and other growth characteristics of fungi in culture are valuable in distinguishing between species. The comparative rates of growth of the fungus mycelium at certain temperatures and in the light and in the dark are also valuable criteria in the identification of certain fungi in culture. Studies similar to these involving several fungi of a species collected from different geographical ranges and different hosts offer opportunity to compare the varying effects of geographical ranges and also of substrata upon the growth of fungi. This thesis attempts to compare these effects and to differentiate between certain geographical forms.

FUNGI USED

The species and forms of fungi selected for study were as follows:

Fomitiporia dryophila Murrill (Strain 1) on Quercus sp. from the Ocald National Forest, Florida.

Fomitiporia dryophila Murrill (Strain 11) on Corylus sp from Lousiana.

Fomes Everhartii (v. Schr.) Sacc. and Syd. on Quercus borealis maxima from Ann Arbor, Michigan.

Fomes Everhartii (v. Schr.) Sacc. and Syd. on Quercus imbricaria from Ann Arbor, Michigan.

Fomes Everhartii (v. Schr.) Sacc. and Syd. on Quercus sp. from Oklahoma.

Poria punctata

Fomes Bakeri

Fomes rimosus

Fomes igniarius

The five forms of Fomitiporia dryophila and Fomes Everhartii were studied both microscopically, macroscopically, and culturally whereas Poria punctata, Fomes Bakeri, Fomes rimosus, and Fomes igniarius were studied only microscopically and macroscopically.

CULTURAL PROCESS

The culture stock of *Fomitiporia dryophila* (Strain 1) and (Strain 11) and of *Fomes Everhartii* on *Quercus imbricaria* from Michigan and on *Quercus borealis maxima* from Michigan was obtained from the laboratory collection of the School of Forestry and Conservation of the University of Michigan, a collection kept in culture continuously in the forest pathology laboratory. *Fomes Everhartii* on *Quercus sp.* from Oklahoma was obtained from a decayed piece of wood collected by Dr. Dow V. Baxter.

The cultural process used in isolating the fungus from Oklahoma may be described in detail.

The block of decayed wood was placed in a vice and sawed to produce a fresh surface. The freshly exposed surface was then gouged with a chisel. The fragments of rotted wood thus gouged out were immediately dipped in a 95% solution of alcohol, flamed, and transferred to a petri dish containing a sterile malt agar medium prepared in the ratio of 20 grams of

malt (Trommer's extract), 25 grams of agar-agar (Bacto granulated), and 1,000 c.c. of distilled water and autoclaved for 20 minutes at 16 pounds pressure. All instruments used in the operation were dipped in a 1 to 1,000 solution of mercuric bichloride to sterilize them and the chisel was flamed each time before gouging.

Approximately a hundred cultures were prepared in this manner and were placed in glass bell jars at a temperature of 16 degrees Centigrade. Low temperatures such as is 16 degrees Centigade retard the growth of fungi/ However, they practically inhibit the growth of bacteria and are used since bacteria are nearly universally present in decayed wood.

After a period of about eight weeks mycelial growth appeared in two of the petri dishes and as soon as the mats were large enough to fill the dishes about one third full subcultures were prepared from them. At the same time subcultures were prepared from the forms obtained from the laboratory collection. Subcultures were prepared by removing a block of the mycelial mat one

centimeter square from the mycelial mat of a culture and transferring it to a sterile agar plate. Blocks one centimeter square were used throughout this experiment in preparing cultures. The cultures prepared for growth measurements were prepared from stock of the same age since older cultures are in a more or less dormant condition and do not grow as quickly following subculturing as do fresh cultures.

All culturing was done in a glass transfer case containing arm holes for the operator, previously sprayed with mercuric chloride solution. The instruments used were also sprayed with mercuric chloride solution in order to reduce the possibility of contaminating the cultures and thus rendering them worthless.

EFFECT OF TEMPERATURE UPON RATE OF GROWTH

Fungi may be placed in classes, arranged according to their reaction in culture. The classes have been standardized for the resupinate polypores as a group.

Those fungi which exhibit a growth of 5 mm. or more at fourteen days over a range of more than 21 degrees Centigrade are said to have a large temperature range whereas those exhibiting a range of less than 21 degrees Centigrade are considered to have a small temperature range.

A. Range of temperature at which growth occurred.

(a) Large range.

