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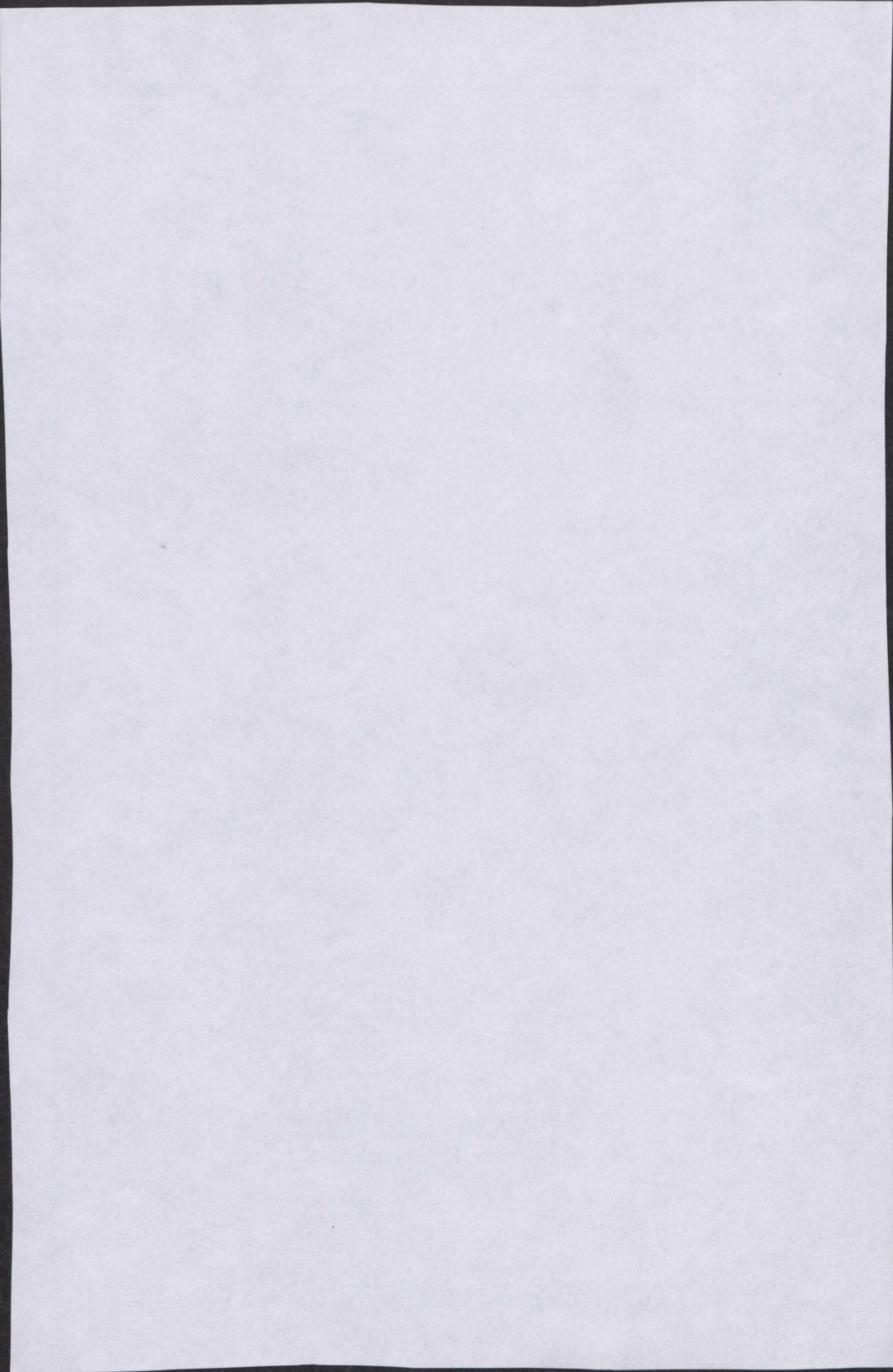
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CONTENTS

VARIATION IN FOMES IGNIARIUS (L.) GILL.[1]

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INTRODUCTION

Many species of fungi, especially among the rusts, smuts, certain Ascomycetes, and Fungi Imperfecti, are known to comprise physiologic forms, differing in pathogenicity, cultural characteristics, and, to a lesser extent, in morphology (14). In any comparative work it is absolutely necessary to know how much the fungi under consideration vary within the species in the characters being studied. However, as considerable cultural work is being done with wood-destroying fungi from local sources, it is desirable to know how much a given species varies, especially in regard to rate of growth on artificial media, rate of decaying wood, resistance to toxic substances, and general cultural appearance.

The present study was undertaken to determine the degree of variation in *Fomes igniarius* (L.) Gill., the fungus commonly causing heart rot of many species of hardwoods. This fungus was chosen because of its wide geographical and host range and because the external appearance of the fruit-body (8) and the cultural characteristics of the organism (1, 12) were known to vary somewhat.

SOURCE OF MATERIAL

The fruiting bodies used in this study were collected during 1931, 1932, and 1933 from eight localities in Minnesota and one in Colorado. The last collection was made by Paul F. Shope of the University of Colorado, who was kind enough to send specimens to the writer. Pertinent data regarding fruiting bodies and cultures made from them are given in Table 1. The "tissue cultures" were made by transferring small pieces of the interior of the fruiting bodies to agar slants. Spores were collected on glass slides, placed under fruiting bodies in moist chambers, and smeared on agar drops on cover slips. Single spores were isolated from these drops with a glass needle and micromanipulator and transferred to sterile agar drops on cover slips. The agar drops with single spores were then transferred to agar slants in test tubes and the spores

allowed to germinate. The usual method of germinating spores on agar drops in Van Tiegham cells was unsatisfactory because of the long time required for some spores to germinate. Most of the spores that germinated did so within 6 days, altho some lay dormant on the agar for as long as 23 days before germinating. Only 8 per cent of the isolated spores germinated. Precooling at 10° and 0° C., incubating at 20° C. and at 28° C., and placing spores on moist wood shavings or on wood decoction agars did not cause increase in the percentage of germination. The period of spore discharge during 1932 appeared to be limited to two weeks (August 6 to 21); consequently, large numbers of spores were not available for further spore germination tests.

A fruiting body is designated by capital letter and number; but, if more than one fruiting body is taken from an individual tree, small letters are added to the designation. For example, B8 and B8a are two fruiting bodies from the same white birch tree. Single spore cultures are designated by a number following the designation of the fruiting body from which they were shed. For example, B8-1 indicates a single-spore culture from the fruiting body B8.

ANATOMY OF THE FRUITING BODIES

Gross Anatomy

Considerable variation in the general appearance of fruiting bodies of *Fomes igniarius* has long been recognized, as is shown by the common recognition of the variety *nigricans* Fries (8), which has even been considered a distinct species, *F. nigricans* (Fries) Gill. (8). There are, however, such intergradations of characters that many specimens can not readily be placed in either taxonomic category.

Overholts (9) gives the following differences between the typical *F. igniarius* and the var. *nigricans:*

> *F. igniarius:* "Pileus convex or ungulate, 3-10 x 5-20 x 2-10 cm., grayish black or black, rarely rimose with age; . . . the older layers conspicuously white-stuffed or incrusted; . . . setae present though sometimes rare."
>
> Var. *nigricans:* "Pileus plane to convex, 3-10 x 3-15 x 2-7 cm., black, sometimes shining black, the surface often cracking in both directions but not becoming roughly rimose; . . . tubes . . . decidedly white incrusted; . . . the setae often abundant."

After careful macroscopic and microscopic examination of my specimens, and from cultural characteristics, it seems that three groups are represented: I, those from *Populus;* II, those from *Betula;* and III, those from other tree species. Overholts identified both typical *igniarius* and variety *nigricans* in each of groups II and III and identified as var. *nigricans* the one specimen from group I sent to him.

Descriptions of the types recognized by the writer are given below.

I. The aspen type. It usually is possible to recognize at a glance fruiting bodies from aspen *(P. tremuloides* and *P. grandidentata).* They are usually smaller than others, and the pore surface often is at a large angle from the horizontal (Fig. 3). The line of the tube layers does not curve upward at the margin so strongly as in the other two groups. The top surface checks but is seldom rimose, probably chiefly because the fruiting bodies seldom live long enough on aspen to attain large size. However, fruiting bodies with a rimose top are found occasionally on large aspen trees.

Table 1. Data Regarding Fruiting Bodies and Isolates Used

Designation	Date collected	Host	Kind of culture	Location
B1*	October, 1931	*Betula papyrifera*	Tissue	Cloquet, Minn.
B2	August, 1931	do	do	Cass Lake, Minn.
B3	August, 1931	do	do	Cloquet, Minn.
B4 and B4a	October 10, 1931	do	do	Forest Lake, Minn.
B 6 and B6a	October, 1931	do	do	Cloquet, Minn.
B7 and B7a	October, 1931	do	do	do
B8, B8a, B8b	August, 1932	do	do	Afton, Minn.
B8-1 and 2	August, 1932	do	Single spore	do
B8a-1, 2, 3	August, 1932	do	do	do
YB1	October 10, 1931	*Betula lutea*	Tissue	Forest Lake, Minn.
YB2†	October 5, 1931	do	do	Ottisville, Minn.
YB3	August, 1932	do	do	St. Paul, Minn.
But. 1†	October 5, 1931	*Juglans cinerca*	Tissue	Afton, Minn.
H1	do	*Carya cordiformis*	do	Franconia, Minn.
H1a†	do	do	do	do
I1	do	*Ostrya virginiana*	do	Afton, Minn.
I2*	do	do	do	do
I3†	do	do	do	do
I4	August 17, 1932	do	do	do
I4-1, 2, 3	do	do	Single spore	do
A1* to 7	October, 1931	*Populus tremuloides*	Tissue	Cloquet, Minn.
A8 and A8a	do	do	do	do
A10, 11, 12	October 5, 1931	do	do	Forest Lake, Minn.
A13	October 19, 1931	do	do	Afton, Minn.
A14, A14a	August 10, 1932	*P. grandidentata*	do	St. Paul, Minn.
A15, 16, 17	September 9, 1932	*P. tremuloides*	do	Grand Lake, Colo.
A19	October, 1931	do	do	Cloquet, Minn.
A20	August 10, 1932	*P. grandidentata*	do	St. Paul, Minn.
A18	August 14, 1932	*P. tremuloides*	do	Forest Lake, Minn.
A18-1	do	do	Single spore	do
A14-1, 2	August 10, 1932	*P. grandidentata*	do	St. Paul, Minn.
A14a-1, 2	do	do	do	do

* Identified by L. O. Overholts as variety *nigricans*.
† Identified by L. O. Overholts as typical *F. igniarius*.

