DECAY IN BASSWOOD
CAUSED BY PHOLIOTA ADIPOSA FR.

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John L. Arend

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By

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INTRODUCTION

American basswood (Tilia americana) is one of the most important timber species in the Lake States region. Common defects of basswood are hollow butt logs and decay caused principally by a heart rotting fungus, Pholiota adiposa. No detailed studies have been made of this pathogen as a source of defect in basswood. This paper reports the results of a study made of rot defect in basswood caused by Pholiota adiposa in Michigan during the years 1935 and 1936. Described are the prevalence of decay in standing basswood trees, rate of spread of rot in decked logs, longevity of viable mycelium in dry wood, and laboratory studies with Pholiota adiposa in culture.

IMPORTANCE OF BASSWOOD

Basswood is found in practically every state east of the Mississippi River. Because of the desirable working qualities of the wood and its relative abundance in Michigan and Wisconsin, basswood is one of the important commercial hardwood trees in the eastern United States.

1/ I wish to acknowledge obligations to Dr. D. V. Baxter for helpful suggestions during this study. I also wish to express my appreciation to the various faculty members of the Forestry School and Conservation and Dr. L. E. Wehmeyer, Botany Department, for their advice during this investigation.

Since basswood is easily worked and can be used for a variety of products, the annual cut has always been large. During the years of 1906, 1907, and 1909, the annual cut of basswood in the United States was 400 million board feet (1). Due to the depressional years and decreasing supply, the annual cut in 1933 was 37,282,000 board feet (1). The lumber is used for boxes and crates, furniture and fixtures, cabinets, and more recently veneer and venetian blinds. Brush (3) lists 670 products for which basswood is used.

Sudworth (9) lists 30 species of basswood growing naturally within the limits of the United States. All species are rapid growers and may be propagated as easily from sprouts as from seed. Basswood matures in 90 to 140 years, often attaining a height of 80 to 150 feet and 4 feet in diameter. The U. S. Forest Service (1) has estimated the present stand of basswood to be 9 billion board feet growing in the United States, of which 5 million are in the Lake States.

THE STUDY AREA

A number of mixed hardwood stands containing basswood sawtimber were examined for defect and cull in Iron and Washtenaw Counties, Michigan, during this study.

The logs for the rate of cull spread portion of the study came from a 10-acre farm woods in the southwestern part of Washtenaw County in southern Michigan. This particular stand has been grazed for many years by livestock and, as a result, tree reproduction is sparse. Most of the merchantable oak and maple have been removed for fuel wood and the stand is now predominantely basswood.

FIELD METHODS

General observations were made in several of the remaining old-growth northern hardwood stands of Iron County in the Upper Peninsula of Michigan during the summer of 1935 as part of the more detailed study carried out in second growth mixed hardwoods of southern Michigan. Numerous basswood logs were also examined for cull and defect at different mills in the Upper Peninsula.

In southern Michigan a total of 100 sample basswood trees, 8.0 inches at d.b.h. and larger, were systematically selected in three different farm woods for detailed examination in regard to defect. The sampling method consisted of running a strip 33 feet wide through the center of each farm woods and studying each of the first 100 basswood that exceeded 8 inches at d.b.h.

Each sample tree was bored with an increment borer to determine the proportion of the diameter at that point which was sound or free from defect. The crown of each tree was classed as either (1) good, (2) average, or (3) poor, to learn if a correlation could be established between character of the crown and incidence of decay in the butt log. The relative terms used for classifying the crowns are described as follows:

Good - A symmetrical crown which is well developed and balanced, contains no dead or dying branches, and appears normal in all respects.

Average - A crown which is less than one-third the height of the tree, smaller than a good crown, and contains no dead or dying limbs.

Poor - A crown that is stag-headed, unsymmetrical, poorly developed, has dead or dying branches, and dead or broken tops.

Each tree diameter was taken at breast high and a boring was made to its center providing the wood was solid. The apparently sound portion of each core was recorded in inches to determine the proportion of solid or decayed wood. The cores were placed in bottles containing sterilized distilled water, numbered, and taken to the laboratory for culturing purposes.

Five basswood trees were felled in the spring of 1935 to study the progress of decay and longevity of the life of the mycelium in the wood after felling. All the trees cut exhibited decay in various parts of the bole. These trees were bucked in 12-foot lengths, and careful diagrams drawn of each cross section showing the outline and extent of visible decay.

The individual logs were not split to determine the longitudinal extent of decay since it was intended to store them under the same usual conditions that logs are decked in the forest. Cross sections about one inch in thickness were also taken at each cut for future comparisons.

The logs were placed on blocks in the woods so that the underside of the logs would not be in contact with the ground. Specimens of decayed wood were taken to the laboratory for culturing purposes to aid in the identification of the causal pathogen.

The diameters of the logs were measured to the nearest tenth of an inch at each cut. The outline of each decayed cross sectional area was drawn on graph paper. Since the outline obtained was usually very irregular, the area was measured by the use of a planimeter. Similar measurements were taken at 4- and 10-month intervals. After 12 to 15 months of storage the ends of the logs became very dry and the decayed area failed to show any appreciable increase in size and the measurements were discontinued.

RESULTS

The proportion of basswood trees exhibiting decay in the butt log gradually increased with diameter as was to be expected. About 80 percent of the trees exceeding 20 inches at d.b.h. had heart rot in the butt log, except for the 22- to 24-inch class which contained only two trees for a sample (Table 1). However, all of the trees, regardless of diameter class, exhibited a surprisingly high proportion of decay in the butt log. In fact, 60 percent of the trees 14 inches and less at d.b.h. showed similar defect.

Table 1.--Proportion of basswood trees exhibiting decay in butt log by diameter classes.

D.b.h. class	Total number trees	Trees sound	Trees defective	Proportion of trees	
				Sound	Defective
Inches	No.	No.	No.	Percent	Percent
8-10	13	5	8	38	62
10-12	10	4	6	40	60
12-14	28	11	17	39	61
14-16	15	5	10	33	77
16-18	16	7	9	44	56
18-20	10	3	7	30	70
20-22	6	1	5	17	83
22-24	2	1	1	50	50
Total	100	37	63		

In general, the vigor and health of a tree is usually reflected by the general appearance of its crown. However, this can be misleading for basswood having good, vigorous crowns, but poor appearing crowns usually reflect decay in the butt log. For example, 50 percent of the trees having crowns classed as "good" were sound in the butt log whereas only 15 percent of the trees with "poor" crowns had sound butt logs (Table 2).

Table 2.--Proportion of basswood trees exhibiting decay in butt log by crown classes.

Crown class	Total number trees	Proportion of trees	
		Sound	Defective
	No.	Percent	Percent
Good	41	50	50
Average	39	36	64
Poor	20	15	85

The Progress of Decay in Basswood Trees After Felling

In studying the progress of decay in felled logs several variables enter into the field methods which may have some effect on the results obtained. After felling the cross sections at the ends of the logs have a lower moisture content than the central portion of the logs. The ends of the logs are also subjected to sunlight and abrupt changes in temperature. There is also the possibility that other fungi may infect the logs while in storage. The latter factor was watched by making cultures from the logs at each periodic measurement. The former factors all tend to produce more conservative results than otherwise would have been obtained had all conditions remained uniform.

The progress of decay in basswood after felling was fairly uniform over a period of 10 months (see Graph I). The increased percentage of decay during the first five months was about equal to the percent of increase during the second five months in spite of the fact that the amount of moisture was gradually being reduced.

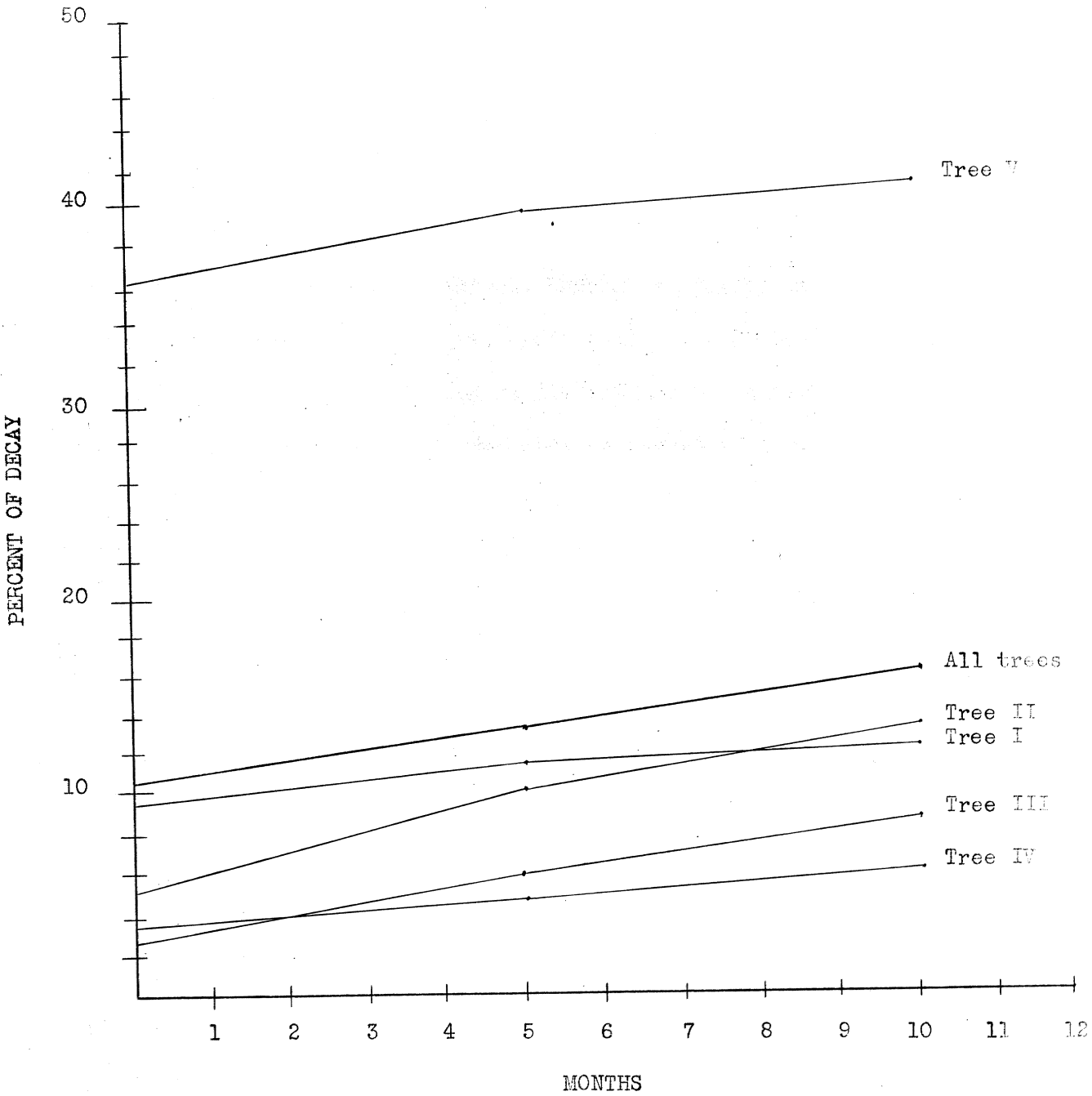
The total increase of decay area measured at the log ends during the first five months averaged 2.6 percent and 2.0 percent for the second five months, or a total of 4.6 percent increase in decayed area in 10 months (Tables 3 and 4). In other terms, the decay in basswood logs caused by Pholiota adiposa increased at the rate of 0.5 percent per month after felling.