Fomitiporia dryophila (Strain 1).

Fomitiporia dryophila (Strain 11).

Fomes Everhartii on Q. imbricaria from Michigan.

Fomes Everhartii on Q. borealis maxima from Michigan.

Fomes Everhartii on Q. sp. from Oklahoma.

(b) Small range.

None.

Fungi may also be classed according to their optimum temperature for growth. Those which make the best growth at 30 degrees Centigrade or over within a period of fourteen days are placed in the high temperature group; those which produce the best growth between 21 degrees Centigade and 30 degrees Centigrade are placed in the medium temperature group; those producing the best growth at 20 degrees Centigrade or under are placed in the low temperature group.

B. Temperature for optimum growth.

(a) High temperature.

Fomitiporia dryphila (Strain1).

Fomitiporia dryophila (Strain 11)

Fomes Everhartii on *Q. imbricaria* from Michigan.

Fomes Everhartii on *Q. borealis* maxima from Michigan.

Fomes Everhartii on Q. sp.from Oklahoma.

(b) Medium growth.

None.

(c) Low temperature.

None.

Fungi may also be placed in classes based upon rate of growth. Those which fill petri dishes in fourteen days at the temperature of their optimum growth are placed in the rapid growth group whereas those which do not are placed in the slow growth class.

C. Rate of growth.

(a) Rapid growth.

Fomitiporia dryophila (Strain 11).

Fomes Everhartii on Q. imbricaria from Michigan.

Fomes Everhartii on Q. borealis maxima from Michigan.

Fomes Everhartii on Q. sp. from Oklahoma.

(b) Slow growth.

Fomitiporia dryophila (Strain 1).

The effect of temperature upon the growth rate upon the five forms of fungi are indicated graphically in figure 1. The results are based upon the growth of seven cultures of each fungus grown at temperatures of 5 degrees to 40 degrees Centigrade at intervals of 5 degrees.

Table 1

Effect of temperature upon growth

Fungus	Temperature in degrees Centigrade								
	5	10	15	20	25	30	35	40	
Fomitiporia dryophila (Strain 1)	0	0	6	8	12	17	0	0	7 days
	0	0	5	18	28	36	0	0	14 days
Fomitiporia dryophila (Strain 11)	0	0	0	22	28	30	35	41	7 days
	2	3	15	41	41	41	41	41	14 days
Fomes Everhartii on Q. imbricaria from Michigan.	0	0	2	7	13	18	10	0	7 days
	0	1	4	17	31	40	20	0	14 days
Fomes Everhartii on Q. borealis maxima from Michigan.	0	0	2	8	10	14	5	0	7 days
	0	0	6	23	26	41	9	0	14 days
Fomes Everhartii on Q. sp. from Okla.	0	0	2	8	13	17	8	0	7 days
	0	0	6	23	30	41	15	0	14 days

EFFECT OF LIGHT UPON RATE OF GROWTH

Fungi may be placed in classes based upon the effect of light upon the rate of growth. Those exhibiting a difference in radial growth of less than 2 mm. are said to grow equally well in the light and in the dark. Those which exhibit a growth radially which differ by more than 2 mm. are said to grow best in the light or in the dark as the case may be.

D. Effect of light.

(a) Growth equal in light and dark.
Fomitiporia drvophila (Strain 1).
Fomes Everhartii on Q. imbricaria from Michigan.

(b) Best growth in dark.
Fomitiporia drvphila (Strain 11).
Fomes Everhartii on Q. borealis maxima from Michigan.

(c) Growth best in light.
None.

The effect of light upon growth given above and shown graphically in figures 3 and 4 is based upon the growth of 5 cultures of each form of fungus grown in the light at room temperature and the growth simultaneously of 5 cultures of each form of fungus grown in the dark at room temperature.

Table 2

Effect of light upon growth

Fungus	In light 7days	In light 14 days	In dark 7days	In dark 14 days
Fomitiporia dryophila (Strain I)	23	12	7	19
Fomitiporia dryophila (Strain II)	13	41	14	41
Fomes Everhartii on Q. imbricaria form Michigan.	6	16	6	17
Fomes Everhartii on Q. borealis max. from Michigan.	3	9	3	16
Fomes Everhartii on Q. sp. from Okla.	4	12	8	23

Growth figures are in millimeters radially and are rounded off to the nearest whole number.