II. The birch type. This type seems to agree with the var. *nigricans* described by Overholts (8) and is best illustrated by the usual form on dead white birch (Figs. 1, 2, and 3). In this group the top surface is black, shiny black before weathering, with checks in two directions, but not rimose with age, and the whole fruiting body is distinctly shelving

Fig. 1. Top Views of Fruiting Bodies of *Fomes igniarius*

A. From white birch (B8). Usual applanate form.
B. From white birch (B8a). Thicker form with rolled margin.
C. From quaking aspen.
D. From quaking aspen.
E. From yellow birch (YB1).
F. From ironwood (I 4).
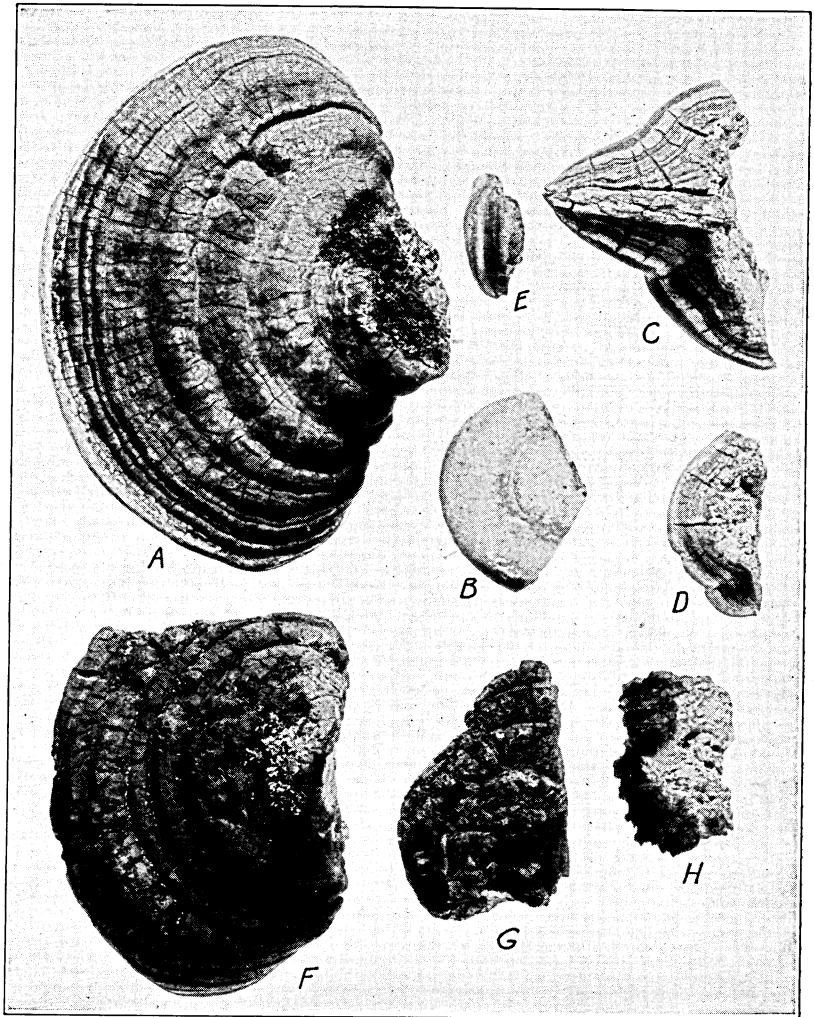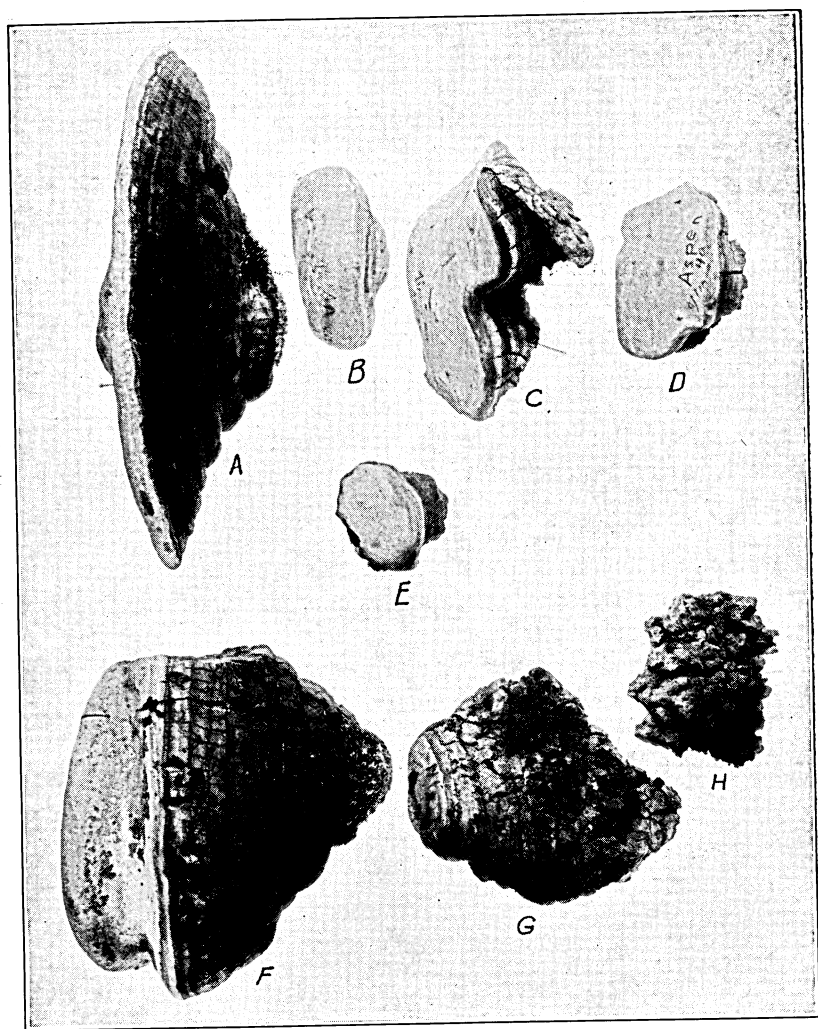G. From ironwood (I 1).
H. From yellow birch. Sterile abortion.

FIG. 2. HORIZONTAL VIEWS OF FRUITING BODIES OF *Fomes igniarius*

A. From white birch (B8). Usual applanate form.

B. From white birch (B8a). Thicker form with rolled margin.

C. From quaking aspen.

D. From quaking aspen.

E. From yellow birch (YB1).

F. From ironwood (I 4).

G. From ironwood (I 1).

H. From yellow birch. Sterile abortion.

and applanate (Figs. 2 and 3). When the margin rolls over the top excessively (Figs. 2 and 3), the black top sometimes does not develop until late. The distinctly applanate type and the thicker specimens with the rolled margin both may occur on the same tree and apparently from the same mycelium, since their colonies mix freely in culture without any apparent antagonism. All the specimens from white birch and many from yellow birch fall in this group.

III. The type from the miscellaneous hosts. The sporophores in this group differ from those of the birch type by being hoof-shaped instead of having the applanate form. They agree with most descriptions of the typical *Fomes igniarius,* altho Overholts identified var. *nigricans* specimens from this group. Here the top surface is either smooth or rough, the margin is often rolled, and the size and shape vary greatly. All specimens from ironwood *(Ostrya virginiana),* hickory *(Carya cordiformis),* butternut *(Juglans cinerea),* and some from yellow birch *(Betula lutea)* are of this type (Figs. 1, 2, and 3).

Fruiting bodies on ironwood are often very conspicuous because of their size, especially in comparison to the size of the stems on which they occur. Fruiting bodies 6 to 8 inches wide and 3 to 4 inches high may occur on stems not over 4 inches in diameter. *F. igniarius* seems to be the most common cause of heart rot of ironwood in Minnesota.

The coloring of all specimens is fairly constant, the tube layers being argus brown (10) except for the white incrustations or stuffings; the margin, sudan brown the first season and weathering through various grays to black; the pore surface, buff yellow at first, then becoming argus brown at maturity. There is one exception to the general coloring, viz., in YB1, where the context varies from amber brown to mars yellow, i.e., it has a distinct yellowish tinge.

Sterile, rimose nodules are sometimes present on white and yellow birches. These nodules (Figs. 1, 2, and 4) are colored like normal fruiting bodies but lack tubes, setae, and basidia, and have bands of regular pseudoparenchymatous tissue imbedded in hyphae similar to those composing normal fruiting bodies. Nodules are present chiefly on living white birch, while normal fruiting bodies are found only rarely on dead trees. Both nodules and normal fruiting bodies were found on living yellow birches. All the sterile nodules were associated with sunken areas or wounds in the stem of the host, as has previously been pointed out by Weir (16) and Katayevskaya (5).

Similar sterile nodules have been reported on beech (2) and have proved to be abortive fruiting bodies of *Fomes everhartii.* My cultures from sterile bodies are distinctly *F. igniarius* and not *F. everhartii,* which appears to be limited to oak in Minnesota so far as can be determined

FIG. 3. SECTIONAL VIEWS OF FRUITING BODIES OF *Fomes igniarius*

   A. From white birch.   Usual applanate form.
   B. From white birch.   Thicker form with rolled margin.
   C. From aspen (A4).
   D. From aspen (A2).
   E. From yellow birch (YB1).
   F. From ironwood (I 3).
   G. From butternut (But.1).

from the specimens in the University of Minnesota herbarium and from my own observation. Considering this and the fact that nodules and normal sporophores both occur together on birches that have died recently, it seems that these sterile bodies are *F. igniarius.* Weir (16) also calls them *F. igniarius,* and Vanin (15) and Katayevskaya (5) refer similar sterile bodies on birches in Russia to the same species.

Weir (16) suggests that abortive fruiting bodies of *Fomes igniarius* may be caused by various physical and chemical actions on the fungus associated with the open wounds commonly present with these abortions. However, it seems that they are a response to some effect of the living host and that the open wounds are, in many cases at least, a result of the action of the fungus.

In the early stages of an abortion, just after the bark has been ruptured, the cambium has been dead for several years under the rupture and the main rupturing force seems to be due to an increase of hyphae in the region of the phloem. The continued growth of the cambium in the rest of the tree results in a sunken area near the abortion. It is quite apparent from Figures 4A and B that the fungus is continually killing back the living portions of the stem and increasing the size of the wound. It seems that the action of the living host changes the normal physiology of the fungus in such a way as to prevent normal fruiting and, instead, sterile, rimose bodies containing bands of definite pseudoparenchyma are formed (Fig. 4); and, with the death of the host and the removal of this inhibiting influence, normal fruiting follows.

When a number of fruiting bodies of *Fomes igniarius* from various hosts are grouped together, the great variation in the general appearance is apparent. Much of this variation is purely ecological and not inherent, because the extremes of the white birch type (Fig. 1, A and B) came from the same tree and apparently from the same mycelium, since their colonies intermingle without antagonism. Nevertheless, there are three fairly distinct groups: the aspen type, the white birch type, and the type from miscellaneous hosts. The aspen type is the most distinct. Certain specimens of the other groups intergrade and may be difficult to separate.

This grouping does not recognize the variety *nigricans,* as commonly recognized by mycologists. Overholts identified the variety in all three groups and the typical species among those from yellow birch, butternut, ironwood, and hickory. Shope (13) refers the aspen type to the typical, while Neuman (7) suggests that the aspen type be given the name *Fomes nigricans* var. *populinus.* I do not suggest any solution to the question of nomenclature but merely point out that there are three groups in my collection and that this grouping is justified by the other evidence in this bulletin.
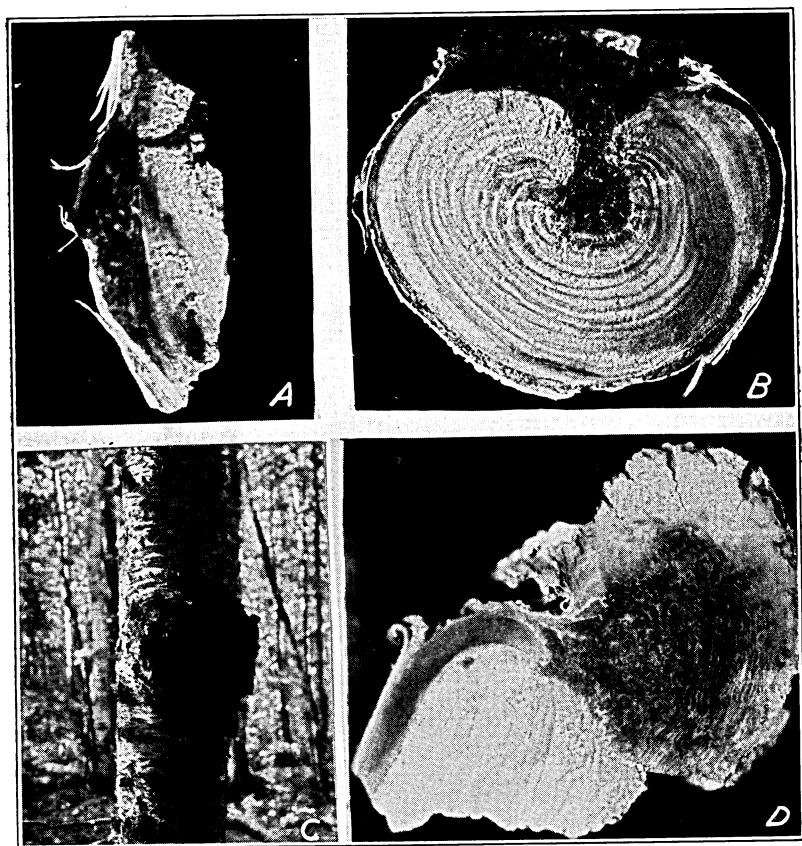
FIG. 4. THE DEVELOPMENT OF ABORTIVE FRUITING BODIES OF *Fomes igniarius*
ON LIVING WHITE BIRCH

A. Early stage. Aggregation of hyphae in the phloem rupturing the outer bark.
B. Abortion extending to the pith. The progressive killing of the cambium
is shown by the annual rings.
C. Late stage. Open wound present with the abortion limited to its margin.
D. Section through late stage showing bands of pseudoparenchymatous tissue.