Table 3.--Progress of decay in stored basswood logs.

Tree No.	Total cross sectional area	Cross sectional area decayed at felling		Cross sectional area decayed after Five months		Ten months	
	Sq. In.	Sq. In.	Percent	Sq. In.	Percent	Sq. In.	Percent
I	254.8	24.1	9.5	29.9	11.8	31.7	12.5
II	218.9	15.5	7.1	21.4	9.8	29.0	13.3
III	279.3	7.2	2.6	16.3	5.8	21.6	7.8
IV	74.8	2.7	3.6	3.2	4.3	3.7	5.0
V	148.4	53.7	36.2	58.0	39.2	61.7	41.6
Total	976.2	103.2	10.6	128.8	13.2	147.7	15.2

Table 4.--Percentage increase of decay in stored basswood logs.

Total trees	Total logs	Cross sectional area exhibiting decay at: Time of felling	Five months after felling	Ten months after felling	Total increase in 10 months
No.	No.	Percent	Percent	Percent	Percent
5	19	10.6	13.2	15.2	4.6



Graph I. The progress of decay in percent for the logs of each tree measured at 5-month intervals. The heavy line is the average progress of decay in percent for all the trees.

Character of Heart Rot in Basswood Caused by Pholiota Adiposa

The common heart rot of basswood caused by Pholiota adiposa is also found in many broadleaf trees such as oak, maple, birch, and beech and to a certain extent on many conifers. Weir and Hubert (12) list grand fir, alpine fir, western hemlock, mountain hemlock, Engelmann spruce, and western white pine as common hosts of this fungus. Weir (11) again lists it as a serious enemy of Abies grandis. The fungus is distributed throughout the United States, Canada, and Europe (10). No detailed report of this disease has ever been issued in the United States although, as this study shows, the heart rot is very destructive in certain stands of timber, notably the basswood forests. Scattered reference to this fungus and frequent misleading descriptions are found in the literature.

The early stage of decay in basswood as observed during this study is hair brown and extends 4 to 15 feet in a longitudinal direction ahead of the late stage or typical decay (Pl. IV, Figs. 2 and 3). The early stage of decay is usually surrounded by an olive green band (0.5 to 2 cm. wide) which I termed the invasion zone. Oftentimes the characteristic invasion zone is in turn surrounded by a very narrow red to yellowish orange band (0.1 to 0.5 cm.). The olive green invasion zone is more pronounced in early years of infection, although it is usually present in trees badly decayed.

Small white patches of decayed wood appearing in the hair brown area are forerunners of the late stage of decay. The late stage of decay is yellowish to very light brown with numerous white transverse streaks (Pl. V, Fig. 1). These white areas hollow out and resemble insect burrows. The rot column is not usually uniformly circular in cross section, but is very irregular and assumes all shapes, such as a half moon (Pls. I-IV). The upper end of the rot column is conical (Pl. IV).

Saprophytism

In all the field work it was constantly observed that only the heartwood of living basswood was decayed by Pholiota adiposa. The decay observed was usually irregular in outline during the early years of decay, but as the rot progresses and nears the sapwood it assumed a uniform circular outline (see Pls. I-III). This same characteristic is very pronounced in longitudinal sections of infected logs (see Pls. IV and V). The decay as it approaches the sapwood in trees that have been infected for a number of years appears to stop. No mycelium was observed in living sapwood from microscopic studies or were cultures of Pholiota adiposa secured from the living sapwood. However, before it can be definitely concluded that this fungus is strictly saprophytic or an obligate saprophyte, artificial inoculation of the sapwood of living trees needs to be tested.

The Apparent Scarcity of Fruit Bodies on Pholiota Adiposa on Living Trees

The writer was unable to find fruit bodies of Pholiota adiposa on living trees. This does not imply that sporophore formation is restricted to dead trees or logs. However, the scarcity of fruit bodies on living trees seems to be common experience with many mycologists and pathologists while working in the field.

Fruit bodies were found on standing dead basswood trees and logs. In one case (Pl. VI, Fig. 1) fruit bodies were found on a large basswood limb about one month after it had been broken off by the wind; however, no fruit bodies appeared on the living trunk.

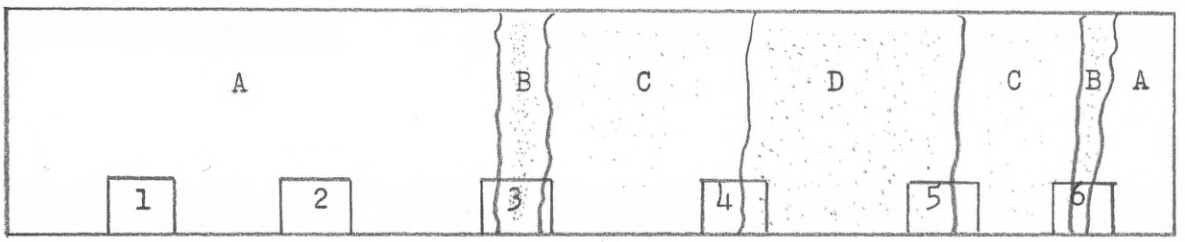


Fig. 1.--A section cut from the decayed cross section shown in Plate VI. A - sound area; B - greenish invasion zone; C - early stage of decay; D - typical or late stage of decay. Slides were made from the areas indicated by the numbered squares.

Sample sections were selected from wood blocks or discs which showed the characteristic decay caused by Pholiota adiposa. Small blocks[1/] 1/4" by 1/4" were chosen for sectioning at the points indicated on the wood sections illustrated in Fig. 1.

Both unstained and stained slides were made to determine the effect of the mycelium on the wood. Radial, tangential, and cross sections were mounted from each block by running them through the alcoholic series, clearing in

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1/ These blocks were boiled in equal parts of glisterine, alcohol and distilled water until the blocks sank, indicating that most of the air was expelled. Radial, tangential and cross sections were cut from these ranging from 15 to 40 u in thickness. It was found that sections 29 to 30 u thick gave the most satisfactory results.

xylol and mounting in Balsam. Two double stains Safranin-Delafield's haematoxylin[1/] and Safranin-picro-aniline blue[2/] were found to be very satisfactory stains and were used entirely in this study. In the latter stain the wood elements take a reddish stain and the hyphae a deep blue, while in the former the wood sections take a reddish to purplish stain and the hyphae a brownish color.

Microscopic Description of Normal Basswood

Basswood is a soft, light, diffused porous wood of uniform texture. The wood rays are homogeneous (rarely heterogeneous) and of two sizes, namely uniseriate and 10-20 cells high, and multiseriate and 50-100 cells high. The ray cells are thin walled and contain numerous crystals. Vessels have numerous spiral thickenings, perforations simple, intervascular pitting alternate, and tyloses are absent. Wood parenchyma is of the metatracheal type or arrangement (arrangement largely independent of the pores) and appears throughout the growth ring in numerous uniseriate lines and in a single terminal row.

1/ The sections were stained over night in safranin. The lignin of the wood elements stains a faster and deeper red than the cellulose. Pour off safranin and add 50 percent alcohol. This dissolves the excess stain and removes the stain rapidly from the cellulose parts of the wood elements. When various parts of the sections became a very light pink they were placed in tap water and thoroughly washed for about 5 min. If the alcohol fails to reduce the deep red stain, add a drop of dil. HCL before washing to speed the reaction. The sections were then stained in Delafield's haematoxylin for 30-45 min. This stain has little or no effect on lignified structures. Transfer to tap water containing a drop of dil. HCL. The reddish color is replaced by a rich purple. As soon as sections begin to appear reddish, pour off acidified water and wash in tap water until acid is washed out. Place sections then in 50 percent alcohol for 1 min., then in 95 percent alcohol for 1 min., 100 percent alcohol for 5 min., transfer to xylol for 1-5 min. Mount in Balsam.

2/ Stain sections in 1 percent aqueous safranin for 1 min., wash in water until the sections appear a distinct pink (either tap or distilled water); place sections in picro-aniline blue made up of 25 cc sat. aqueous solution of aniline blue and 100 cc of sat. aqueous solution of picric acid. Heat stain for a few minutes until it simmers, then remove the wood sections. Wash in running water until dark blue color has disappeared. Clear in clove oil 2-4 min., xylol 5 min., then mount in Balsam. The wood elements take a reddish stain while the fungous hyphae take a deep blue stain.

Microscopic Description of Decayed Wood

The first microscopic evidence of decay in basswood caused by Pholiota adiposa is the disappearance of the primary wall in the vessels. The width of the walls of the wood elements appears to thicken, but this is due to decomposition products in the lumen of the cells surrounding the cell wall. In the late stage of decay the wood elements are filled with perforations made by the hyphae in passing through the cell walls. The middle lamella, wood rays, parenchyma cells and parts of vessels and wood fibers are left in badly decayed wood.