EFFECT OF THE BAVENDAMM OXIDASE TEST

The Bavendamm Oxidase Test, a test which differentiates between "white rotting" and "brown rotting" fungi was applied to the five forms of fungi. Their reactions to the test may be recorded as strong when the discoloration extends some distance beyond the mycelial mat, medium when it extends to the edges of the mat, and weak when it extends only beneath the center of the mat. All of the fungi which produce discoloration are said to be "white rotting fungi" and those which do not are said to be "brown rotting fungi". The medium used in the Bavendamm Oxidase Test is malt agar and tannic acid in the proportion of 9 to 1.

E. Reaction to Bavendamm Oxidase Test.

(a) Strong reaction.

Fomitiporia dryophila (Strain 1).

Fomitiporia dryophila (Strain 11)

Fomes Everhartii on Q. imbricaria from Michigan.

Fomes Everhartii on Q. borealis maxima from Michigan.

Fomes Everhartii on Q. sp. from Okla.

(b) Medium reaction.

None.

The results of the Bavendamm Oxidase Test given above and shown in Plates 1, 2, and 3 are based upon the reaction of two cultures of each of the five forms of fungi. The test was carried out at room temperature.

Table 3

Growth characteristics on malt agar

Fungus	In light				In dark				
	Color (Ridg.)	Texture	Agar Discol-oration	Pore Formation	Margin	Color (Ridg.)	Texture	Agar Discol-oration	Pore Formation	Margin
Fomitiporia dryophila (Strain 1)	yellow ocher to raw sienna to cream buff	cottony concentric	present	none	silky	honey yellow to deep colonial buff to cartridge buff to masicot yellow	cottony slightly nodulose	present	none	no border
Fomitiporia dryophila (Strain 11)	yellow ocher to buckthorn brown to sorghum brown (dried blood red)	nodulose chamois	present	none	no border	deep colonial buff to buckthorn brown	nodulose chamois	present	none	no border
Fomes Everhartii on Q. imbricaria from Michigan.	chamois	cottony only slight tendency to be concentric	present	none	no border	chamois	cottony	present	none	no border
Fomes Everhartii on Q. borealis var. from Michigan.	cream buff to chamois to ochraceous buff	cottony only slight tendency to be concentric	present	none	silky	cream buff to clay color	cottony slight tendency to be concentric	present	none	no border
Fomes Everhartii on Q. sp. from Okla.	cream buff to chamois	cottony concentric	present	none	no border	cream buff	cottony slight tendency to be concentric	present	none	no border

MORPHOLOGICAL CHARACTERISTICS

Terms used in the study of fungi in culture may be defined as follows:

Concentric ring growth. - An uneven growth of mycelium in a culture, which appears in the form of circles.

Cottony. - Describes mycelium that is erect and intertwined into a thick mass of short fibrous threads resembling cotton.

Agar discoloration. - A noticeable darkening of the whole medium.

Definite border. - A distinct growth of mycelium at the margin of the culture that is distinguishable from the rest of the mycelium by the color or by culture.

Indefinite border. - No distinct growth of the hyphae at the margin of a culture which can be separated from the rest of the mycelium by color or by texture.

Exudations. - colorless drops of resin-like liquid which appear on the surface of a culture.

Nodulose. - Describes the more or less rounded masses or lumps of interwoven mycelium which characterize a culture.

The fungi may be separated from one another by the following key:

Key to Morphological Characteristics

1 Texture of mycelium cottony with a slight tendency to concentric growth. (2)

1 Texture of mycelium not cottony nor with tendency to concentric growth but with texture nodulose or nodulose chamois. (4)

2 Color of mycelium "cream buff".

Fomes Everhartii on Q. sp. from Okla.

2 Color of mycelium not "cream buff" but darker. (3)

3 Color of mycelium "cream buff" to "clay color" Growth most rapid in the dark.

Fomes Everhartii on Q. borealis max. Mich.

3 Color of mycelium "chamois". Growth equal in light and dark.

Fomes Everhartii on Q. imbricaria, Mich.

4 Color of mycelium "deep colonial buff" to "buckthorn brown". Belongs to the rapid growth group. Growth is best in the dark.

Fomitiporia dryophila (Strain 11).