Microscopic Anatomy

The microscopic structures were studied from free-hand sections cut
from pieces of sporophores, using the methods described by Over-
holts (8). In any given specimen, 100 measurements of each element
were made except in a few cases where 50, or, with setae 5 to 25,
measurements were all that could be made. Pores and dissepiments were
measured perpendicularly to the tubes. In measuring the diameter of
the hyphae of the context, small groups of hyphae were teased from the
sterile margins of the fruiting bodies, the margins being moistened first

with a drop of 8 per cent KOH. This is the only place where comparable hyphae can be secured, because in the aspen specimens the thin context elsewhere soon becomes woody and unsuited to a study of this kind. In addition, the great variation in the hyphae of the dissepiments makes it difficult to get the average diameter. The results are tabulated in Table 2.

Diameter of the pores.—The pore diameters of all specimens examined from white birch and all from aspen, except A4 and A15, are nearly equal. The two exceptions have distinctly larger pores. The A15 specimen is from Colorado, and rough measurements of six others from the same locality indicate that they are the same as regards diameter of pores. Shope (13) gives 3-4 pores per millimeter for *F. igniarius* in Colorado, while Overholts (9) gives 4-5 for the species in the midwestern states. This agrees with the smaller pore diameter of most specimens from Minnesota, altho all from ironwood, butternut, and yellow birch have distinctly larger pores. There does not seem to be a gradual series from small to large pores but rather two distinct groups, except for the single specimen, A4, which is somewhat intermediate and has pores significantly larger than the average for the aspen group.

Thickness of the dissepiments.—With the exception of A14a, A4, and YB2, the dissepiments are of fairly uniform average thickness. The two specimens, A14 and A14a, from the same individual host tree and probably the same mycelium, are near the lower and upper limits of variation. The great variation in most fruiting bodies is shown by the large probable errors. What variation in dissepiments occurs between different sporophores apparently is not correlated with host, variety, or any other characteristic studied and is probably dependent largely on ecological factors.

The length and width of setae.—The setae, with respect to length, form a well-distributed series from 11.9μ to 16.5μ, with those from one specimen, A15, at 19.0μ. The setae in the specimens from *Populus* are with one exception, A6, longer than in the other two groups. The difference in width is less pronounced, altho those of the aspen type are mostly wider.

The outstanding difference in setae is in their numbers. In B4 and B8 they are very abundant, in A14 and I2 apparently absent, while in the others they are scarce to moderately abundant. It is interesting to note that A14 without setae and A14a with setae are apparently from the same mycelium.

In the matter of diameter of the hyphae of the context, there are two distinct groups—those from *Populus* and those from other hosts. In the

aspen type they average 4.45 $\pm$ .024μ, while the others average 3.26 $\pm$ .017μ. Smaller samples taken from other specimens in the University of Minnesota herbarium show that there is the same relationship between specimens from widely separated regions in Minnesota and Wisconsin and collected over a period of 25 years. The differences within the groups probably are not very significant, since differences between A14 and A14a, apparently from the same mycelium in an aspen, and between B8 and B8a, apparently from one mycelium in birch, are near the limits for the two groups.

Summary of Microscopic Characteristics

The white birch and the aspen types are similar with respect to diameter of the pores but are dissimilar in all the other characteristics studied. The length of the setae, and, to a less extent, width, and the diameter of the hyphae of the context separate the aspen type from the other two. Thus, the separation into three types by macroscopic characters of the fruiting bodies is substantiated by the microscopic characters. Variations within the groups, with the possible exception of A4 and A15, probably are due largely to ecological factors rather than to genetic differences.

CULTURAL CHARACTERISTICS

Within a given isolate the variations encountered in ordinary cultural work, under different environments, in such characteristics as color or topography seem to be merely due to a different rate of change to a final character. For example, there may be considerable variation in the color of any culture, depending on the conditions under which it is grown. The colors, however, form a series from white to brown, and the final color is brown. The conditions merely change the rate at which the color change occurs. Grown on a favorable medium under ordinary room conditions, the final typical characters are usually developed within 14 days. No constant variation in any isolate has been observed during repeated subculturing, even after two years in some cases. That is, the isolates seem to be genetically constant.

For comparison, isolates were grown on malt agar (2.5 per cent malt, 1.5 per cent agar) under ordinary room conditions in 250-cc. flasks or in petri dishes. The gradations between isolates are so imperceptible that detailed descriptions of individual cultures are impracticable. However, the cultures as a whole are easily separable into three groups.

Table 2. Dimensions, in Microns, of Certain Microscopic Structures in the Sporophores of Fomes igniarius

Fruiting body	Diameter of pores	Thickness of dissepiments	Length of setae	Width of setae	Diameter of context hyphae
B4	104.3 ± .60*	52.6 ± 1.43	13.6 ± .11	4.9 ± .061	3.38 ± .055
B6	99.8 ± .57	47.0 ± 1.02	14.0 ± .16	4.9 ± .070	3.05 ± .050
B8	105.1 ± .45	47.3 ± 1.81	12.8 ± .14	4.6 ± .099	3.27 ± .052
B8a	104.1 ± .63	47.9 ± 1.33	12.8 ± .12	5.0 ± .063	2.93 ± .049
Average of white birch	102.9 ± .33	48.7 ± .71	13.4 ± .07	4.8 ± .038	3.19 ± .026
A14	101.5 ± .57	46.4 ± 1.11	†	†	4.21 ± .046
A14a	106.1 ± .44	61.2 ± 2.04	15.2 ± .58	6.0 ± .255	4.49 ± .058
A6	103.0 ± .88	59.6 ± 2.09	11.9 ± .20	5.1 ± .072	4.49 ± .062
A2	102.8 ± .56	51.6 ± .90	16.2 ± .38	5.8 ± .117	4.10 ± .066
A18	99.7 ± 1.20	48.7 ± 2.38	16.5 ± .23	5.8 ± .046	4.79 ± .111
A4	124.5 ± 1.33	63.3 ± 2.24	15.3 ± .29	6.4 ± .102	4.56 ± .053
A19	97.5 ± .51	51.7 ± 1.46	15.4 ± .23	6.4 ± .070	4.21 ± .075
A15	145.6 ± 1.73	48.4 ± .67	19.0 ± .34	6.9 ± .128	4.71 ± .071
Average of aspen	100.8 ± .36	53.5 ± .61	15.8 ± .13	6.3 ± .048	4.45 ± .024
I3	138.4 ± 1.00	49.7 ± 1.92	13.9 ± .43	6.4 ± .192	3.31 ± .058
I2	143.6 ± 1.08	54.6 ± 1.66	†	†	3.31 ± .050
I4	136.2 ± .57	54.5 ± 1.43	12.4 ± .24	5.1 ± .082	3.28 ± .048
But.1	141.7 ± .54	50.6 ± 2.43	†	†	3.20 ± .062
YB1	149.8 ± .67	48.4 ± 2.23	14.1 ± .35	4.6 ± .142	2.70 ± .015
YB2	150.4 ± .94	71.2 ± 2.43	‡	‡	3.25 ± .057
Average of misc. group	149.2 ± .82	56.6 ± 2.05	13.1 ± .35	5.1 ± .146	3.18 ± .051

* Probable error of the mean is given after each figure, $PE_m = .6745 \dfrac{\sigma}{\sqrt{N}}$.

† Setae apparently absent.

‡ One seta found.

Group I.—In this group are included most of the aspen isolates. They are comparatively slow growers, produce a strong odor of methyl salicylate on malt agar, and have a tendency to produce radial ridges in the culture mat when aging (Fig. 9B). The mats do not become particularly leathery with age and are usually irregular at the margin (Fig. 5). The first growth is white, changing through yellows to dresden brown or even mummy brown, i.e., they are distinctly darker when mature than the cultures from any other host. Under the room conditions of this study the production of thick mats by the aspen cultures is more common than reported by Fritz (1). Also, the mats are composed largely of fiber-like hyphae as are the cultures from other hosts.

Aspen isolates A7, A8, A10, A11, A15, A16, A17, and A18 constitute a variation of Group I. These isolates at first produce only a small appressed colony, almost black, and stain the agar near the culture (A15 in Fig. 5). Gradually they form a thin, velvety mat of dresden brown (Fig. 6). After two to three weeks some part of such cultures may produce a typical mat of aerial mycelium, altho the margins remain of the appressed type. Occasionally even the cultures typical of Group I have this appressed type of growth (Fig. 7). The irregular growth curves in Fig. 10 are mostly due to this.
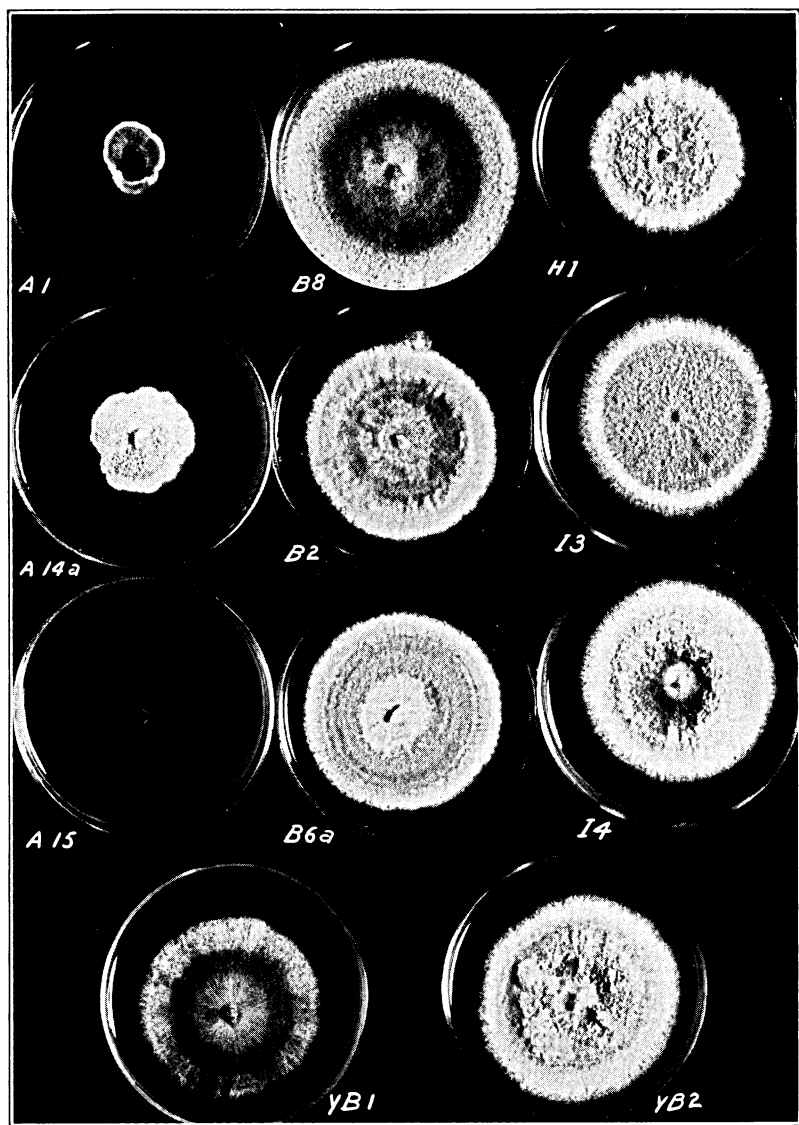
Fig. 5. Tissue Cultures of *Fomes igniarius* on Malt Agar after 13 Days at Room Temperature