Fungous hyphae were found abundantly in the different elements throughout the decayed wood although they were most easily seen in the vessels. This mycelium was less abundant in the early stages of decay. Radial sections showed the mycelium to the best advantage. Two kinds of hyphae were discernible: large irregular thickened, branching hyphae with numerous clamp connections; and very fine unbranched hyphae upon which clamp connections were few.

The hyphae penetrated the walls of the elements in all directions. They did not necessarily pass through the pits, since the hyphae often was observed to penetrate a cell wall within a very short distance from a bordered pit. The hyphae also showed slight constrictions when passing through a cell wall, although this feature was not pronounced.

Greenish Invasion Zone

The olive-green zone usually separating the decayed from the apparently sound wood is made up of wood elements which are occluded with a greenish deposit consisting, apparently, of partially digested cell substance, oxidation products or decomposition substances. The wood parenchyma cells show the first affects. The cell walls appear to become roughened and seem blurred under the microscope even with careful focusing. First the lumen of the vessels then the fibers appear to be filled with this greenish substances. As the walls of the elements appear to swell, the lumina of the cells are filled with deposits which in turn form a yellowish green to a dark green. The method of its formation or decomposition is not clear. Fungal enzymes acting upon the wall substance are probably responsible for these deposits. These deposited substances are insoluble in 72 percent sulphuric acid and even in concentrated sulphuric acid. This leads one to believe that the fungus cannot use these products for food. However, on the inner side of the olive-green zone bordering late decay, the colored substance begins to break down and finally disappear, due either to further digestion or to absorption by the hyphae. The deposits stained a deep blue to purplish color (rarely red) with Safranin-Delafields Haematozylin. This would account for the disappearance of the deposits in the later stages of decay, but does not account for their insolubility in concentrated sulphuric acid. On the other hand, the enzymic action on the wood cells may produce exudation of lignified substances in the lumina which are insoluble in concentrated sulphuric acid, but this does not account for their disappearance in late stages of decay.

LABORATORY METHODS

Cultures were isolated from the sections of decayed basswood brought to the laboratory. A sterile scalpel was used to remove exterior portions of the wood sections and small bits of wood tissue were transferred to malt-agar plates. In a course of a week or so, growing mycelium appeared on the malt-agar. The outer edge of the mycelial growth was transferred to a second agar plate to assure pure cultures. This mycelium was allowed to grow over the plate and was used as stock material for sub-cultures.

If drying out of the diseased wood had occurred or if the small bits of wood tissue taken from the infected wood was likely to occur before mycelial growth started, they were first placed in sterile distilled water for a short period before being placed on the malt agar. This phase of the method was particularly valuable when dried pieces of infected wood were tested for viable mycelium.

Isolations were also made from increment borings brought to the laboratory in sterile distilled water. The cores were removed with sterile tweezers and small portions $\frac{1}{4}$" to $\frac{1}{2}$" long were broken off, flamed for a second in alcohol and then placed in malt agar plates. Later the increment borer was sterilized in an autoclave for 20 minutes under 15 pounds of pressure and 50 cultures were made from a small basswood log infected with Pholiota adiposa. A sterilized axe was used to chop a clean surface to start the boring.

Inasmuch as no sporophores were originally found on the infected trees, various attempts were made to produce these in the laboratory in order to identify the cause of the decay in the wood.

Small portions of the growing mycelium were transferred from the [illegible] petri-dish cultures to wide mouth Erlenmeyer liter flasks. Each of these flasks contained a slant of malt-extract agar.[1] Slants were prepared by pouring 275 cc. of this medium into each liter flask, after which the flasks were placed in a leaning position until the medium solidified. The layer of malt agar averaged about 2 inches in thickness at the base. The inoculated flasks were placed in the light and in the dark at room temperatures. These were examined twice a week.

A second series of flasks was prepared in the same manner, except before sterilizing 30 grams of saw dust were mixed with the malt-agar and the same procedure followed as in the last method. The species of saw dust used were Douglas fir, Soft maple and White oak. These flasks were inoculated and placed in the light and in the dark at room temperatures.

A modification of Etter's medium was also prepared.[2] Corn meal, corn starch and the powdered wood were mixed together, and enough distilled water added to make the mixture well moistened. Three or four grams of malt-extract were added to this medium in the flasks before they were sterilized. The powdered wood was prepared by grinding on an emery belt the species of wood used, which were Douglas fir, Ponderosa pine, soft maple, and basswood.

Tissue cultures were made from a portion of the diseased wood brought into the laboratory every few months in order to determine the longevity of the life of the mycelium of Pholiota adiposa in basswood after felling. Mycelial growth which was secured from these cultures was transferred to a malt-agar slant in a wide mouth Erlenmeyer flask to see if a sporophore would develop in order to be certain of the identity of the fungus.

1/ 25 gms. agar agar, 20 gms. malt extract, and 1000 cc distilled water.

2/ Corn meal (White Quaker) . . . . . 48 gms.
Corn starch (Kingsford) . . . . . 16 gms.
Powdered wood . . . . . 8 gms.

PHOLIOTA ADIPOSA FR. IN CULTURE

Pholiota adiposa grew successfully on the various kinds of wood tested as well on several other substrata. The medium used to study the habit, rate, and character of mycelial growth was malt agar in Petri dishes. (Table 5)

Habit of Growth.--The advancing hyphae either penetrated the agar or spread rather slowly over the surface. The advancing zone is white, cottony, and in most cases has a uniform margin, depending on the age of the inoculum. If 14-day inocula or older are used, vegetative hyphae may appear in spots ahead of the advancing zone. This is undoubtedly due to the dissemination of secondary spores, for oidia and chlamydospores are present in 14- to 21-day cultures and conidial-like structures in 30-day cultures. The central portion of the mycelial growth becomes wooly in about 14 days and felty to sodden in 20 to 30 days and takes on a yellowish color, deepening with age. There is little difference in texture of the mycelial growth under dark and light conditions.

Color.--The margin of the vegetative growth is always pure white while the central portion becomes cream colored, and later yellow, following the margin in the order named. Cultures grown in light had a deeper yellow color than those grown in the dark.

Rate of Growth.--Mycelial growth averaged 10 mm. for the first 7 days under light conditions and 8 mm. under dark conditions. About 25 days are required to cover a petri dish (45 mm. in diameter) in the light and about 28 days in the absence of light. The average rate of hyphal growth was calculated for a number of hyphae over a period of several days at 75° F. and was found to average 1.3 microns per minute.

Microscopic Features.--The advancing hyphae were delicate, hyaline, rarely branched and with a few inconspicuous clamp connections. The hyphae averaged about 2 u in width. The older hyphae were 2 to 3 u wide, much branched, and had numerous clamp connections. In 14-day cultures, many hyphae began to break up into a chain-like appearance, forming oidia.[1/] All chlamydospores observed were terminal. Conidial-like bodies were observed in 30-day cultures very similar to the conidia of Pholiota aurvella (8), which is very similar to P. adiposa and differs only in arrangement of scales on the stipe.

1/ The hyphae assumed a chain-like appearance, formed cross walls, and broke up into individual spores which were called oidia, while larger, thick-walled cells that appeared at the tips of various hyphae were called chlamydospores.

Table 5 CULTURAL CHARACTERS AND AVERAGE RADIAL GROWTH OF PHOLIOTA ADIPOSA

Age Days	Average Radial Growth	Texture	Color	Description of Mycelium
		Under Artificial Light		
7	10 mm.	Floccose to cottony	White	Hyphae 2 to 3 microns wide, thin walled, delicate, rarely branched, septations few, clamp connections numerous.
14	25 mm.	Appressed to cottony	White margin with central portion yellowish	Hyphae 2 to 3 microns wide, walls thicker than 7-day cultures; septations few, clamp connections numerous.
21	45 mm.	Appressed to cottony	White margin; central portion yellowish	Hyphae much branched, clamp connections numerous; some hyphae breaking up into oidia; few terminal chlamydospores.
		Under Continuous Dark Conditions		
7	8 mm.	Floccose to cottony	White	Hyphae thin walled, 1.5 to 2 microns wide, hyaline, delicate; clamp connections few; branching hyphae fork-like.
14	23 mm.	Cottony to wodly, downy	White	Hyphae hyaline, delicate, walls slightly roughened, 1 to 3 microns wide, branching numerous.
21	40 mm.	Cottony to woolly, felty	White to cream colored	Hyphae 2 to 3 microns wide, walls slightly roughened, much branched. Oidia and chlamydospores present.

MYCELIAL GROWTH OF PHOLIOTA ADIPOSA ON DIFFERENT SUBSTRATA

The mycelial growth of this fungus was studied on several different media including malt agar, malt agar and maple sawdust, malt agar and Douglas fir sawdust, malt agar and white oak sawdust. Sporophores developed on the various media tested within 30-45 days under artificial light conditions except on the malt agar-white oak medium, where fruit bodies were produced in 60 days. In fact, all of the malt agar wood mixtures tended to produce more typical sporophores than plain malt agar but required a longer period of time.

Sporophores of this fungus, as indicated by this study, require light for their best development. This characteristic corresponds to the results obtained by other investigations for other fungi of Long and Harsch (7), Baxter (2, p. 542), and Buller (4). Sporophores developed in darkness tend to have a long stipe, usually about 6 inches long, and a small pileus about 1/2 inch in diameter. These fruit bodies are not viscid and the scaley appearance is less pronounced. Identification of fruit bodies developed in total darkness would be very difficult, if not impossible, to identify as this species.

All fruit bodies developed under light conditions, or subjected to sunlight, were strongly positive heliotropic from the very beginning of their formation. Gravity seemed to have little or no effect on the sporophores, as they always pointed toward the light. In darkness the fruit body usually had a coiled stipe. Sometimes the stipe would curl in such a direction that the pileus would be forced down into the medium.