4 Color of mycelium "honey yellow". Belongs to the slow growth group. Growth is equal in the light and in the dark. Resin drops are conspicuous.

Fomitiporia dryophila (Strain 1).

The cultural characteristis of the five fungi may be summarised for easy distinction from one another. They are as follows:

Fomitiporia dryophila (Strain 1). - This fungus is the brightest in color of the group. It also differs in color from the rest in having yellow ocher and in being the most greenish yellow of the group. It is the only one of the forms which belongs to the slow growth group and has the greatest tendency to concentrid ring growth in the light.

Fomitiporia dryophila (Strain 11). - This fungus is the most even in color of the group and is of a brown similar to the color of the context of Polyporus gilvus. It lacks concentric growth and is nodulose in texture. It grows much faster than does any other member of the group and reaches its optimum growth at 45°, a temperature at which the other members of the group grow very little. The tendency of the cultures to form red color at the age of two weeks is also very distinctive.

Fomes Everhartii on Q. sp. from Oklahoma. - The color of this fungus is very definitely the lightest of the five forms studied. Otherwise it is difficult to distinguish from the others of the species Fomes Everhartii its growth is

being only slightly more rapid than that of the other two forms of Fomes Everhartii and the growth being only slightly more concentric. Fomes Everhartii on Q. imbricaria from Michigan and Fomes Everhartii on Q. borealis maxima from Michigan are the most nearly alike of the group in color, in form, and in growth in light and dark. A minor distinction is that the later has a silky margin whereas the former does not.

Microscopic and macroscopic characteristics of the five forms of fungi and of several species which are similar in appearance to them.

Fungus	Tubes (old layers)	setae	hyphae	spore	Remarks
Fomes igniarius	white stuffed (L.0.0.)	present sometimes rare (L.0.0.)	3-4 m (L.0.0.)	4-6u hyaline (L.0)	The poplar form of Fomes igniarius is rimose upon the upper surface and so differs from Fomes Bakeri.
Fomes Bakeri	not white stuffed (L.0.0.)	absent (L.0.0.)	4-6 m (L.0.0.)	5-6u hyaline (L.0)	Fomes Bakeri most nearly resembles Fomes igniarius nigricans but is distinguished from Fomes Bakeri by width of hyphae, in Fomes Bakeri 4-6 m (L.0.0.) and in Fomes igniarius nigricans 3-4 (L.0.).
Fomes rimosus		absent (L.0.0.)	3-4 m (L.0.0.)	4-5u hyaline (L.0)	Conspicuously rimose (L.0.0.).
Fomes igniarius laevigata	somewhat whitish stuffed (Baxter)	not common (Baxter)	2-4u (Baxter)	3-5u (Rom)	
Poria punctata	white stuffed but not consp. (Baxter)	present not brown (Baxter)		5-8u hyaline (Bax)	Poria punctata most nearly resembles Fomitiporia dryophila (Strain 1) but differs in that it does not exhibit the tendency to become undulate in growth and has a less red margin.
Fomitiporia dryophila (Strain 11) Log Town Miss. type	present but rare brown	present but rare brown (Metcalf)	3-4u (Metc.)	4-5 hyaline (Met	Is resupinate, and has the characteristic "hematite red" margin as in strain 1 but differs in that the pore surface is glistening as in Fomes Everhartii and not dull as in Fomes igniarius.
Fomitiporia dryophila (Strain 1)	white stuffed but not consp. (Baxter)	absent (Metcalf)	3-4u (Met.)	4-5 (Met	The fungus is undulate as in Fomes Everhartii from Okla. or entirely resupinate. The colors of the two are identical the chief difference being smooth rather than glistening undersurface. It differs from Fomes Bakeri in having red margins and in being more undulate.

	Tubes (old layers)	Setae	Hyphae	Spores	Remarks
Fomes Everhartii from Michigan.		present (Metcalf)	3-6u (Met.)	4-5 brown (Met.,	Pileus becomes rough rimose and blackish. (L.O.O.) It occassionally forms black abortive knots and is occassionally found on logs. (L.O.O.).
Fomes Everhartii from Oklahoma.		present (Metcalf)	3-6u (Met.)	4-5 brown (Met.)	The undersurface is glistening as in the Lake States Fomes Everhartii but the top surface differs in that it is smooth not rimose. The upper surface exhibits reddish colors i. e. "Hematite red" and "carob brown" and darkens somewhat behind to blackish. The colors resemble those of Fomitiporia dryophila. It also forms black rimose knots as in the Lake States Fomes Everhartii.