Culture group   I—A1, A14a, and A15 from aspen.
                 II—B8, B2, and B6a from white birch.
               III—H1 from hickory, I 3 and I 4 from ironwood, YB1 and YB2 from yellow birch.
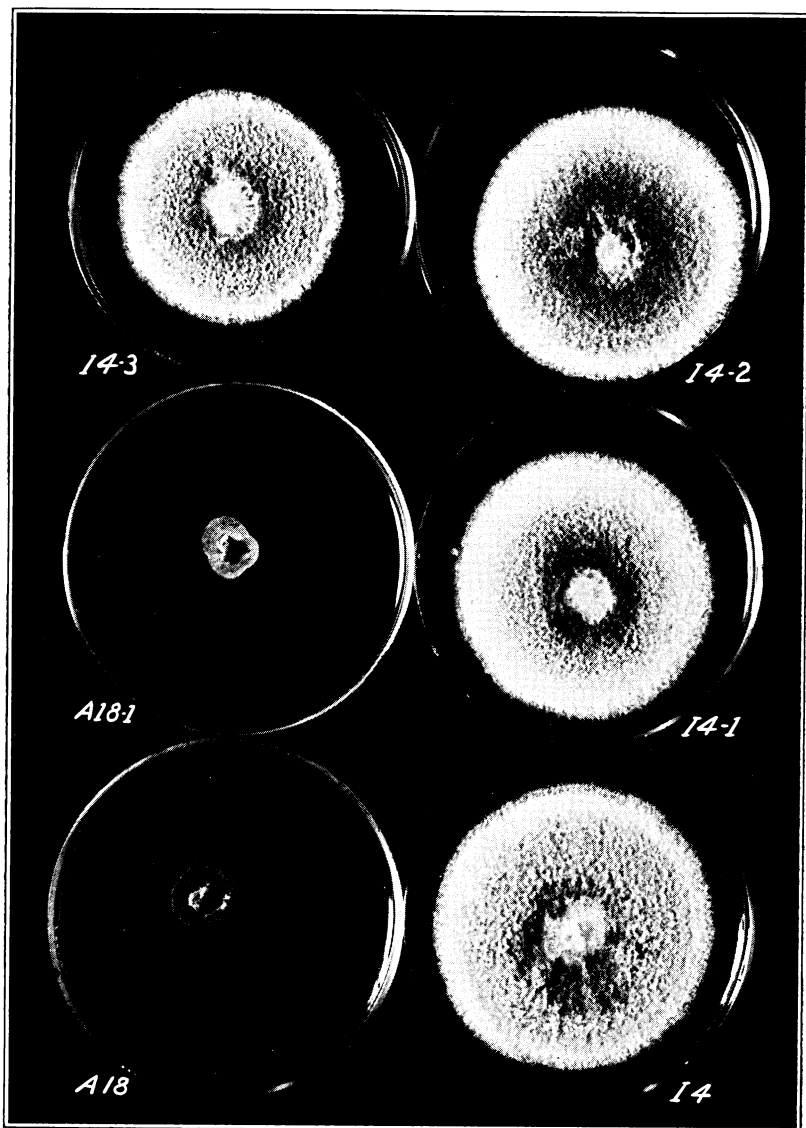
FIG. 6. MONOSPOROUS AND TISSUE CULTURES OF FRUITING BODIES FROM ASPEN
AND IRONWOOD, ON MALT AGAR AFTER 13 DAYS AT ROOM TEMPERATURE

A18, tissue culture of fruiting body from aspen.
I 4, tissue culture of fruiting body from ironwood.
A18-1, monosporous culture from fruiting body A18.
I 4-1, I 4-2, and I 4-3, monosporous cultures from fruiting body I 4.
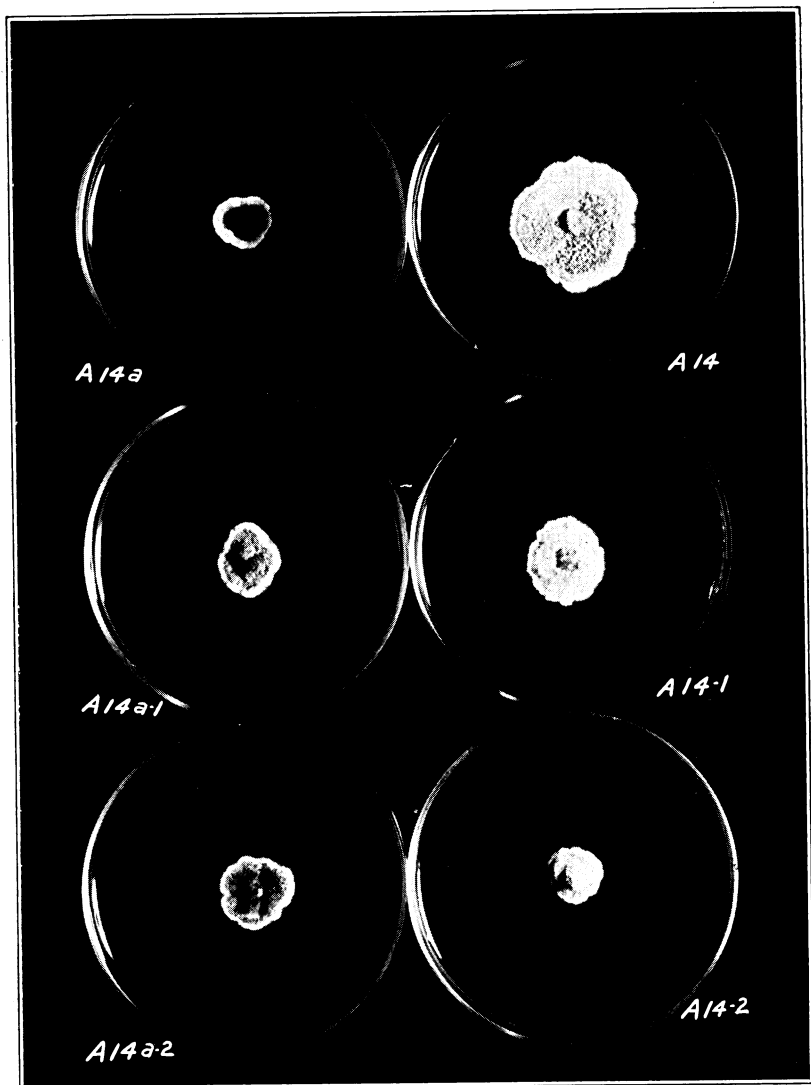
Fig 7. Monosporous and Tissue Cultures of Fruiting Bodies from Aspen, on Malt Agar after 13 Days at Room Temperature

A14a, and A14, tissue cultures.
A14a-1 and A14a-2, monosporous cultures from fruiting body A14a.
A14-1 and A14-2, monosporous cultures from fruiting body A14.
Cultures A14 and A14a are apparently from the same mycelium.
The occasional slow-growing, appressed colony is evident in A14a.
See Figure 11 for the usual rate of growth of this culture.

FIG. 8. MONOSPOROUS AND TISSUE CULTURES OF FRUITING BODIES FROM
WHITE BIRCH, ON MALT AGAR AFTER 13 DAYS AT ROOM TEMPERATURE

B8 and B8a, tissue cultures.
B8a-1 and B8a-2, monosporous cultures from fruiting body B8a.
B8-1, B8-2, and B8-3, monosporous cultures from fruiting body B8.
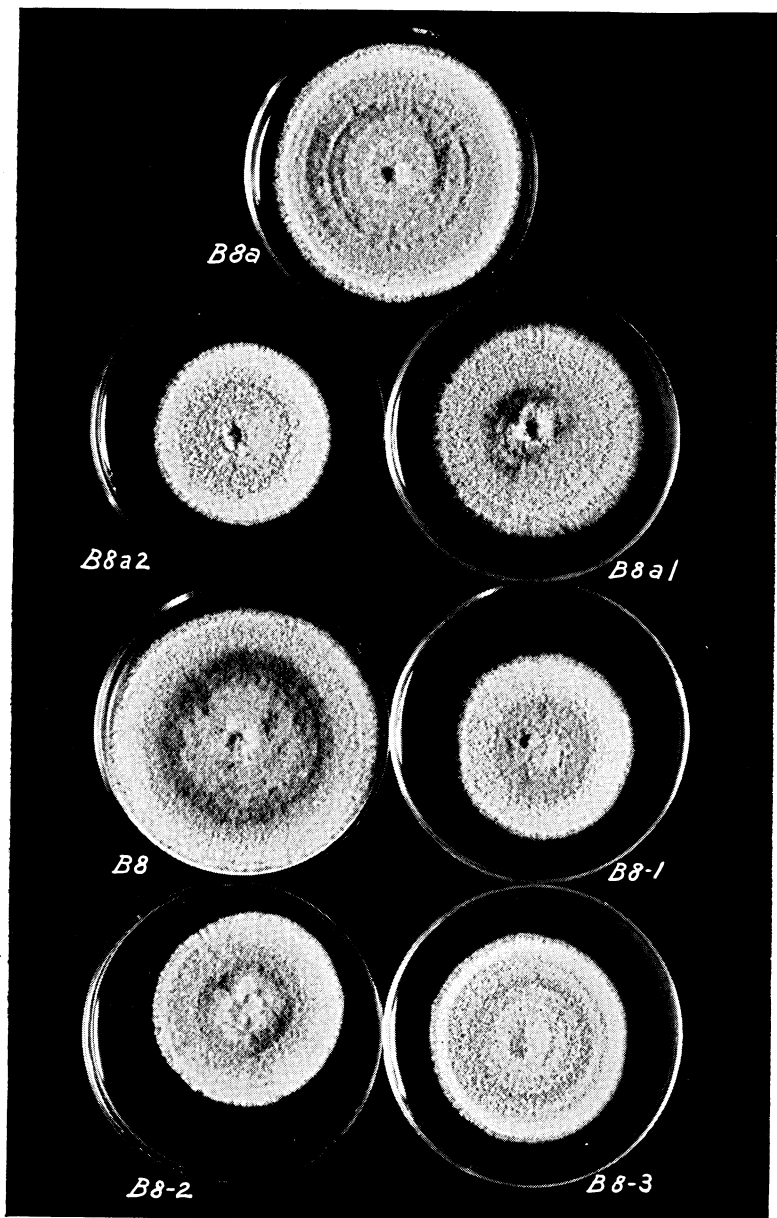
Group II.—In this group are included all the isolates from white birch. These cultures are rapid growers (Fig. 10) and produce a tougher mat than do those of Group I. They have no tendency to radial markings but sometimes form circular zones, even when young (Fig. 5). They do not produce a methyl salicylate odor (except faintly in B8 and B8a and not consistently here). The color does not become deeper than a buckthorn brown; in fact they are mostly so light a brown as to give the general impression of being yellow.

Group III.—The cultures of this group resemble those of Group II in rate of growth, consistency of mat, topography, and in not regularly producing a methyl salicylate odor, but they differ enough to be distinguished easily.

Isolate YB1 is slow in producing a typical mat, first forming a thin, appressed mat, but the cultures do not stain the agar as do appressed mats of the aspen cultures. The rate of growth is not slower than for Group II (YB1 in Fig. 5).

Isolates YB2, H1, and H1a are nearly identical with cultures from white birch until three or four weeks old when they form thick tufts of aerial mycelium near the center of the colonies (Fig. 9C). The isolates from ironwood, I 1, I 2, I 3, and I 4, produce the same tufts and also are darker. Sometimes isolate I 4 produces a faint odor of wintergreen.
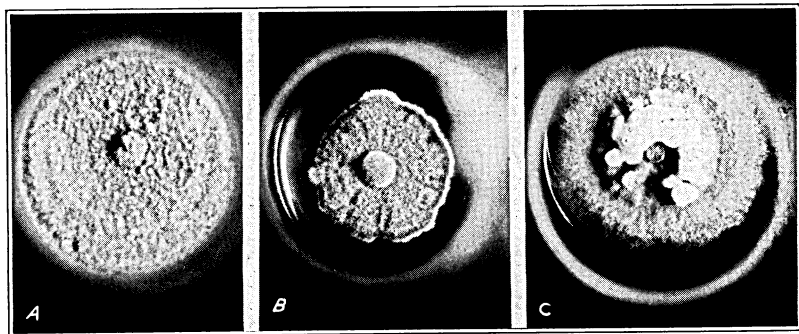
FIG. 9. CULTURES OF *Fomes igniarius* ON MALT AGAR, FIVE WEEKS OLD

A. Isolate B8 from white birch. (Culture group II)
B. Isolate A14a from aspen. (Culture group I)
C. Isolate YB2 from yellow birch. (Culture group III)

Isolates from the sterile bodies on birch usually resemble those from the normal sporophores from the same host. Katayevskaya (6) says they differ by fructifying in culture. Only two of six of my isolations produced the daedaloid pores on the surface of the agar (malt). The setae, hyphae, and the few spores that formed seemed typical of the normal fruiting bodies.