The point of location of sphorophores on the slant varies according to the observations made by different investigators. Long and Harsch (7, p. 79) found that more than 95 percent of the sporophores developed on the upper

half of the slant. They attributed this characteristic to the drying of the medium in the upper thin portions faster than in the lower part. From Table 6 it can be seen that no sporophores developed on the upper half of the slant. The majority of the fruit bodies developed in the thickened portions of the slant or the lower half. These results are similar to the findings of Baxter (2, p. 542) who tried the drying effect upon fruit-body formation of Polyporus hispidus and received negative results.

In the tests undertaken to study the starvation effects upon Pholiota adiposa in regard to sporophore development, the mycelium grew out around the inoculum on the agar but no fruit body was developed after a period of 60 days.

The proper conditions for sporophore development have been widely studied and many results drawn. Various specific conditions have been used which induced sporophore formation by a certain fungus, but when these same conditions were imposed on other fungi they failed to produce fruit bodies. Fungi are variable organisms in nature and oftentimes produce the same variability in artificial culture. Some investigators advocate that as long as there is food and room for the vegetative mycelium to grow, sporophore formation will be absent. In the culture medium of malt agar and Douglas fir, a fruit body was developed before the mycelium had covered the slant.

The most satisfactory media used for sporophore development was the modified Etter's medium. About 30 days are required for the formation of sporophores. Usually 2 to 6 very typical fruit bodies occur in this time. Sporophores appeared several times on these media before the vegetative mycelial growth had covered the substratum.

From the results of the various substrata it appears that the modified Etter's medium placed in the light provides the best conditions for sporophore formation by Pholiota adiposa. Typical fruit bodies were not produced in the absence of light on any of the various substrata used. The character of the substratum appears to play an important role in sporophore initiation, although Long and Harsch (7) concluded that the substratum plays only a minor role in sporophore formation. The drying of the medium or lack of food and moisture has little or no effect on sporophore formation by Pholiota adiposa.

SPOROPHORES OF PHOLIOTA ADIPOSA FR. IN CULTURE

Identity and Technical Description

Pileus 3-12 cm. broad, hemispheric or convex to plane, golden yellow to zinc-orange, very viscid, covered with dark brown concentric scales, margin even; gills adnate becoming emarginate, 2-8 mm. broad, grayish brown then thick, solid or rarely with a small hollow, usually curved or erect, yellow becoming dark brown, scales yellowish or tawny increasing in number upward; spores 7-8 X 4-5 u, ellipsoid or oblong-ellipsoid, smooth, ferruginous; cystidia like bodies present, not conspicuous, projecting only slightly if at all, dark brown.

In the field Pholiota adiposa is found on both deciduous and coniferous stumps, logs, and trees and may be confused with P. aurivella, its closest relative. The latter has similar microscopic characters but differs slightly in microscopic characters. P. aurivella has a darker colored pileus, and slightly larger scales than Pholiota adiposa. The scales on the stipe increase in number downward while the scales of Pholiota adiposa increase in number upward.

Table 6 CHARACTER OF MYCELIAL GROWTH OF PHOLIOTA ADIPOSA ON VARIOUS MEDIA

Media	15 Days	30 Days	45 Days
Malt-agar	Light: Slant ½ covered with mycelial growth; zonate; margin white, cottony; center cream colored. Dark: Slant ½ covered with cottony mycelial growth.	Light: Slant covered, growing up sides of flask, cream colored. Small fruit body partly developed at base of slant. Dark: Slant almost covered, margin uneven; small abortive fruit body at base of slant.	Light: Two fruit bodies. Stipe 2" long, cap 1" dia., viscid, scaley. An abundance of brownish spores. Dark: 1 fruit body; stipe curled, 6" long, cap ½" dia. Brown spore print on flask under the gills. Several abortives ones.
Malt-agar and Maple Sawdust	Light: Slant covered with cottony mycelial growth. Tufted patches of mycelium. Dark: Slant ½ covered; mycelium white, cottony to downy.	Light: Slant covered with dense mycelial growth. Sporophore 1" high on center of slant. Dark: Slant covered, sodden-like mass of mycelium.	Light: 2 fruit bodies & numerous small ones at base of slant. Stipe 2"long, cap 1½" dia., scaley, viscid. Numerous spores. Dark: Mycelium growing up sides of flask; tufted-like, cream colored. Few abortive fruit bodies at base of slant.
Malt-agar and Douglas Fir	Light: Slant ¼ covered with white mycelium. Cottony. Dark: *----------	Light: 1 fruit body in center of slant, small but very characteristic. Slant ½ covered. Dark: *----------	Light: Slant 3/4 covered; snow white mycelium. Very typical fruit body at base of slant. Dark: *----------
Malt-agar and White Oak	Light: Slant ¼ covered with white, cottony to felty mycelial growth. Difficult to distinquish from D. fir medium. Dark: Slant ¼ covered with a snow white, cottony mycelial growth.	Light: Slant ½ covered; white zonate. Medium is discolored a dark brown at margin of mycelial growth. Dark: Slant ½ covered. Mycelium white, zonate, downy.	Light: Slant 3/4 covered with white, cottony, zonate mycelium. Medium stained dark brown ahead of marginal growth. Dark: Slant 3/4 covered. Mycelium yellowish color. No fruit bodies.

* Culture lost through contamination.

Table 7 CHARACTER OF MYCELIAL GROWTH OF PHOLIOTA ADIPOSA ON MODIFIED ETTER'S MEDIUM

Species of Wood	15 Days	30 Days	45 Days
Maple	Light: Substratum 3/4 covered with a white mycelial growth Dark: Substratum ½ covered with white cottony to woolly mycelial growth.	Light: Small fruit body in center of flask. Mycelium cream colored. Dark: Substratum 3/4 covered with mycelial growth	Light: 6 sporophores. Stipe 2-4" long, cap 1" dia. Spores covering the mycelium. Dark: Several coiled stipes with no pilei. 3/4 of medium covered with mycelium.
Ponderosa Pine	Light: Substratum covered with cream colored mycelial growth. Dark: Substratum ½ covered with creamish mycelium.	Light: One typical fruit body; mycelium brownish to cream colored; woolly to downy. Dark: Substratum covered with mycelial growth; several coiled stipes with no developed pilei.	Light: 2 fruit bodies. Stipe 2-4" long, cap 1-1½" dia. An abundance of spores produced. Dark: Several stipes curled up at bottom of flasks.
Douglas Fir	Light: Substratum less than ½ covered with white mycelium. Dark: Substratum ¼ covered with white mycelial growth.	Light: 1 typical sporophore developing. Mycelium has not yet covered the substratum. Dark: Substratum ½ covered with white mycelial growth. 1 fruit body; stipe 5" long, cap ¼" dia.	Light: 1 large typical sporophore. Stipe 3-5" long, pileus 2" dia. Flask is colored by brownish spores. Dark: Substratum 3/4 covered with mycelium; 1 curled sporophore with long, curled stipe and small pileus.
Basswood	Light: Substratum ½ covered with a white mycelial growth. Dark: Substratum ¼ covered with a white mycelial growth.	Light: Substratum covered with a white mycelial growth. No fruit bodies. Dark: Substratum 3/4 covered with white to creamish mycelium. No sporophores.	Light: 2 sporophores, small but typical; stipe 2" long, pilei 3/4" dia. Dark: No sporophores.

LONGEVITY OF THE MYCELIUM IN BASSWOOD

A wood section was taken from one of the sample logs one year after felling and stored in the laboratory. As the summer of 1936 was exceptionally hot and dry, the temperature in this same laboratory was so high that cultures placed on a table 12 feet back from an east window were killed. In spite of these conditions, on November 21, 1936, or 17 months after the tree was felled, cultures of active growing mycelium were secured from this section of the log. The moisture content of this same section of log was 6.5 percent[1/] at the time the cultures were made in comparison to 91.7 percent moisture content of the wood at the time of cutting.

Table 8.--Longevity of mycelium in basswood after felling.

Date tissue cultures were started	Number of months after felling	Fungous growth		Sporophores developed	
		Yes	No	Yes	No
April 15, 1935	0	X		X	
Sept. 18, 1935	5	X		X	
April 15, 1936	12	X		X	
Nov. 21, 1936	17	X		X	

Since viable mycelium was isolated from basswood logs which had a moisture content of 6.5 percent 17 months after felling, the mycelium can become active in wood after many months of service and can cause serious economic damage under favorable moisture conditions.

The low moisture content at which the mycelium of Pholiota adiposa can remain viable has more significance when the average moisture content of wood products under actual use is examined as determined by Henderson (5).

1/ Based on oven dry weight.

Only 14 out of 100 wood products listed have a moisture content less than 6 percent. These 14 include such products as musical instruments, patterns and pulleys. While important wood products such as aeroplanes, automobile bodies, baseball bats, cooperage products, furniture, doors, plywood, etc. all have at least 6 percent moisture content or higher.

Other investigators (6) have revived mycelium of this same fungus from lowland white fir, Abies grandis, after a period of 6 years in dry storage. Viable mycelium was isolated from basswood in dry storage after a period of 2 years by Hubert (6).

THE INCREMENT BORER AS AN INSTRUMENT FOR ISOLATING HEART-ROTTING FUNGI FROM LIVING TREES

Successful cultures of heart-rotting fungi were obtained from increment cores taken in the field. From 46 test cultures made, 17 exhibited bacteria and only 1 was contaminated with Penicillium or Aspergillus. The remaining 28 or 62 percent of the cultures remained uncontaminated. Pholiota adiposa appeared in 10 cultures, and in 2 cultures an unidentified fungus with a white, stringy mycelium (see Pl. VI, Fig. 2), and the remaining 16 uncontaminated cultures showed no growth.

From these results it seems possible that the increment borer can be used satisfactorily in determining the causal pathogen and to some extent the degree of defectiveness without chopping into the tree or injuring it mechanically when trying to secure a tissue culture of heart-rotting fungus. It is also very obvious that this method fails to detect decay unless it is confined or at least extends down to 4 or 5 feet from the base of the tree.

SUMMARY AND CONCLUSIONS

American basswood (Tilia americana) is one of the most important sawtimber trees in the Lake States region. One of the most prevalent fungal organisms causing defect in basswood is Pholiota adiposa.

This paper describes the results obtained from a study of basswood trees and logs in southern Michigan in relation to decay caused by Pholiota adiposa.