SUMMARY

Of the fungi tested from different geographical ranges clear distinctions were shown in growth in culture. The differences of the geographically distinct fungi were nearly as great as those between the two species used. The differences due to the different substrata as exhibited by Fomes Everhartii from Michigan were very slight being practically negligible. The characters of the Fomes Everhartii from Oklahoma were sufficiently distinct to establish it as a new form, dryophila, since it resembles Fomitiporia dryophila in many respects.

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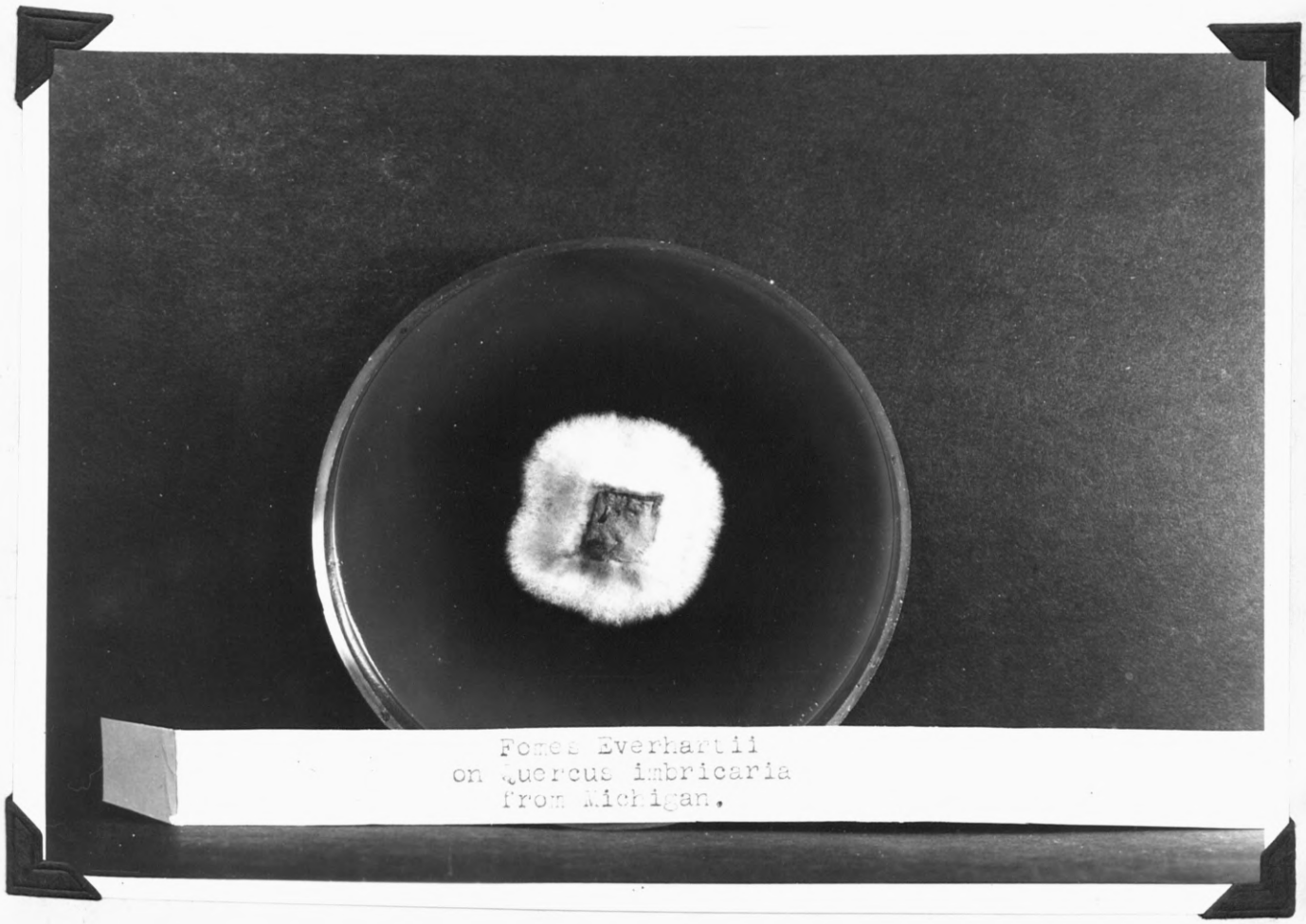
PLATE 1



Fomes Everhartii
on Quercus imbricaria
from Michigan.