The monosporous cultures, altho resembling the tissue cultures from the parent sporophore, differ in the following respects:

(1) The mats are much thinner in the monosporous cultures from white birch, the tissue culture being two to three times thicker than the monosporous. This is not true of the cultures from ironwood and aspen.

(2) The color develops more slowly, and the final color is usually lighter.

(3) The rate of growth is less, both on agar (Figs. 6, 7, 8, and 11) and as measured by the amount of wood decay caused.

Among the monosporous cultures all the types of the tissue cultures appear. Both the typical and the appressed types of the aspen cultures are represented (Figs. 6 and 7). The appressed monosporous culture came from a sporophore producing a similar culture (Fig. 6), showing that basidiospores apparently carry the factor or factors for such growth.

There is no appreciable difference in the general appearance or rate of growth of the monosporous cultures from ironwood. Among the birch haploid cultures, B8a-2 grows considerably faster. Among the aspen haploid cultures, A14a-1 grows faster and, like A14a-2, is lighter in color than the others. A18-1 differs from the others in always remaining appressed.

Summary of Cultural Characteristics

There are three cultural groups, corresponding with the three groups of fruiting bodies. The isolates from aspen are distinct in rate of growth, color, and the production of wintergreen odor. The other two groups are more intergrading in characteristics, as are the fruiting bodies from which they came. Within each group is an infinite variation in minor degree.

The only cultures in my collection that are indistinguishable are those that intermingle in culture without any apparent antagonism and presumably are from the same mycelium in nature. This, of course, applies only when the cultures are grown on a favorable medium near the optimum temperature. On unfavorable media, or near the extremes of temperature permitting growth, the differences are toned down and two cultures may closely resemble each other, even tho they differ under optimum conditions.

TEMPERATURE RELATIONS

Temperature tests were made in 90-mm. petri dishes on malt agar (2.5 per cent malt and 1.5 per cent agar). Incubation was for 10 days at 11, 22.5, 24.5, 27.5, 30, and 36° C. All inoculum blocks were cut from the margins of actively growing cultures by means of a 3.5-mm. cork

borer. Each culture was run in duplicate, and there always was close agreement between the dishes of each culture except in some of the slow-growing aspen cultures. The results are presented in Figures 10 and 11.

Aside from the differences in the rate of growth of the different cultures, there appears to be little difference between the reactions of various strains to different temperatures. The irregularities of the aspen cultures are due to the great variation between duplicate plates, but from
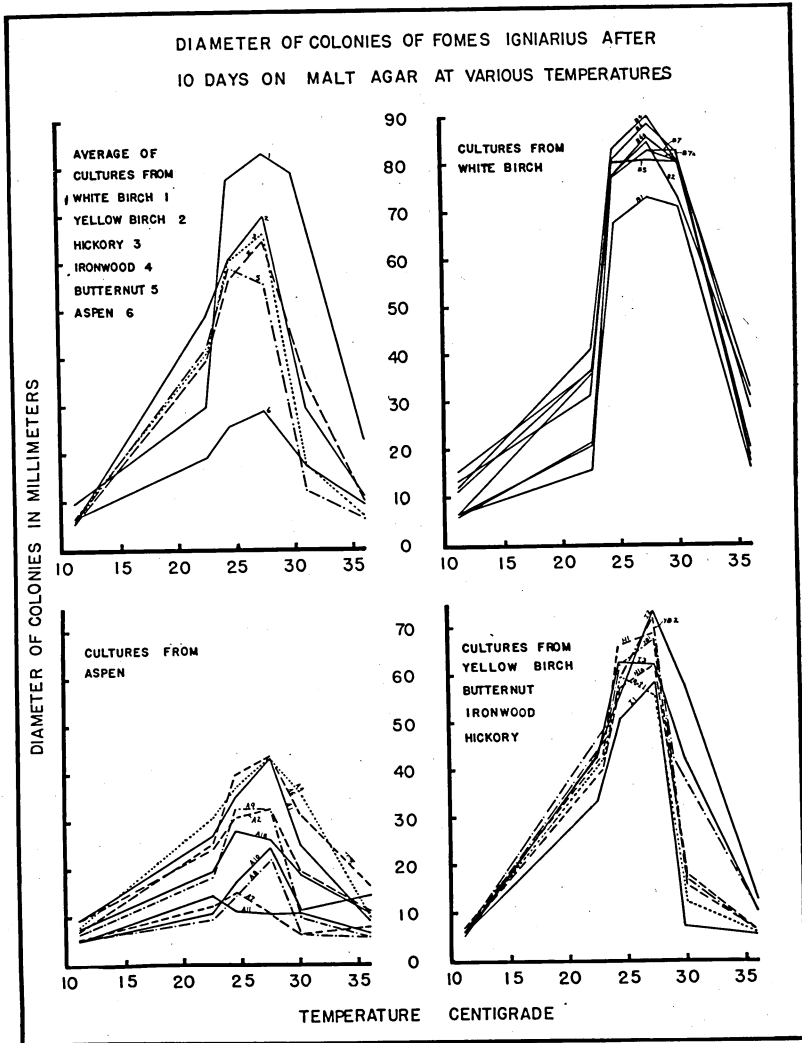
FIG. 10. THE EFFECT OF TEMPERATURE ON THE GROWTH OF *Fomes igniarius* ON MALT AGAR
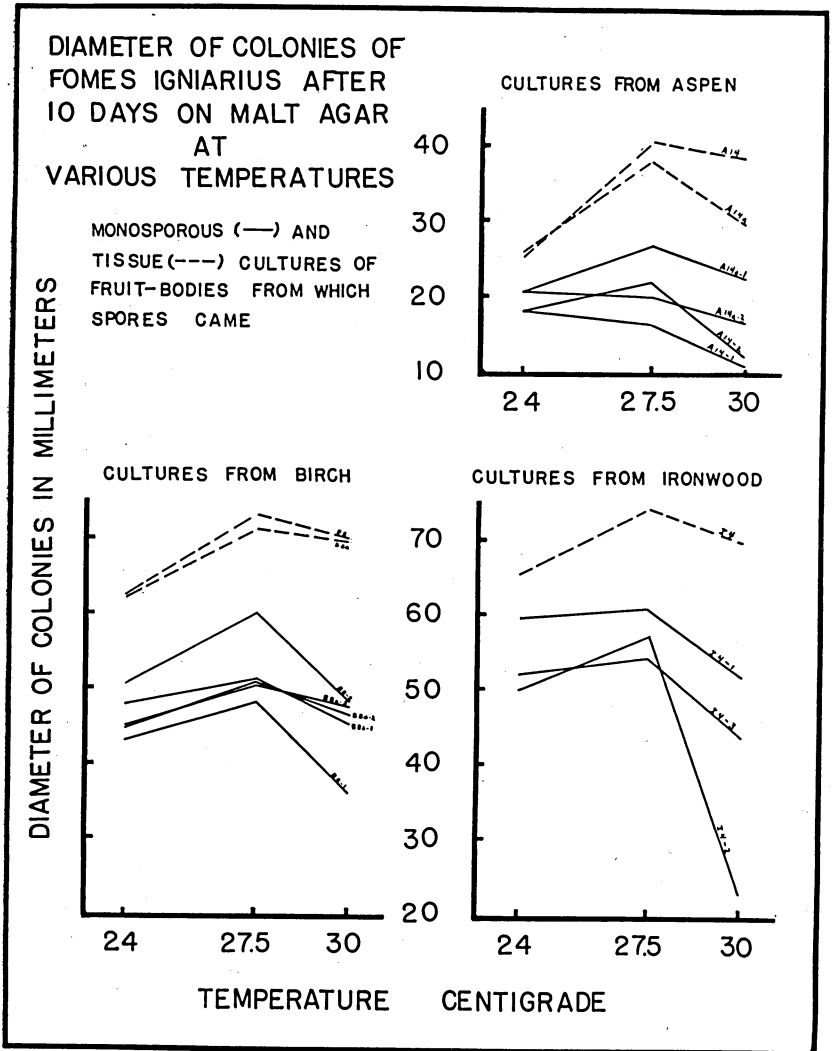
Fig. 11. The Effect of Temperature on the Growth of Monosporous and Tissue Cultures of *Fomes igniarius* on Malt Agar

the general shapes of the curves it is likely that the true optimum for all is near 27.5° C., as is true for most of the other cultures. There appears to be some factor in the usual medium and cultural surroundings that is finely balanced for the aspen cultures, and minute differences that do not affect the other cultures appreciably influence the rate of growth of the aspen cultures. Among the other cultures, I 3 and But.1 may have an optimum slightly below that of the others, but this would fall somewhere

between 24.5 and 27.5° C. and could not be determined with the equipment available.

Hubert (3) gives the optimum temperature for *Fomes igniarius* as 30° C. but does not mention on what medium, and recently Humphrey and Siggers (4) reported the optimum on a beef extract malt agar as 30° C. (inhibited at 42° C.) and on malt agar as 32° C. (inhibited at 40° C.). Both of these optimum temperatures are considerably higher than any I found in my tests.

The inhibiting temperature in my tests seems to be about 40° C. At 38 to 39° there is a trace of growth for all except a few of the slower-growing aspen cultures (A10, 11, 13, 7, and 8). At 44 to 45° all were killed in 23 days.

At 6 to 7° C. all cultures showed a trace of growth in 15 days, and at 2 to 4° C. I 2 and five of the aspen cultures (A10, 11, 13, 6, and 7) were inhibited.

From all the data it seems that at most there are only minor differences between isolates, as concerns optimum temperature. As concerns inhibiting points, there may be a slight difference between the slower-growing aspen cultures and the others, but this would be only a degree or two. It is possible that in the slower-growing cultures the rate of activity at the lower and higher temperatures is as great compared to that at the optimum as with the fast-growing cultures and that no visible trace of growth occurs before the cultures dry out. At least it seems safe to conclude that there are no major differences in the temperature relations of the cultures studied.

THE PRODUCTION OF A METHYL-SALICYLATE ODOR IN CULTURE

Certain isolates of *F. igniarius* synthesize on certain media a substance that resembles methyl salicylate (wintergreen) in odor. Fritz (1), using cultures of *F. igniarius* from *Fagus americana, Ulmus americana, Betula lutea, B. alba, Acer saccharum, Ostrya virginiana, Populus tremuloides,* and *P. grandidentata,* found that all produced a wintergreen odor on Czapek's synthetic agar, but she does not mention it on malt agar, which she also used. With my isolates, only those from aspen *(P. tremuloides* and *P. grandidentata)* consistently produce a strong wintergreen odor on malt and to a less extent on Czapek's synthetic agar. Occasionally isolates I 2 (from ironwood) and YB2 (from yellow birch), and the single spore and tissue cultures from white birch fruiting bodies, B8 and B8a, produce a faint odor of wintergreen on malt agar, altho this is not constant for any of them.

To test the effect of methyl salicylate on the growth of various cultures of *F. igniarius,* enough chemically pure methyl salicylate was added

to sterilized 2.5 per cent malt agar to bring the concentration of the chemical to 0.50 per cent by volume. The concentration was undoubtedly lowered during the cooling of the agar in the petri dishes. Six isolates from white birch, one from hickory, two from ironwood, and six from aspen were completely inhibited by the chemical; and, when the blocks of inoculum were transferred to salicylate-free agar after six days, only cultures A8 and A10 revived, the others remaining inactive during the three weeks that they were observed (Table 3).

Table 3. The Effect of Methyl Salicylate on the Growth of Fomes igniarius on Malt Agar

Isolate	Diameter of colony expressed as percentage of colony on plain malt agar		
	0.50% salicylate	0.15% salicylate*	0.03% salicylate
A1	dead	75.0	100.0
A4	do	not used	71.4
A8	alive	115.0	140.0
A10	do	100.0	144.4
A14	not used	78.5	183.3
A15	dead	130.8	66.7
A9	do	66.7	188.9
B1	do	45.4	30.8
B2	do	19.6	53.3
B4	do	17.5	33.3
B7	do	29.5	51.3
B8	do	14.4	44.1
I2	do	9.5	25.2
H1a	do	11.0	56.1
YB1	do	26.7	43.1
YB2	do	9.6	40.0
Average of aspen cultures		88.6	103.8
Average of other cultures		19.1	41.6

* One drop of chemical to 20 cc. of agar. Estimated 0.15 per cent methyl salicylate.