The rate of spread of the decay and longevity of viable mycelium in basswood was studied in both the field and laboratory. The study extended over a period of 2 years, 1935 and 1936. Some of the important results from the study are as follows:

(1) From an examination of 100 basswood trees 8 inches and larger at d.b.h. in 3 different woodlots in Washtenaw County, Michigan, only 37 percent of the trees appeared sound at the point bored with an increment borer.

(2) The proportion of basswood trees exhibiting decay in the butt log increased with diameter. Over 80 percent of the trees exceeding 20 inches at d.b.h. showed heart rot in the butt log compared to 60 percent.

(3) A good healthy appearing basswood crown is a poor indicator of soundness of the butt log. Only 50 percent of trees with crowns classed as "good" had sound butt logs. However, only 15 percent of the trees with crowns classed as "poor" had sound butt logs.

(4) The radial spread of visible decay in basswood logs after felling was found to increase in area at the rate of 2.6 percent the first 5 months and 2.0 percent the second 5 months for a total of nearly 5 percent of the first year.

(5) Since fruiting bodies of Pholiota adiposa are seldom found on infected standing trees, a method and technique was developed for identifying the fungus in standing trees. This consisted of culturing the fungus in the laboratory from increment cores taken from infected trees in the field and growing the culture on a modification of Etter's medium. Normally developed sporophores were grown on this medium in the laboratory.

(6) The mycelium of Pholiota adiposa remains viable in dry basswood logs for nearly two years after felling. In fact, successful cultures were made from a portion of a basswood log stored in the laboratory when the moisture content of the wood was 6.5 percent 17 months after felling.

(7) The macroscopic and microscopic features of the mycelium of this fungus are described when grown under different environmental conditions in the laboratory.

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Appendix Table 1 Diameter and Crown Conditions of Basswood Trees Examined

Tree No.	Diameter D.B.H.	Proportion of Diameter Decayed 1/	Condition of Crown
	Inches	Percent	
1	14.7	29	Average
2	10.5	0	Good
3	8.6	0	Good
4	9.9	10	Average
5	14.4	14	Average*
6	12.5	0	Good*
7	12.2	Discolored	Good*
8	14.1	0	Good*
9	16.4	50	Good
10	12.3	17	Average
11	13.2	14	Average
12	16.7	50	Poor
13	16.3	13	Average
14	12.5	0	Average
15	18.7	0	Average
16	9.8	50	Poor
17	12.9	17	Average
18	17.6	89	Average
19	13.1	0	Average
20	15.4	13	Average*
21	12.2	33	Average*
22	13.8	50	Poor
23	8.7	87	Poor
24	14.6	43	Poor
25	11.2	60	Poor
26	13.0	29	Poor
27	13.0	0	Good
28	15.0	38	Average
29	14.0	29	Average
30	13.1	67	Average
31	16.3	0	Average
32	12.2	0	Good
33	12.8	0	Good
34	16.9	0	Good
35	18.3	78	Average
36	8.8	0	Good
37	11.6	0	Good
38	12.7	0	Good
39	16.7	25	Good
40	19.2	20	Good
41	12.5	17	Poor
42	18.0	44	Good
43	12.1	67	Good
44	21.0	30	Average
45	10.0	40	Good

1/ Based on proportion of core showing defect. Each tree was bored to its center usually at a point breast high.

* Sprout origin.

Table 1 (Continued)

Tree No.	Diameter D.B.H.	Proportion of Diameter Decayed[1]	Condition of Crown
	Inches	Percent	
46	9.2	80	Average*
47	9.0	25	Average*
48	9.7	0	Average
49	13.2	57	Average
50	13.2	43	Average
51	11.2	33	Average
52	14.8	43	Poor
53	9.8	Discolored	Poor
54	17.6	0	Good
55	19.4	10	Good
56	12.0	0	Good
57	12.0	50	Poor
58	13.9	0	Average
59	16.1	0	Good
60	16.1	13	Good
61	19.1	0	Good
62	10.4	Discolored	Poor
63	16.1	0	Average*
64	14.5	0	Average*
65	14.5	29	Poor*
66	13.2	17	Poor
67	13.2	0	Average*
68	13.1	0	Average*
69	13.1	17	Good*
70	9.2	10	Good*
71	10.5	0	Poor*
72	17.7	0	Average*
73	20.7	20	Poor*
74	21.5	20	Good*
75	12.8	17	Average*
76	14.5	0	Poor*
77	15.9	25	Good*
78	9.5	0	Poor*
79	14.7	0	Average
80	16.9	13	Good
81	13.0	17	Poor
82	18.0	78	Good
83-	18.8	33	Good
84	15.4	14	Good*
85	21.9	30	Good
86	21.5	20	Good
87	14.3	0	Good
88	23.1	0	Good
89	20.8	0	Average
90	19.6	30	Good*
91	12.0	17	Average*
92	17.2	0	Good*
93	14.7	57	Average*
94	8.2	0	Good
95	11.8	60	Good
96	18.5	22	Poor
97	18.3	55	Average*
98	12.6	0	Average
99	18.3	0	Average
100	22.8	16	Good

Appendix Table 2 Progress of Decay in Basswood

Tree No.	Log No.	Diameter of Cross Sections	Basal Area	Area of Decay		
				At Felling	Five Mo. Later	Ten Mo. Later
		Inches	Sq. In.	Sq. In.	Sq. In.	Sq. In.
I	1	12.1	115.2	19.5	23.6	24.1
I	2	9.1	64.8	--@	--	--
I	3	8.1	51.8	1.1	1.6	1.9
I	4	5.3	23.0	3.5	4.7	5.7
I Total			254.8	24.1	29.9	31.7
II	1	12.5	122.4	3.1	5.7	6.8
II	2	8.7	59.0	6.2	8.7	10.8
II	3	7.1	40.3	6.0	6.5	7.0
II	4	2.8	7.2	.25	.50	4.4
II Total			218.9	15.5	21.4	29.0
III	1	12.8	129.6	.6	3.9	5.7
III	2	9.3	67.7	2.8	5.1	8.1
III	3	8.1	51.8	2.5	4.8	5.0
III	4	6.1	30.2	1.3	2.5	2.8
III Total			279.3	7.2	16.3	21.6
IV	1	7.5	44.6	--@	--	--
IV	2	5.2	21.6	2.7	3.2	3.7
IV	3	3.1	8.6	--@	--	--
IV Total			74.8	2.7	3.2	3.7
V	1	11.3	79.4	45.9	47.3	47.5
V	2	7.1	40.3	1.2	2.3	3.9
V	3	4.6	15.8	5.4	6.3	6.6
V	4	4.1	12.9	1.2	2.1	3.7
V Total			148.4	53.7	58.0	61.7

@ No decay or discoloration visible.

Plate I

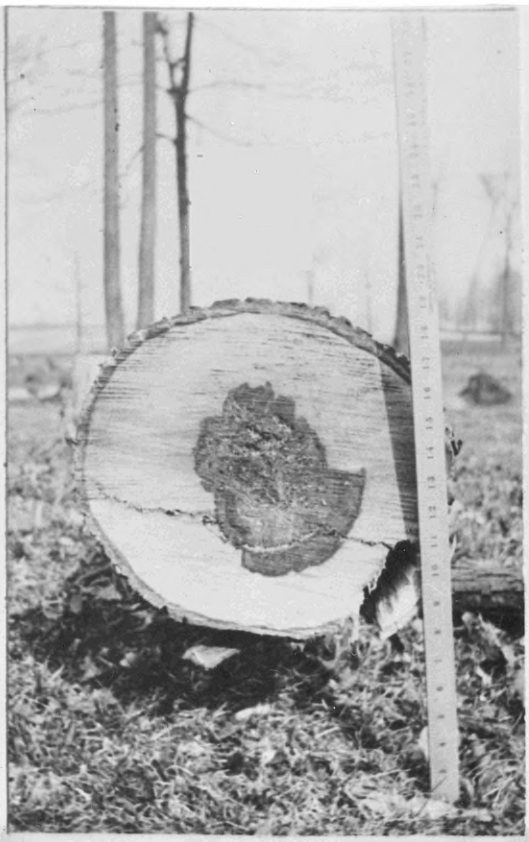

Fig. 1--First 12 ft. log of basswood tree 1 infected with Pholiota adiposa.

Fig. 2--Second 12 ft. log of same tree exhibited no decay or discoloration.

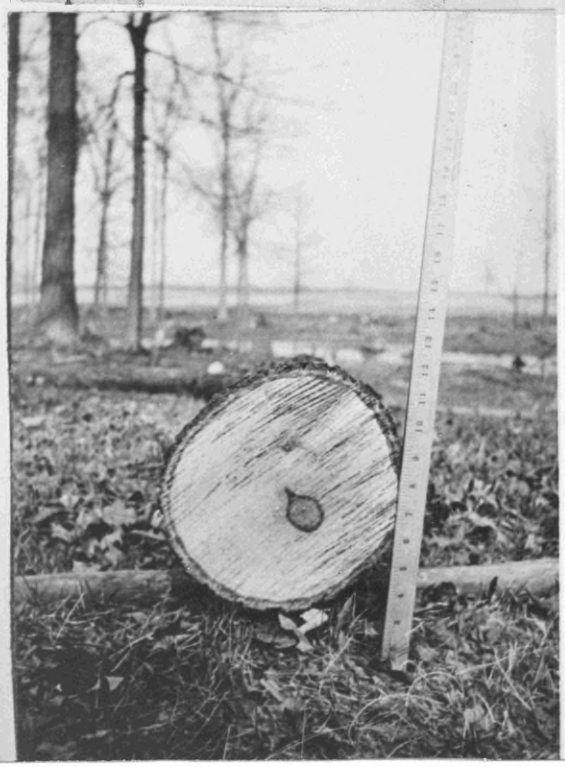

Fig. 3.--Third 12 ft. log had small amount of decay.