Bavendamm Oxidase Test

PLATE 2

Fomes Everhartii
on Quercus borealis maxima
from Michigan.

Fomes Everhartii
on Quercus sp.,
from Oklahoma.

Bavendamm Oxidase Test

PLATE 3

Fomitiporia dryophila
on Corylus sp.
from Louisiana.

Fomitiporia dryophila
on Quercus sp.
from Florida.

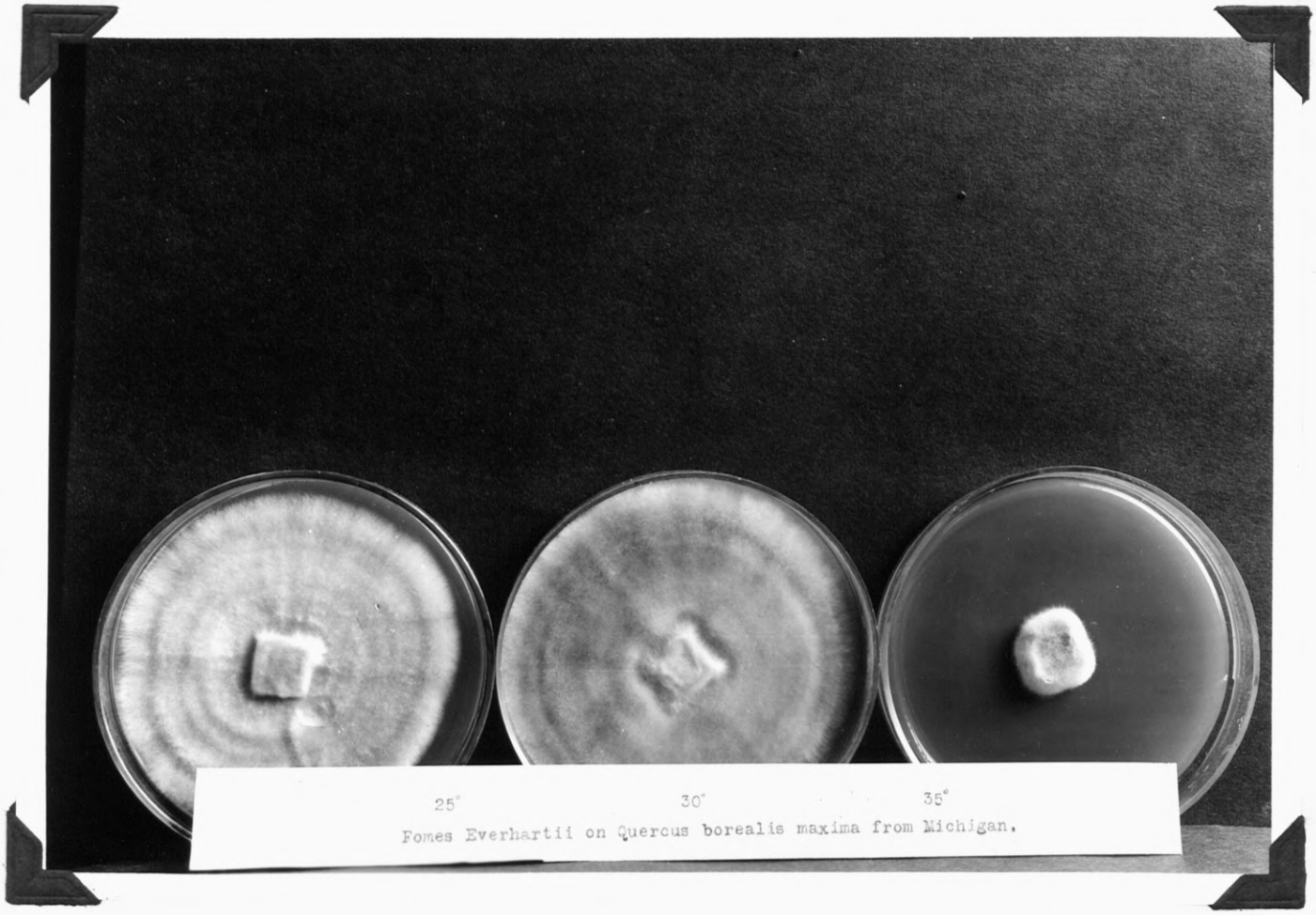
Bavendamm Oxidase Test

PLATE 4

Fomes Everhartii on Quercus imbricaria from Michigan.