On malt agar containing about 0.03 per cent methyl salicylate there is a marked difference in the rate of growth of cultures from aspen compared with those from other hosts. In this series the cultures were grown in 150-cc. flasks, as the methyl salicylate seemed to evaporate less from them than from petri dishes. The concentration was noticeably less, judged by the intensity of the odor, at the end of the test. In Table 3 the diameters of the colonies after 10 days at room temperature were expressed as the percentage of the diameter of the colonies grown on methyl salicylate-free agar. Each isolate was run in duplicate, both on the salicylate and salicylate-free agars. In Table 3 are also the results of a set of approximately 0.15 per cent methyl salicylate agar.

The isolates that naturally produce wintergreen odor on malt agar are stimulated or only slightly inhibited by concentrations of the synthetic methyl salicylate that greatly retard the growth of the isolates that do not synthesize it to any extent themselves.

ANTAGONISM AND COMPATIBILITY OF CULTURES ON ARTIFICIAL MEDIA

The Interaction of Tissue Cultures

The very great diversity of cultures of *Fomes igniarius* is shown by their interaction in culture. When two colonies are grown together on agar their interaction varies from a complete intermingling, without any apparent antagonism, to complete inhibition of growth at the point of meeting. Three principal types of interaction were distinguishable:

(a) Compatible.—Two cultures intermingle completely so that they are indistinguishable and have no line of demarcation between the aerial mats or discolored zone in the agar at the line of meeting (Fig. 12).
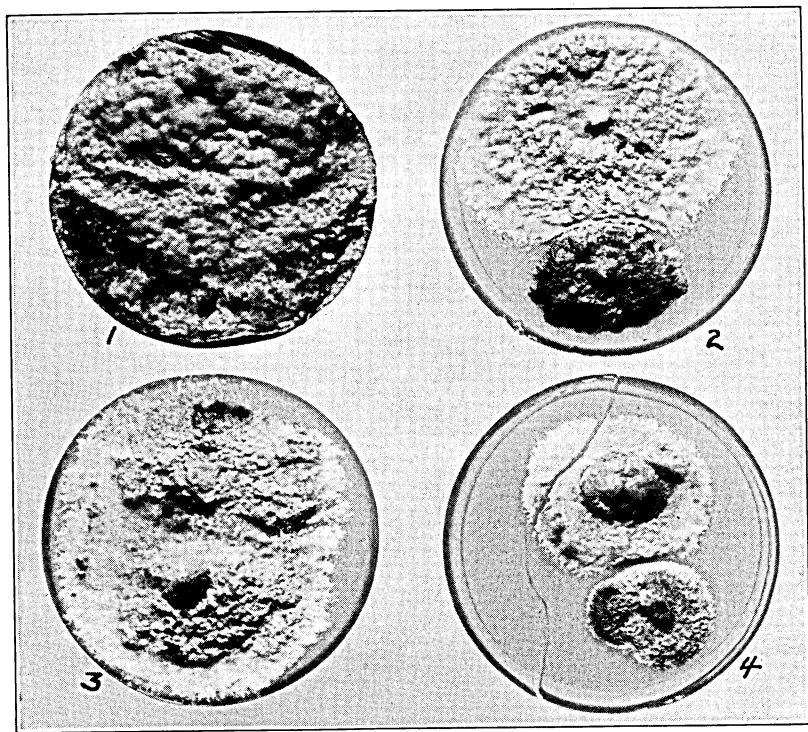
FIG. 12. INTERACTION OF CULTURES OF *Fomes igniarius* ON MALT AGAR 10 TO 14 DAYS AFTER COLONIES MET

1. Dicaryotic mycelium growing over two haploid mycelia that fused (B8a-1 and B8-2).
2. Cultures A4 and B4 touching, but aversion is shown by line of demarcation between aerial mats and by discolored agar.
3. Complete intermingling of cultures H1 and H1a with no apparent aversion.
4. Free agar left between two antagonistic colonies (A1 and B1)

(b) Intermediate.—Two cultures meet and form a dark zone in the agar, stopping the growth of one, while the other grows over the first. This type of interaction is not common, and in all cases overlapping is limited to 2-3 mm. of the margin and stops at the meeting of the definite aerial mats.

(c) Antagonistic.—Two cultures meet and form a dark zone in the agar, a distinct line separating the aerial mats. This is the common type (Figs. 12 and 13).

In a few instances there is a zone free of mycelium except for the invisible submerged mycelium (Fig. 12), but this is not constant for any pair of cultures.

The submerged hyphae intermingle to some extent in all three types.

The phenomena accompanying the interaction of haploid mycelia are discussed later in the bulletin.

Schmitz (11), using four cultures of *Fomes pinicola* Fr., from douglas fir, western hemlock, white fir, and western white

FIG. 13. INVERTED CULTURE FLASK SHOWING DISCOLORED ZONE IN AGAR AT POINT OF MEETING OF CULTURES B6a AND H1

pine, found that any two of these would not intermingle freely in culture but formed a clear line of demarcation. This work was amplified later by Mounce (6), who, using a large number of cultures of *Fomes pinicola* from several hosts and from widely separated localities, found that complete intermingling occurred only under the following conditions:

(a) When a mycelium is paired with itself.

(b) When a mycelium from sporophore tissue is paired with one resulting from mass spores produced by the same sporophore.

(c) Usually when a mycelium from infected wood of a tree is grown with a mycelium from a sporophore which grew upon the tree.

(d) Usually when a mycelium from a mass of spores or sporophore tissue is grown with a mycelium from mass spores or sporophore tissue from a second sporophore from the same tree.

She also found that lines of aversion were common when monosporous cultures were grown together. Frequently a definite line of

demarcation was formed at the meeting of two monosporous mycelia that were sexually compatible. The formation of this line did not seem to depend upon variation in temperature, light, amount or kind of medium used, provided normal mycelial development occurred.

The antagonism and compatibility of cultures of *Fomes igniarius* were tested on 2.5 per cent malt agar in 250-cc. flasks, all pairings being run in duplicate and each set repeated at least once.

In the 300 possible pairings of 24 tissue cultures of *F. igniarius* from seven host species, free intermingling occurred between two transfers of the same isolate and also between the following pairs: B4—B4a, B6—B6a, H1—H1a, A1—A4, A15—A16, and A14—A14a. Except for pairs A1—A4 and A15—A16, each pair of intermingling cultures was obtained from two fruiting bodies from one individual tree and probably from the same mycelium. The two exceptions were from two collections in Colorado and Minnesota, and each pair could have come from an individual tree, altho their history is not known.

In a few instances, isolates B6, B6a, A8, A9, and A10 grew 2-3 millimeters over the appressed margins of cultures of other isolates but never over the definite aerial mats. Colonies touched in practically every case; only in pairs containing a slow-growing aspen culture was there left an area free of mycelium, and this was not constant between replicates.

All possible combinations of six isolates from four large-tooth aspens growing in a clump were made, and the only intermingling cultures were those from two fruiting bodies growing on the same tree.

Tissue isolates from three fruiting bodies growing on a dead white birch intermingled in all combinations. Tissue isolates made from fruiting bodies growing at 2, 5, 7, 10, 15, and 20 feet on a quaking aspen interacted as follows:

	2	5	7	10	15	20
2	0	—	—	—	—	—
5	—	0	0	0	0	0
7	—	0	0	0	0	0
10	—	0	0	0	0	0
15	—	0	0	0	0	0
20	—	0	0	0	0	0

The figures refer to the height at which the fruiting bodies grew.

0 = free intermingling.

— = definite line of aversion.

At three feet, i.e., between fruiting bodies at two and five feet, there was brown discoloration in the rotted wood, but this was not organized into a zone line as definite as those normally occurring in the wood rotted by this species. The isolate from the fruiting body growing at two feet is entirely different from the others, being slower growing and of a darker color. The inference would be that two distinct mycelia were present in this tree.

From the above data it would seem that only cultures from the same mycelium will intermingle freely. With two exceptions, only isolates from the same individual tree show no antagonism. When there is any apparent difference between isolates, even from the same tree, there is antagonism, and between isolates from different trees, whether similar or different in appearance, there is antagonism. Complete histories of the two exceptions are not known, but the intermingling pairs were from the same collections and could have been from the same trees. Mounce (6) found two cases in *F. pinicola* in which different diploid mycelia had no antagonism, one case being between mycelia from different host species, while the other pair could have come from the same tree. It seems that through segregation and recombination almost every mycelium is genetically different with respect to the factor or factors influencing compatability.

The Interaction of Tissue Cultures and Haploid Cultures

Lines of demarcation between the aerial mycelia and discolored zones in the agar also occurred in all pairings of monosporous cultures with tissue cultures of the parent fruiting bodies.

The Interaction of Haploid Cultures

When two haploid cultures meet on agar there are two types of interaction:

(a) No sexual fusion.—When two sexually incompatible cultures meet they are separated by a line of demarcation between the aerial mats and by a darkened zone in the agar. Subcultures taken from the point of meeting usually separate into the two component parts which react as at the original meeting. All haploid lines from different hosts (aspen, ironwood, and white birch) and all haploid lines of the same sex show this type of interaction. Sometimes two haploid lines of the same sex grow together, remaining in the haploid condition. The agar below such mixtures darkens, and the rate of growth may be greater or less than that of the fastest component.

(b) Sexual fusion.—When two sexually compatible haploid cultures meet, there is at first antagonism. However, subcultures from the point of meeting are dicaryotic and show all the characteristics of tissue cultures. Since clamp connections are not produced by this species, sexual fusions are determinable definitely only by nuclear stains. However, the dicaryotic mycelium developing between the two haploid cultures can be discerned as an ovoid colony growing over the haploid colonies (Fig. 12). Also, the difference in the rate of growth between haploid and dicaryotic cultures, when related, is sufficient to enable detection of most fusions by the rate of growth of subcultures taken from the point of meeting.

Both nuclear staining (Haidenhain's iron alum haematoxylin) and rate of growth (Table 4) show that the haploid lines from fruiting body I 4 are of two sex groups, fusing as follows:

	I4-1	I4-2	I4-3
I4-1	—	+	+
I4-2	+	—	—
I4-3	+	—	—

+ = sexual fusion.

The synthetic dicaryons I 4-1 x I 4-2 and I 4-1 x I 4-3 seem to be about the same in general appearance and rate of growth as the tissue isolate I 4 (Table 4). From the material it is not certain whether there are more than two sex groups formed by this fruiting body.

Table 4. The Rate of Growth of Mixtures of Haploid Lines of Fomes igniarius from Ironwood Compared to That of the Haploid Lines Alone

Isolate or mixture	Diameter of colony in mm.		Nuclear condition	Diameter of colony as percentage of fastest component	
	Trial 1*	Trial 2†		Trial 1	Trial 2
I4-1 x I4-2	71.0	66.0	dicaryotic	113.6	113.9
I4-1 x I4-3	68.5	66.0	do	112.3	113.9
I4-2 x I4-3	37.5	42.0	haploid	60.0	73.7
I4-1	61.0	58.0	do		
I4-2	62.5	57.0	do		
I4-3	52.0	43.5	do		
I4		64.0	dicaryotic		

* 10 days at 27.5° C.
† 8.5 days at 27.5° C.

The haploid lines from fruiting bodies B8 and B8a are of four sex groups and fuse as shown below:

	B8-1	B8-2	B8a-1	B8a-2	B8a-3
B8-1	—	—	—	+	—
B8-2	—	—	+	—	—
B8a-1	—	+	—	—	—
B8a-2	+	—	—	—	+
B8a-3	—	—	—	+	—

+ = sexual fusion.

The synthetic dicaryons B8a-1 x B8-2, B8a-2 x B8a-3, and B8-1 x B8a-2 resemble the tissue cultures B8 and B8a, which are probably from the same mycelium in nature. In rate of growth, however, two of them are considerably slower growing than the tissue cultures of the parent fruiting bodies B8 and B8a (Table 5). Thus in the segregation and recombination of the factors within a single mycelium new dicaryons may arise differing at least in rate of growth.