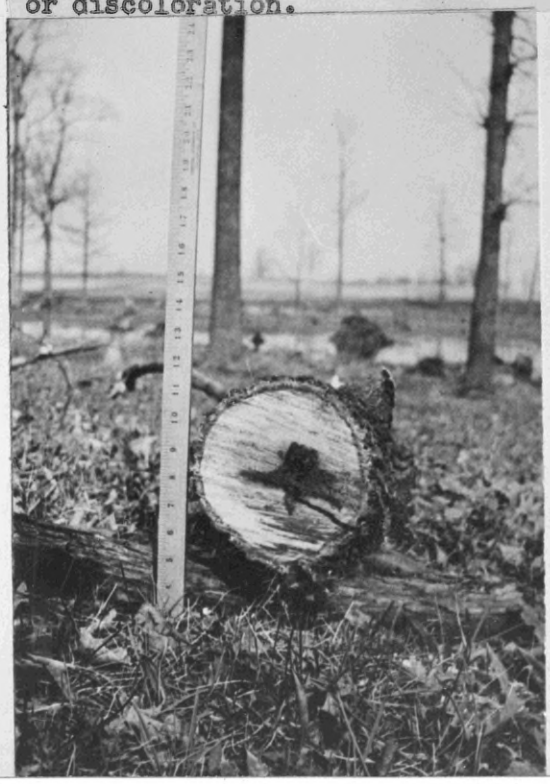

Fig. 4.--Fourth 12 ft. log had larger amount of decay.

Fig. 1.--First 12 ft. log of basswood tree 2 infected with Pholiota adiposa.

Fig. 2.--Second 12 ft. log of same tree.

Fig. 3.--Third 12 ft. log of same tree.

Fig. 1.--First 12 ft. log of basswood tree 5 with characteristic late stage decay of Pholiota adiposa.

Fig. 2.--Second 12 ft. log of the same tree exhibited less decay than the first log.

Fig. 3.--Third 12 ft. log.

Fig. 4.--Fourth 12 ft. log.

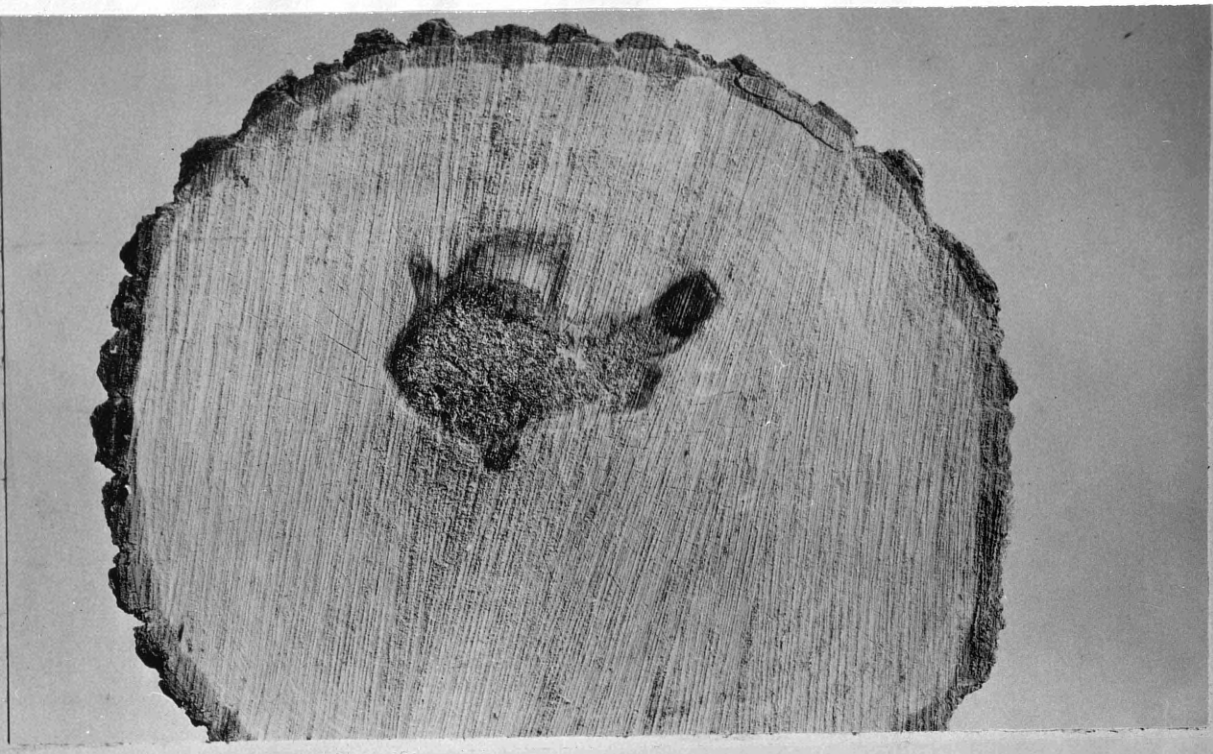

Fig. 1.--Cross section of a basswood log infected with Pholiota adiposa. The dark invasion zone bordering the late stage of decay is very pronounced. Characteristic insect holes are also present.

Fig. 2.--Upper portion of a split basswood tree showing the brownish conical appearance of the early stage of decay caused by Pholiota adiposa.

Fig. 3.--Close up view of the same section.

Plate V

Fig. 1.--Longitudinal section of a basswood log showing all stages of decay caused by Pholiota adiposa. Brownish invasion zone has advanced to the sapwood.

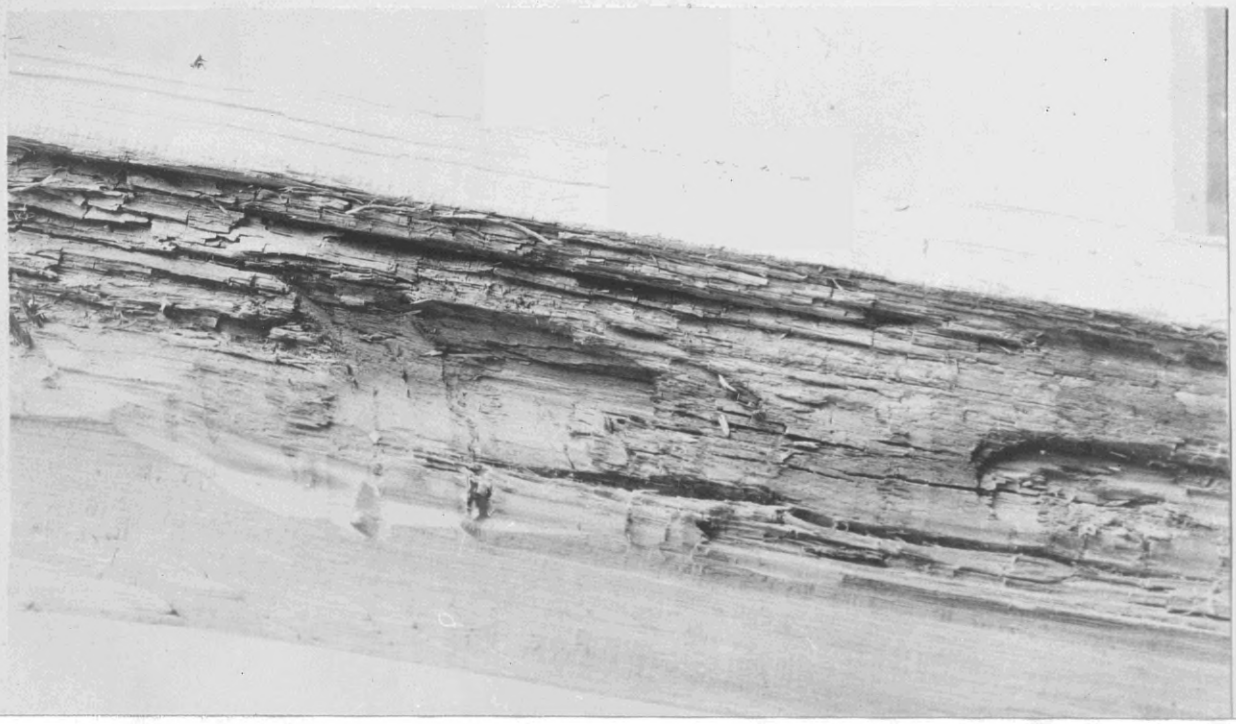

Fig. 2.--Viable cultures of Pholiota adiposa were obtained from a section of basswood 17 months after felling when the moisture content of the wood was reduced to 6.5 percent.

Fig. 1.--Fruit body of Pholiota adiposa on a dead basswood limb. Collected November 15, 1936.

Fig. 2.--An unidentified fungus in culture that was obtained from an increment core taken from a standing basswood tree.

Fig. 1.--Mycelium of Pholiota adiposa growing on a white pine block (left) and a basswood block (right). A small sporophore is developing on the basswood block.