Temperature Test

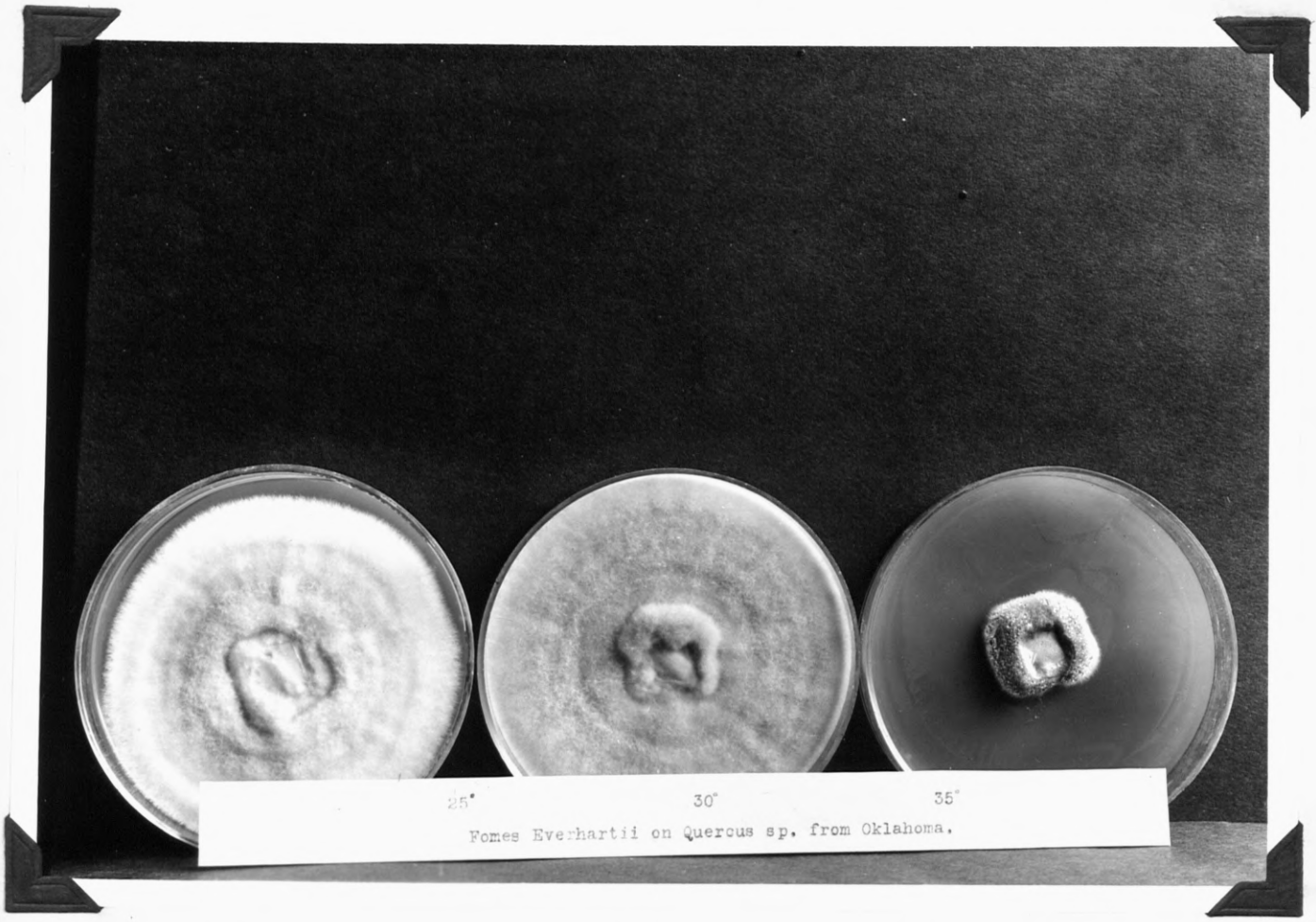
PLATE 5



Fomes Everhartii on Quercus borealis maxima from Michigan.