Table 5. The Rate of Growth of Mixtures of Haploid Lines of
Fomes igniarius from White Birch Compared to That
of the Haploid Lines Alone

Isolate or mixture	Diameter of colony in mm.		Nuclear condition		Diameter of colony as percentage of fastest component	
	Trial 1*	Trial 2†			Trial 1	Trial 2
B8-2 x B8a-1	72.0	68.5	dicaryotic	AB-ab	120.0	121.2
B8a-2 x B8a-3	62.0	65.5	do	Ab-aB	122.8	136.4
B8-1 x B8a-2	60.0	59.0	do	aB-Ab	121.0	122.9
B8a-1 x B8-1	45.5	45.0	haploid	ab, aB	79.1	84.9
B8-1 x B8a-3	50.5	54.0	do	aB, aB	101.0	114.9
B8-2 x B8a-2	45.0	55.0	do	AB, Ab	75.0	97.3
B8-1 x B8-2	61.5	63.0	do	aB, AB	102.5	111.5
B8-2 x B8a-3	59.0	57.5	do	AB, aB	98.3	101.8
B8a-1 x B8a-2	58.5	49.5	do	ab, Ab	101.7	93.4
B8a-1 x B8a-3	56.0	58.0	do	ab, aB	97.4	109.4
B8	65.0		dicaryotic			
B8-1	48.0	47.0	haploid	aB		
B8-2	60.0	56.5	do	AB		
B8a-1	57.5	53.0	do	ab		
B8a-2	50.5	48.0	do	Ab		
B8a-3	50.0	47.0	do	aB		

* 10 days at 27.5° C.
† 9 days at 27.5° C.

The four haploid cultures from fruiting bodies A14 and A14a, apparently from the same mycelium in nature, fuse as follows:

	A14-1	A14-2	A14a-1	A14a-2
A14-1	—	—	—	—
A14-2	—	—	+	+
A14a-1	—	+	—	—
A14a-2	—	+	—	—

+ = sexual fusion.

Thus, three sex groups are represented among the cultures from fruiting bodies A14 and A14a. The synthetic dicaryons from the haploid cultures from the two related fruiting bodies, A14 and A14a, are about the same in rate of growth and appearance as the isolates from the two parent fruiting bodies (Table 6).

Even tho the groups of haploid lines from aspen, ironwood, and white birch do not seem to be interfertile, the groups themselves seem to be heterogeneous, and new forms may appear as a result of various combinations between different lines.

Table 6. The Rate of Growth of Mixtures of Haploid Lines of Fomes igniarius from Aspen Compared to the Haploid Lines Alone

Isolate or mixture	Diameter of colony in mm.		Nuclear condition		Diameter of colony as percentage of fastest component	
	Trial 1*	Trial 2†			Trial 1	Trial 2
A14-1 x A14a-1	17.0	13.0	haploid		94.4	96.3
A14-1 x A14a-2	18.0	15.5	do		105.9	119.2
A14-1 x A14-2	14.0	10.5	do		112.0	110.5
A14a-1 x A14a-2	13.5	10.5	do		75.0	77.8
A14-2 x A14a-2	27.0	20.2	dicaryotic AB-ab		158.8	155.4
A14-2 x A14a-1	27.0	20.5	do		150.0	151.9
A14-1	12.5	9.5	haploid	Ab or aB		
A14-2	11.0	8.5	do	AB		
A14a-1	18.0	13.5	do	ab		
A14a-2	17.0	13.0	do	ab		
A14a	26.5		dicaryotic			

* 8 days at 27.5° C.
† 6 days at 27.5° C.

THE RATE OF DECAY CAUSED BY DIFFERENT ISOLATES

All decay tests were made in 5-ounce glass jars fitted with metal screw caps. Twenty-five cubic centimeters of medium (2.5 per cent malt, 1.5 per cent agar) were placed in each jar and the cultures allowed to grow well over the surface of the agar before the blocks of wood were placed in the jars. This gave an even and abundant inoculum. The blocks were sawed from fresh, green sapwood and were about 2 x 2 x 4.5 centimeters in size. In a given series all were cut, dried, resoaked, sterilized, and incubated together, so that any differences in moisture content were due only to differences in the hygroscopicity of the individual wood blocks or to differences in the ability of individual cultures to convey moisture from the agar to the wood. This method probably eliminated as much as possible all differences in substratum and cultural conditions, so that true differences in the ability of the different strains to cause decay could be brought out. With some fungi, such as *Lentinus lepidius,* more uniform results can be secured than with one like *F. igniarius,* that does not decay a block uniformly throughout.

Series I: The decay of aspen *(P. tremuloides)* wood by tissue cultures

The wood blocks were dried at 70° C. for 48 hours to determine the sound dry weight. They were then soaked in hot water until they sank and sterilized 20 minutes under 15 pounds steam pressure in glass jars, the blocks being in a single layer so that each would have as nearly as possible the same moisture content. When cool, three blocks were placed in each culture jar, the caps screwed on loosely to allow gas exchange, and incubated for 90 days in a room of fairly constant humidity (80-95 per cent) and temperature (80° F.). At the end of the incubation period the surface mycelium was removed and the blocks dried at 70° C. All percentages are based on the sound dry weight of the wood.

The conditions in the culture jars were favorable for the rapid development of the fungus, the jars being completely filled with mycelium at the end of the incubation period (Fig. 14). Zone lines formed on the surface of the glass and in the wood. The former were fine, like the black zone lines formed by the fungus in nature, while those formed in the wood blocks were less definitely organized.

After sterilization, the moisture content of the wood, based on 15 blocks, was about 100 per cent of the sound dry weight, and at the end of the incubation, based on all the test blocks, 135.9 ± 12.06 per cent ($PE_{so} = .6745\sigma$). Differences of 20 to 30 per cent in moisture content of different samples used for one isolate did not seem to affect the amount of decay. At moisture contents higher or lower than those used in this study the differences found probably would have greatly influenced the amount of decay.
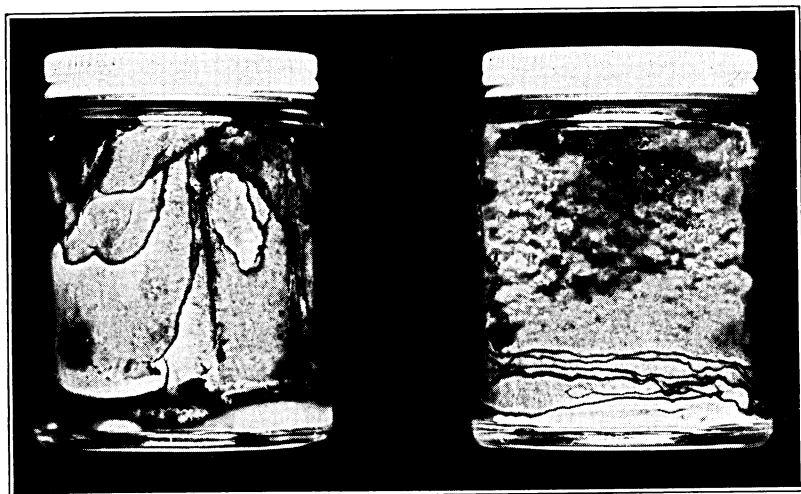
FIG. 14. THE GROWTH OF *Fomes igniarius* ON WOOD BLOCKS DURING
90 DAYS INCUBATION
Zone lines were formed on the surface of the glass culture jar.

In Series I the three blocks in each jar were weighed as one sample
so that only two figures for each isolate were secured on which to base
the loss in weight. Using this method, no good figure for comparing
individual isolates could be secured. However, considering the small
variations between all the isolates and the averages by host species, it
seems likely that there is little difference in the ability of different isolates
to cause decay of aspen wood. In subsequent studies, therefore, red gum
and white birch woods were used and three or four culture jars used
for each isolate. Also, each block was numbered separately so that 9 to
12 individual figures were secured for each isolate and the dispersion

expressed as the probable error of the mean: $(PE = .6745 \frac{\sigma}{\sqrt{N}})$.

Series II: The decay of white birch *(B. papyrifera)* wood by tissue
cultures

These blocks were treated like those in Series I except that for con-
venience they were sterilized in paper, wrapped in groups of three. This
series was incubated for 93 days at room temperature in a corrugated
paper box lined with wax paper.

Series III: The decay of red gum *(Liquidamber styraciflua)* wood by
tissue cultures

In this series, after being dried at 70° C., the blocks were immersed
in boiling water for three minutes to sterilize and add moisture. Other-
wise they were treated as were those in Series I.

In Table 7 the isolates are arranged in the order of the amount of decay caused on the three wood species. On aspen wood the isolates from aspen and white birch are fairly well distributed in the table, while those from the other hosts are interspersed among the lower half. Isolate A13 is the only one showing a marked difference in the ability to decay aspen wood, it being very much lower than any of the others. The large differences between the samples of individual isolates make any but large differences between isolates insignificant.

On birch wood the white birch isolates in general cause greatest decay, the aspen isolates least, and the others an intermediate amount. On red gum the same general relationship holds except that the iron-wood and butternut isolates are more spread out.

In Table 8 the figures of Table 7 are rearranged and expressed as averages by host species, showing the relatively uniform decay rate of all on aspen wood and the large differences on the other woods.

Table 7. The Relative Amount of Decay of Aspen, Birch, and Red Gum Woods by Different Isolates of Fomes igniarius, Expressed as Loss in Weight

Order	Aspen—90 days		Birch—93 days		Red gum—90 days	
	Isolate	Loss in weight	Isolate	Loss in weight	Isolate	Loss in weight
1	A8	31.4	B4	26.54 ± .741	I3	32.0 ± 1.53
2	A6	30.4	A16	24.48 ± 1.563	B8	26.7 ± .90
3	A12	30.3	B6a	23.25 ± .668	B6	23.4 ± 1.01
4	A10	29.6	B2	20.86 ± 1.060	B6a	23.3 ± 2.26
5	B6	29.1	YB1	19.81 ± 1.500	B4	20.6 ± 1.56
6	B2	29.0	B1	19.15 ± .638	BN	15.5 ± 1.50
7	A7	28.8	B8a	18.30 ± .864	A8	10.8 ± 1.28
8	A3	28.2	I4	17.30 ± .800	But.	9.0 ± 1.21
9	B7a	27.6	YB2	16.13 ± .573	A15	7.6 ± .96
10	YB1	27.0	A10	14.08 ± 1.509	I4	7.1 ± 2.77
11	B5	26.6	A8	12.91 ± 1.563	A14a	6.9 ± .54
12	A9	26.4	BN	11.64 ± .301		
13	A5	25.6	I2	11.12 ± .648		
14	B4	25.4	A9	8.94 ± .589		
15	A1	24.9	A1	8.30 ± .298		
16	A6	23.6	A4	7.67 ± .426		
17	I2	23.4	H1a	4.50 ± .540		
18	A11	23.4	checks	0.30 ± .014		
19	I1	22.8				
20	H1a	22.7				
21	B6a	22.6				
22	B1	22.6				
23	B7	21.9				
24	A2	21.7				
25	H1	20.5				
26	YB2	18.3				
27	I3	14.7				
28	But.	14.2				
29	A13	3.9				

Table 8. The Relative Amount of Decay in Aspen, Birch, and Red Gum Wood Caused by Different Isolates of Fomes igniarius Averaged by Host Species

Source of isolates	Loss in weight, in percentage of sound dry weight		
	Aspen	Birch	Red gum
White birch	25.50 ± .822	21.50 ± .362	23.79 ± .765
Aspen	25.31 ± 1.127	12.36 ± .451	8.06 ± .558
Yellow birch	23.03 ± 2.075	17.70 ± .758	
Butternut	14.23 ± 3.342		9.01 ± 1.210
Hickory	21.48 ± 1.989	4.50 ± .540	
Ironwood	21.61 ± 1.649	11.96 ± .837	20.56 ± 1.582

Summary of Decay Studies with Tissue Cultures

Using aspen wood as a substratum, it is seldom that significant differences in decay ability of isolates of the different groups can be found. Using red gum or white birch woods, however, differences are brought out.

In general, the isolates from white birch decay both birch and red gum woods faster than those from other hosts. One aspen isolate falls in the range of the white birch isolates, while the others are much slower decayers. Among the white birch isolates there are minor differences but not so great as among the isolates from other hosts, especially from aspen.

In decay ability, as in the rate of growth on malt agar and in the general appearance of the fruiting bodies and cultures on malt agar, the isolates from aspen and white birch form two fairly distinct groups. The isolates from other hosts, whose fruiting bodies and general cultural characteristics resemble those of the white birch group more closely, also are more intergrading in decay ability.