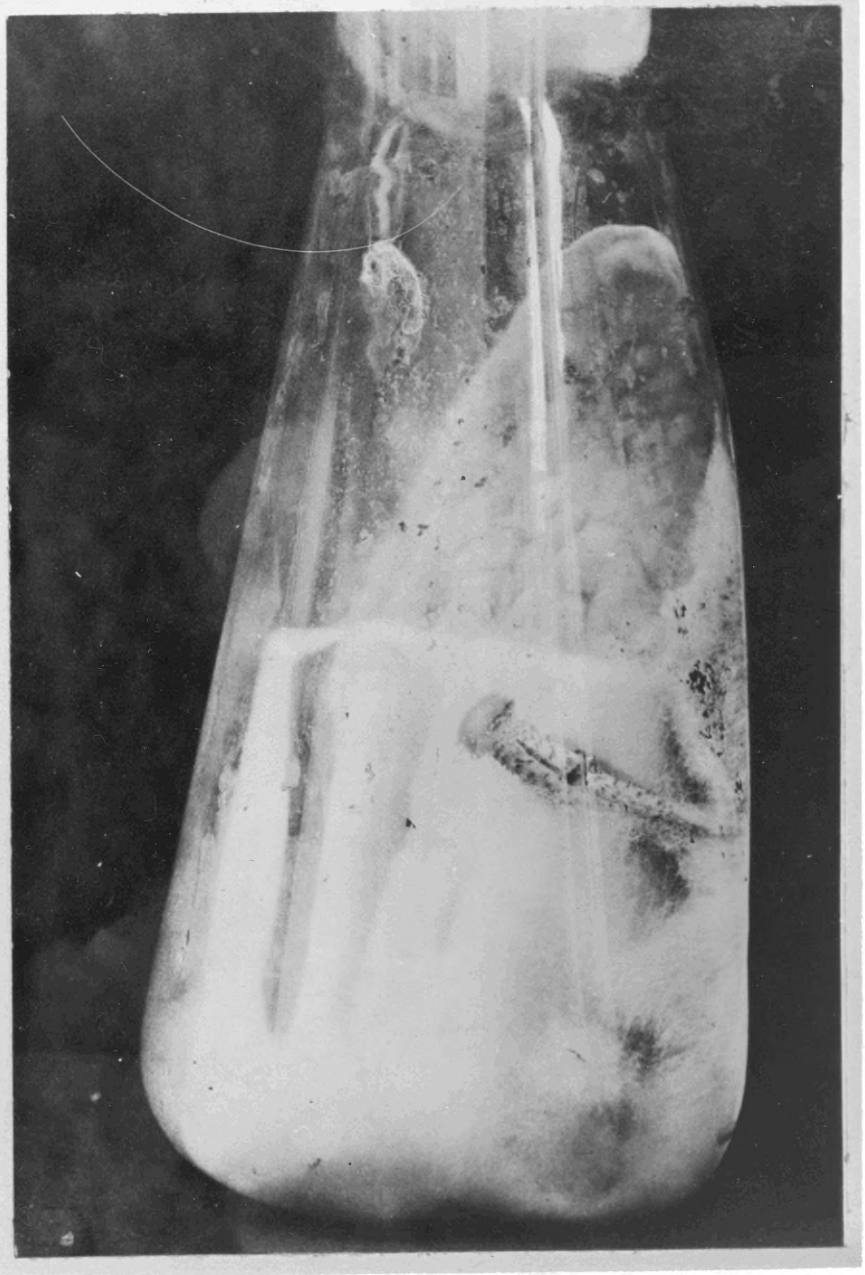

Fig. 2.--Young sporophore of Pholiota adiposa developing on a malt agar slant. See Plate IX, Fig. 2, for later development.

Plate VIII

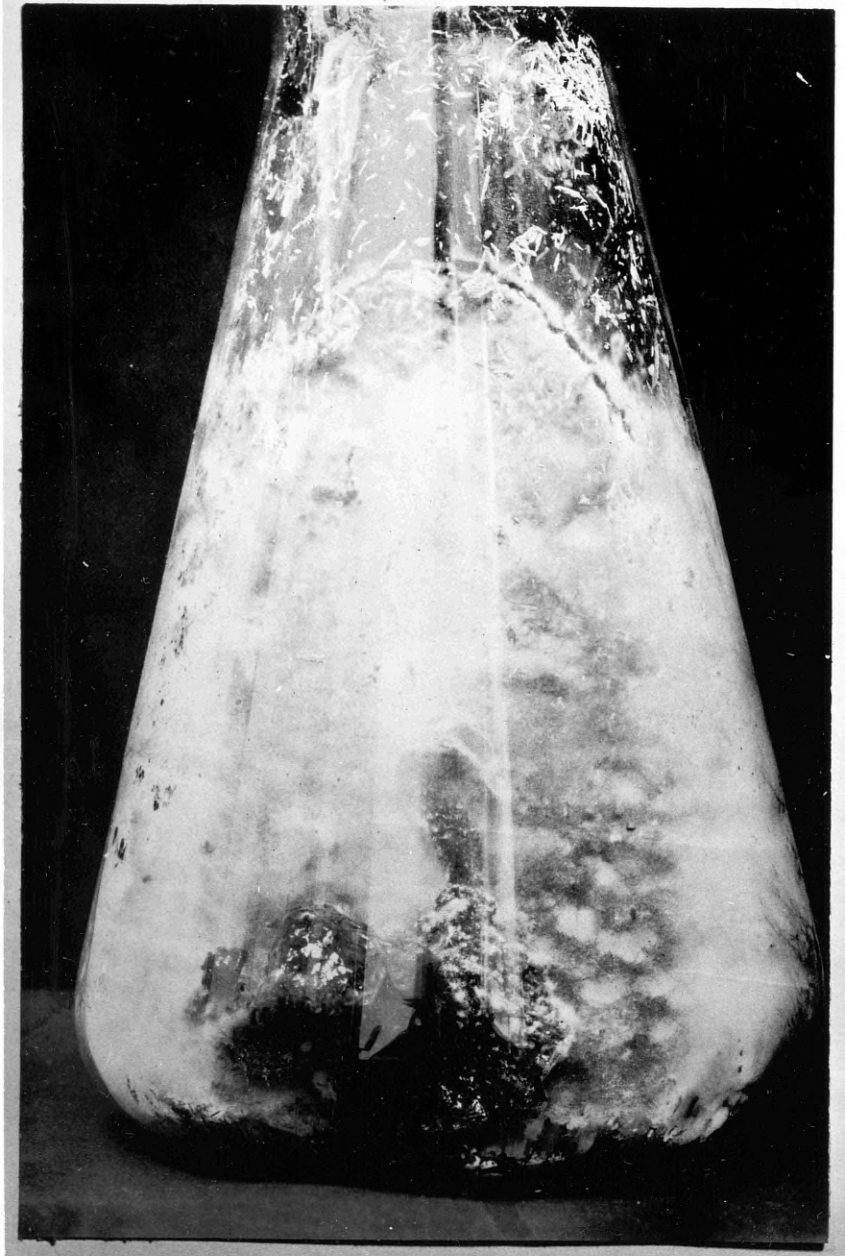

Fig. 1.--Mycelial growth of Pholiota adiposa on malt agar-maple sawdust medium. Note sporophore developing on the thickened portion of the medium. The dark color in the bottom of the flask is from spore discharges.

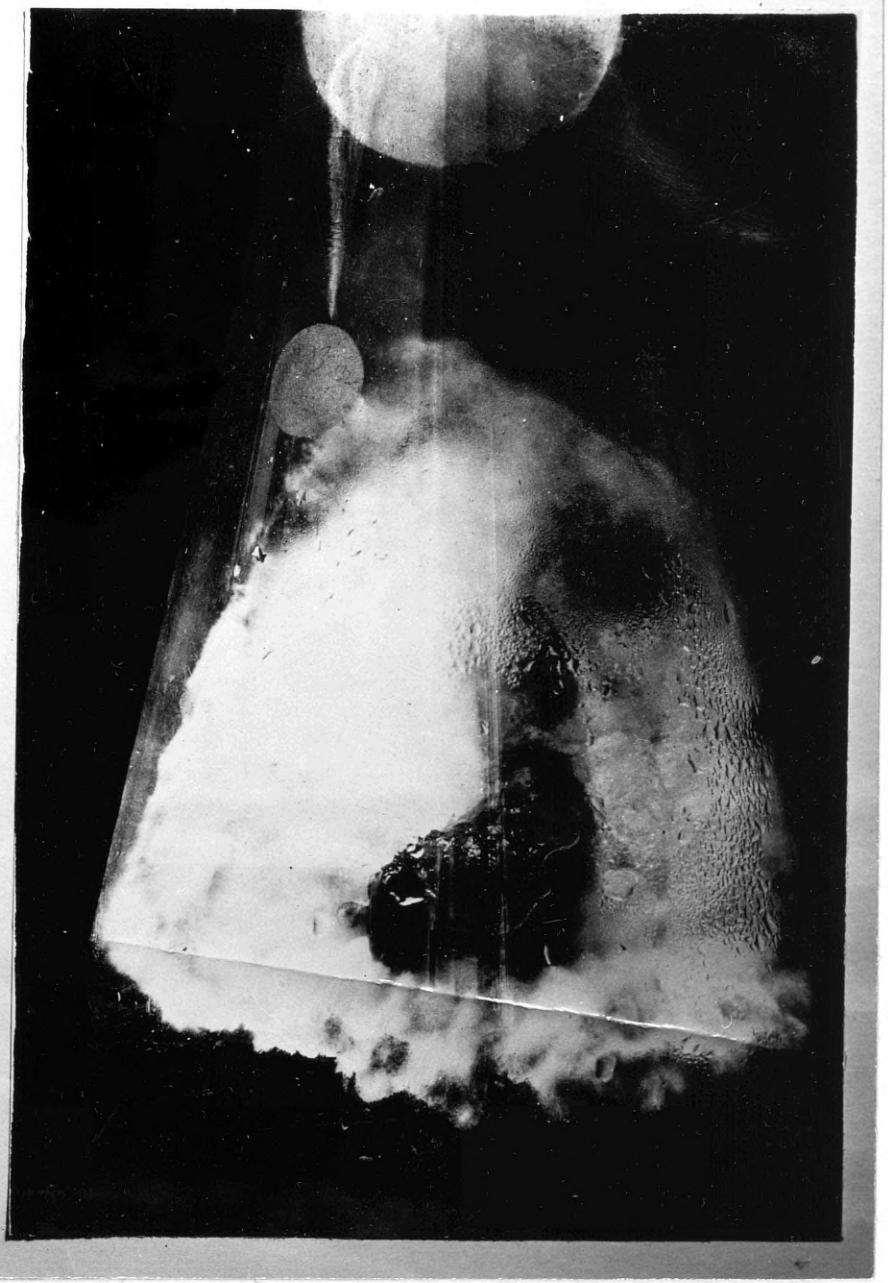

Fig. 2.--Mycelial growth of Pholiota adiposa on malt agar-Douglas fir sawdust. A sporophore developed before the mycelium completely covered the slant.