Temperature Test

PLATE 6



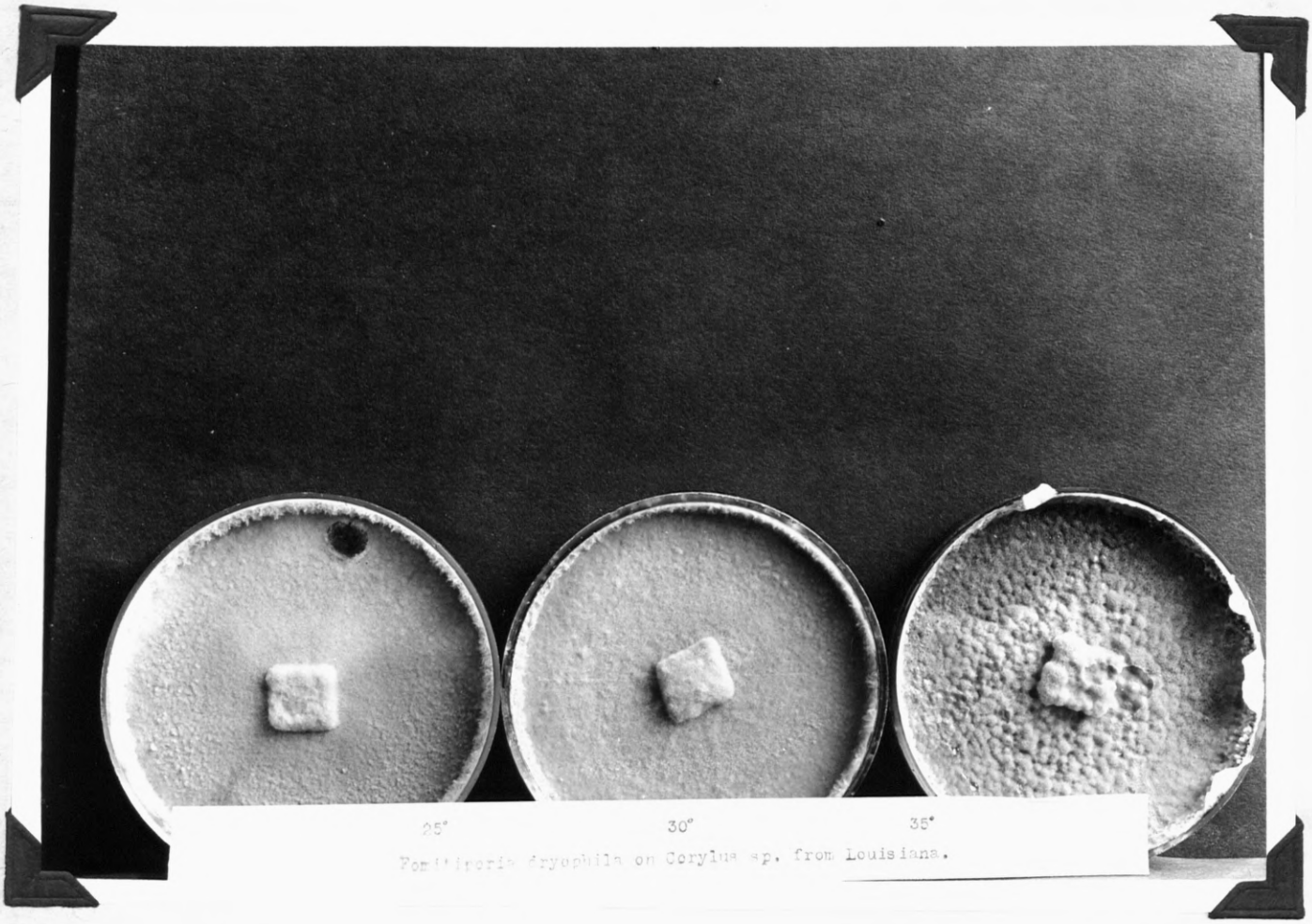
Fomes Everhartii on Quercus sp. from Oklahoma.

Temperature Test

PLATE 7

Temperature Test

PLATE 8



Pomiforia fryophila on Corylus sp. from Louisiana.

Temperature Test