Series IV: The decay of birch *(B. papyrifera)* wood by monosporous cultures and tissue cultures of the parent fruiting bodies

The blocks of this series were dried at 100° C. for 24 hours and autoclaved 20 minutes at 15 pounds pressure before being placed on the culture mats in the jars. They were incubated for 103 days in a small copper incubating chamber kept under room conditions.

In each case the monosporous (haploid) cultures decayed the wood much less rapidly than the tissue cultures (dicaryotic) of the parent sporophores (Table 9). Among the haploid cultures from birch the wood decaying proclivities do not vary significantly, altho there were differences in the rate of growth on malt agar. The haploid cultures from aspen and ironwood, however, vary considerably in the amount of decay caused.

Altho no extensive tests were made with dicaryotic lines produced by the fusion of haploid lines, two were tried on white birch wood, and

in both cases the synthetic dicaryons caused decay at about the same rate as did the tissue cultures of the parent fruiting bodies:

1. A14-2 x A14a-1 .... 22.99 ± .923 per cent loss in weight in 103 days
    A14 .... 23.09 ± 1.178 per cent loss in weight in 103 days
    A14a .... 22.90 ± 1.070 per cent loss in weight in 103 days

2. B8a-2 x B8a-3 .... 21.74 ± .673 per cent loss in weight in 103 days
    B8a .... 21.91 ± 1.947 per cent loss in weight in 103 days

Table 9. The Amount of Decay of White Birch Wood, Expressed as Loss in Weight (Percentage of Sound Dry Weight), by Monosporous Cultures and Tissue Cultures of the Parent Fruiting Bodies After 103 Days

Fungus*	Percentage loss in weight
B8	20.18 ± 1.373
B8-1	8.92 ± .184
B8-2	8.01 ± .326
B8a	21.91 ± 1.947
B8a-1	8.57 ± .463
B8a-2	8.77 ± .249
B8a-3	8.01 ± .354
A14	23.09 ± 1.178
A14-1	10.34 ± 1.095
A14-2	11.32 ± .808
A14a	22.90 ± 1.070
A14a-1	12.46 ± .493
A14a-2	9.39 ± .189
I4	18.05 ± 1.172
I4-1	5.80 ± 1.177
I4-2	7.33 ± 1.391
I4-3	9.82 ± .985

* The first isolate in each group is a tissue culture from the fruiting body, the others are monosporous cultures from spores shed by the fruiting body.

THE REACTION OF DIFFERENT ISOLATES TO ZINC CHLORIDE

The toxic and stimulating effect of methyl salicylate has been discussed elsewhere. No attempt was made to make standard toxicity tests with this chemical, because its excessive volatility makes it impossible to control the concentration over a period of time. Only the differences between the large groups are clearly shown, and most other differences are thought not to be significant.

Toxicity tests were made, using zinc chloride, to determine if the different isolates reacted differently to a toxic substance. An inorganic salt was used because of the ease of handling. All tests were made with the same stock solution of zinc chloride, which tested by gravimetric analysis ($AgCl_2$ precipitated out by adding an excess of $AgNO_3$) 4.54 per cent zinc chloride. The media were made up by adding enough of the zinc chloride and sterile distilled water to 264 cubic centimeters of

sterilized agar (2.5 per cent malt and 1.5 per cent agar) to make 360 cubic centimeters of medium of each concentration. This was thoroly mixed by shaking and was then poured into petri dishes. Each isolate was run in duplicate and incubated at 25° C. for 10 days, the time required for the fastest growing isolate to nearly cover the surface of the check plates. Blocks of inoculum were cut from the margins of actively growing cultures by means of a 3.5-millimeter cork borer. After the incubation period the inhibited blocks were transferred to plain malt agar and the time required to revive was recorded. Those that showed no indication of growth after 14 days on the plain agar were considered dead. The concentrations of zinc chloride used were: 0, 0.03, 0.06, 0.08, 0.11, 0.14, 0.21, 0.28, 0.42, 0.56, 0.69, 0.83, and 0.97 per cent.

In all three trials the concentration causing total inhibition was about the same for each isolate, but the concentration required to kill the isolates varied greatly between trials and apparently is not a very useful figure for comparison (Table 10). Several times isolates revived at high concentrations while there were several other concentrations between this and the next lower one that did not kill the cultures.

Table 10. The Toxic Effect of Zinc Chloride in Malt Agar on the Different Isolates of Fomes igniarius

Isolate	Concentration of zinc chloride (per cent by weight)					
	Concentration totally inhibiting			Concentration killing		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
A4	.08–.11*	.08	.08	.21(.42)†	.42	.42–.56
A8	.08	.11	.11	.42+‡	.56–.69(.83)	.42–.56
A9	.11	......	......	.42	......	....
A10	.08	......	......	.42+	......	
A15	.08	.08	.08	.42+	.83	.14(.69)
A14a	.11	.11	.11	.42+	.56	.42–.56
BN	.14	.14	.14	.28	.56	.28–.56
YB2	.14	......	......	.42	......	....
YB1	.14–.28	.21	.21	.56	.56	.69
I2	.14	.14	.14	.42	.56	.56–.69
I4	.14	......	......	.42	......	....
H1a	.14	.14	.21	.56	.28–.42	.42–.69
B8	.14	.14	.14	.28	.28–.42(.69)	.42–.56
B1	.14	......	......	.42	......	....
B7	.14	.14	.14	.42	.42(.83)	.42
B2	.14–.28	......	......	.42	......	—
B4	.14	.14	.14	.69–.97	.42(.56, .83)	.83+

* At lowest concentration one inoculum block grew. At the highest none grew.
† Figures in brackets show irregular revival at higher concentrations.
‡ Highest concentration used.

The most important fact brought out by the toxicity tests is that the aspen isolates are totally inhibited at concentrations of 0.08 to 0.11 per cent, while all the others grew on concentrations up to 0.14 to 0.21 per cent. Within the groups the only constant differences are YB1 and A14a,

which grew on concentrations slightly higher than the others of their respective groups.

DISCUSSION

From the literature it is quite apparent that there is considerable confusion in the taxonomic separation of the variety *nigricans* from the typical *Fomes igniarius*. The separation is obscure and uncertain. From the general appearance and shape of the fruiting bodies, from the pore diameters, the length of the setae, and the diameter of the hyphae of the context, the sporophores in my collection can be separated into three groups that do not conform to the recognized taxonomic classification but are correlated with certain host species. One group seems to be limited to *Populus;* a second to *Betula,* especially *B. papyrifera;* and the third to certain other tree genera, altho they occur on certain species of *Betula.* Sporophores of the aspen group are usually smaller, and the pore surface is often at a large angle from the horizontal; those of the birch group are larger, more applanate, and the top surface fairly smooth; and those of the other group are larger, like those of the birch type, but are more ungulate and often have a rimose top. In pore diameter the aspen and birch groups are similar (100.8 and 102.9 μ respectively), while those of the third group are much larger (149.2 μ). There are but two exceptions to this, two of the aspen specimens being larger (124.5 and 145.6 μ). With one exception, the setae of the aspen group are longer (15.8 μ) than those of the other two groups (13.4 and 13.1 μ). The aspen specimens likewise have larger hyphae in the context (4.45 μ) than the other two groups (3.19 and 3.18 μ). There is some variation within each group, but most of this is probably due to ecological factors.

In cultural characteristics the aspen group is very distinct, all the isolates being slower growers and producing a methyl salicylate odor on malt agar, while all the isolates from other hosts are faster growers and none produce a methyl salicylate odor on malt agar, except two which sometimes produce a faint odor. There is also a difference in reaction to synthetic methyl salicylate, the aspen group being stimulated, or less retarded in growth, on concentrations that greatly retard the other isolates. The birch and miscellaneous host groups can not always be separated by cultural characteristics, but, in general, the birch type is faster growing and the miscellaneous group often produces thick, dense tufts at the center of aging cultures. Within each cultural group there is variation in color, topography, and rate of growth between nearly all isolates. This almost infinite variation is also expressed in antagonism between all isolates known to have come from different mycelia in nature; between haploid cultures, even those sexually compatible, and between haploid cultures and dicaryotic isolates of the parent sporophores.

The optimum temperature, which is considerably lower than previously reported, seems to be about the same for all isolates of all three groups. Slight differences in the optimum and in the high and low inhibiting and killing temperatures may occur but could not be tested with certainty with the equipment available.

From the decay tests made there seems to be only one isolate, A13, that differs markedly in its ability to decay aspen wood. The differences between the other isolates are largely nullified by the large differences between samples of individual isolates. On white birch and red gum woods, however, more differences are apparent, the aspen isolates being in general slow, the white birch isolates fast, and the others intermediate decayers. There is considerable variation between isolates in each of the three groups in decay ability on both white birch and red gum woods, and all three groups overlap.

Haploid cultures are slower growing and decay wood more slowly than the dicaryotic tissue cultures of the parent fruiting bodies. In rate of growth there are differences, some large, between haploid cultures from the same fruiting body. The synthetic dicaryons are usually of about the same general appearance and rate of growth as the parent dicaryons, but some are much slower in growth.

Fomes igniarius appears to be heterothallic. Haploid cultures from white birch comprise four sex groups, the three from ironwood comprise two sex groups, and the aspen cultures from two related fruiting bodies comprise three sex groups. No sexual fusions could be detected between haploid cultures from different tree species, by using a nuclear stain, or by the appearance of a new colony at the point of meeting, or by rate of growth of transfers from this point.

As an expression of the toxic effect of zinc chloride on the various isolates, the concentration totally inhibiting growth on malt agar seems to be more constant than the killing concentration. The isolates from aspen are totally inhibited by concentrations of 0.08 to 0.11 per cent, those from white birch at 0.14 per cent, except that one may be slightly higher, and those from other hosts at 0.14 to 0.21 per cent. The concentrations killing the isolates vary considerably between different tests, and, altho there appear to be individual differences within each group, there are no general differences between the various cultural groups.

The variations here reported show the necessity of using standard cultures in making comparative tests but at the same time remembering, when drawing conclusions, that considerable variation in a given characteristic may occur between the standard used and certain other isolates. Especially in identifying species by cultural characteristics, a wide range of known isolates may be necessary.

If certain groups of *Fomes igniarius* are limited to certain host species, as appears to be true, the presence of fruiting bodies on some tree species may not be a serious source of inoculum for other tree species. For example, birches bearing sporophores of *F. igniarius* may not be a source of inoculum for aspen; hence in a sanitation cutting a few diseased birch trees could be ignored. However, in order to establish this host relationship conclusively, it would be necessary to inoculate various host species with isolates from other hosts and determine the effect of a given host on each fungus group. If there were any effect, it is not likely that it would permanently affect cultural characteristics; therefore it is very unlikely that the birch and miscellaneous groups infect aspen to any extent, since the aspen type is so very distinct culturally. The differences between the birch group and that from hosts other than aspen are not so great as between the aspen group and the others. The birch and miscellaneous groups appear to be much more closely related to each other than to the aspen group.

SUMMARY

The species *Fomes igniarius* seems to comprise three fairly distinct groups that differ in the morphology of their sporophores, in rate of growth, general appearance, and the production of methyl salicylate odor on artificial media; rate of decaying some wood species; and in reaction to zinc chloride. As concerns some of these characteristics, there is overlapping of the groups; but, considering all characteristics, the three groups seem quite distinct. The groups do not agree with taxonomic separation into typical *igniarius* and variety *nigricans* but are correlated with species of host trees.

Each isolate differs in some major or minor respect from all others, with the exception of a few that appear to, and may, have come from the same mycelium in nature. The differences within the groups are both morphological, as color and consistency of the cultural mats, and physiological, as exemplified by antagonism on artificial media, reaction to zinc chloride, and rate of growth. The temperature relations of all isolates on malt agar appear to be about the same. The optimum does not agree with previous reports.

Haploid lines grow more slowly on artificial media and decay wood at a much slower rate than do the dicaryotic isolates of the parent fruiting bodies. Haploid lines from the same fruiting body may vary in rate of growth and in general appearance.

No sexual fusions between the three groups were observed. New dicaryons differing in rate of growth from isolates of parent fruiting bodies were produced by mating lines from related fruiting bodies.

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