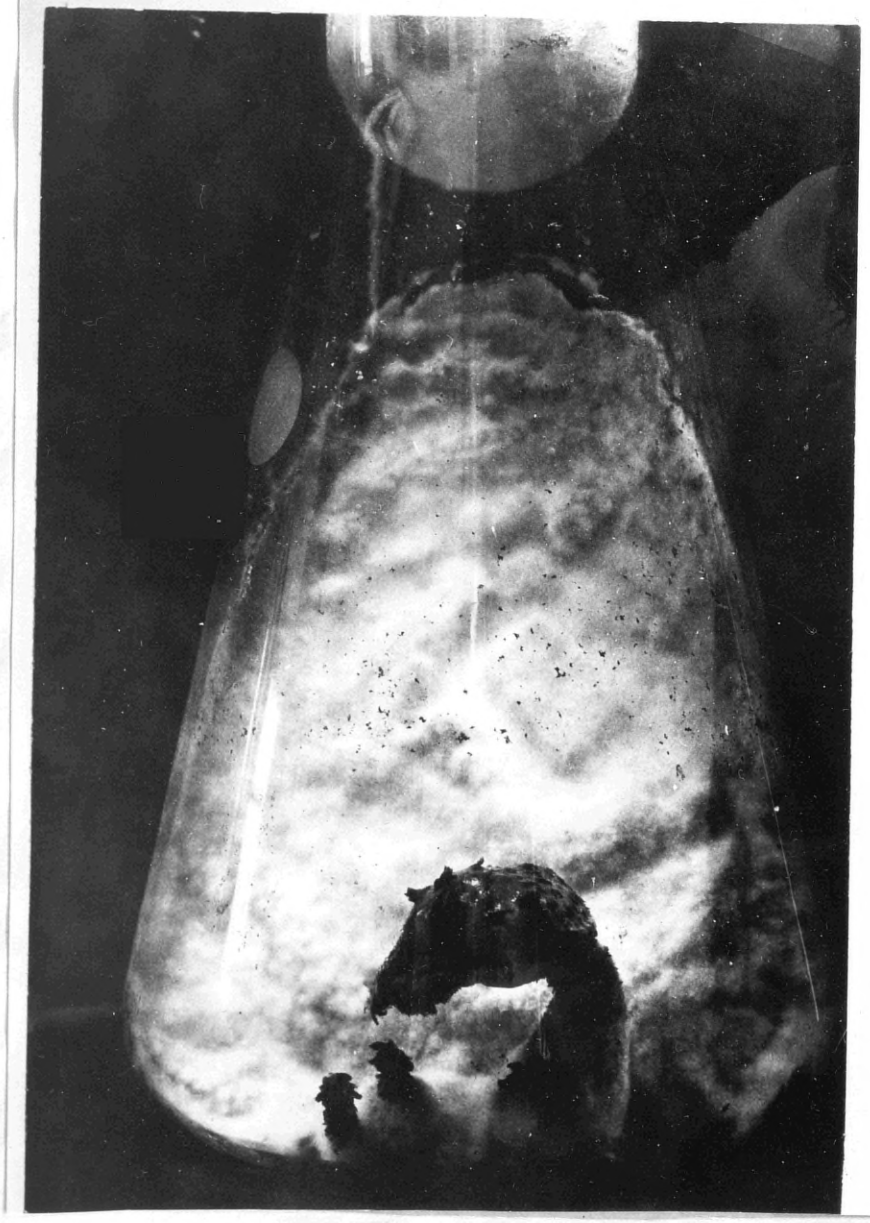

Fig. 1.--Mycelial growth of Pholiota adiposa on malt agar-white oak sawdust. One relatively large sporophore and several small ones are growing at the bottom of the slant. Age of culture 60 days.

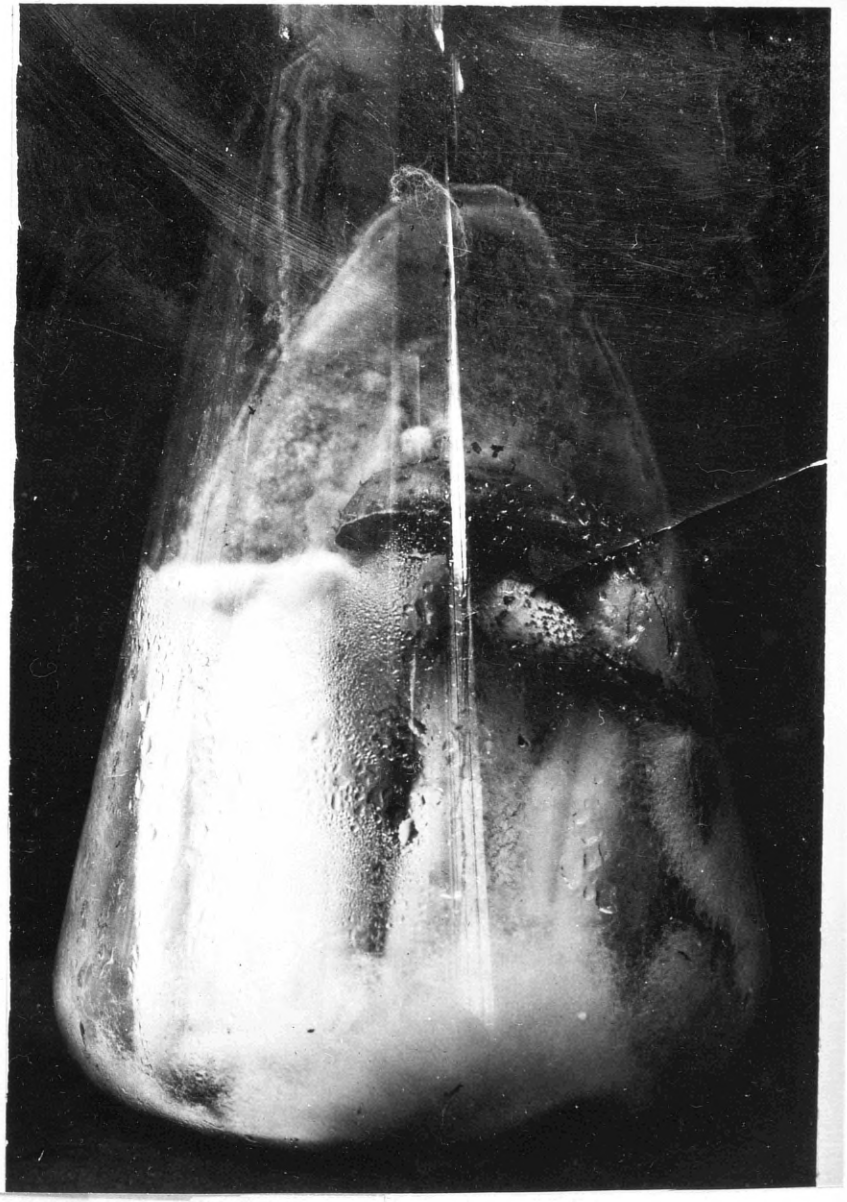

Fig. 2.--Same sporophore shown in Plate VII, Fig. 2, seven days later.

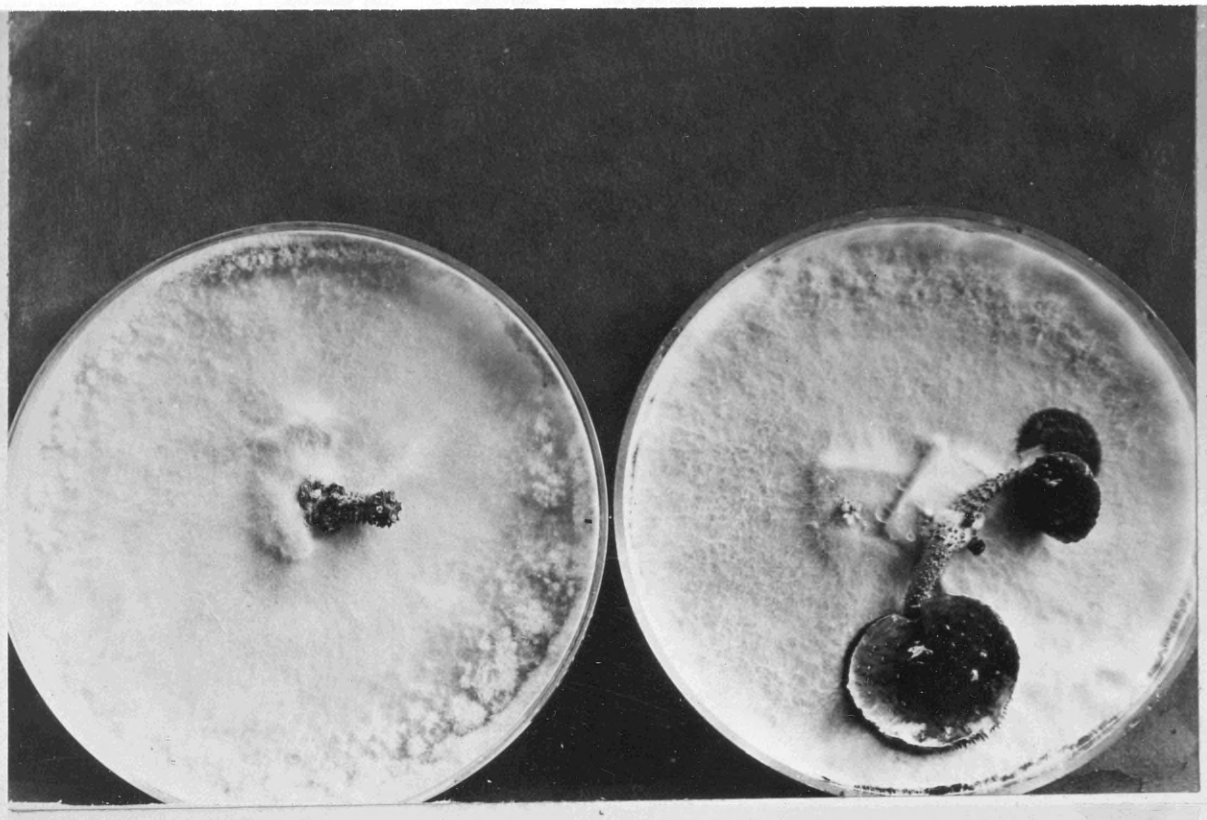

Fig. 1.--Third-day old cultures of Pholiota adiposa. Fruiting bodies are common occurrence in 25- to 30-day old cultures. Note the dark spore markings on the mycelium.

Fig. 2.--Fruit bodies of Pholiota adiposa grown in light (left) and dark (right).