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TITLE OF THESIS....."THE DEVELOPMENT OF MYCELIUM OF Fomes igniarius (Fries) Kickx. in Culture and in host tissue."

UNIVERSITY.....of ALBERTA.....

DEGREE FOR WHICH THESIS WAS PRESENTED....Ph.D.....

YEAR THIS DEGREE GRANTED.....1971.....

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(Signed).....Shukla.....

PERMANENT ADDRESS:

Village - Gajepur
P.O. - Sewapuri
Dist. Varanasi, U.P.
INDIA

DATED...Dec. 9,.....1970

THE UNIVERSITY OF ALBERTA

THE DEVELOPMENT OF THE MYCELIUM OF *Fomes igniarius* (Fries) Kickx. IN CULTURE AND IN HOST TISSUE

by

© PRABHAKAR SHUKLA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

EDMONTON, ALBERTA

SPRING, 1971

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "THE DEVELOPMENT OF THE MYCELIUM OF *Fomes igniarius* (Fries) Kickx. IN CULTURE AND IN HOST TISSUE", submitted by Prabhakar Shukla in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Hans E. Green
External Examiner

Edwin A. Cossins

Supervisor

Date December 4, 1970.

ABSTRACT

The structure and development of the mycelium of *Fomes igniarius* (Fries) Kickx. is described on the basis of results obtained from comparative studies of the variety *populinus* occurring on aspen poplar and the variety *laevigatus* occurring on birch. It has been found that the mycelium has a distinctive morphology which is expressed in both the host tissue and in culture.

The mycelium of *F. igniarius* consists of three kinds of hyphae: thin-walled hyphae, fiber hyphae and cuticular cells. The thin-walled hyphae are frequently branched and lack clamp connections. The type of branching is monopodial and branches form in acropetal succession. The branches arise mostly at acute angles, but branching at right angles and in whorls occurs occasionally. Hyphal anastomosis is frequent in thin-walled hyphae. Fiber hyphae and cuticular cells originate from the thin-walled hyphae with a septum at the differentiation point. The distinctive characters of the variety *laevigatus* are the presence of thin-walled, broad hyphae and development of hyphal rings. In variety *populinus*, the presence of half septa, cell wall constrictions and invaginations are the distinguishing features.

The maximum mycelial growth takes place at 27°C in both solid and liquid media. Diffusion zones are produced by *F. igniarius* on gallic and tannic acid agars. *F. igniarius* var. *populinus* is a slow growing fungus. The advancing zone becomes bayed after three to four weeks on malt-agar medium.

The color of the mycelium becomes dark brown at later stages of growth. The fungus produces a strong wintergreen odor on malt-agar medium. On the same medium variety *laevigatus*, comparatively faster growing, is cinnamon-orange in color at later stages of growth. The advancing zone is hyaline with the hyphae well separated from each other. The wintergreen odor is mild in this variety.

Ultrastructure studies of the hyphae have shown that there is dolipore type of septum in the various kinds of hyphae composing the mycelium. The hyphal cell wall in *F. igniarius* was observed to be either single or multilayered and was usually triple layered. Cell organelles such as endoplasmic reticulum, mitochondria and vesicles were found in abundance in the growing hyphae. As the fiber hyphae and cuticular cells developed, the cell organelles disintegrated and at maturity only diffuse cytoplasm was observed suggesting the possible utilization of these organelles in the thickening of the cell wall. A characteristic feature of this fungus was the presence of complex concentric membranes. The lomasomes were seen to bud off from these membranes.

The morphological and physiological characters of the mycelium are evaluated with respect to their usefulness in classification.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Dr. L. L. Kennedy for her thoughtful guidance, constructive criticism and unfailing help in the presentation of this thesis.

I would like to express my thanks to Dr. W. N. Stewart and Dr. N. Colotelo for their valuable suggestions given to me from time to time. Appreciation is expressed to Dr. S. K. Malhotra for training in the use of electron microscope. I acknowledge gratefully the technical assistance provided by Mrs. Sita Prasad during the course of electron microscopic study. Thanks are also due to other members of my examining committee for their helpful suggestions. I wish to thank Mrs. Betty Ford for her help in the typing of this thesis.

Sincere appreciation is extended to my brother, Mr. Sudhakar S. Shukla for looking after my family in India in my absence.

Financial support for this research was provided by a Graduate Research Assistantship and a Graduate Teaching Assistantship in the Department of Botany and from a grant to Dr. Kennedy from the National Research Council of Canada.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Fomes igniarius (Fries) Kickx. is a member of the Polyporaceae a large and economically important group of Basidiomycetes. The fungus is readily recognizable by its woody, sessile sporocarp with a black pileus and a dark brown pore surface. White trunk rot, caused by *F. igniarius* is a serious limitation in hardwood utilization in temperate regions of the world. This fungus is an important cause of decay in standing trees but is not a cause of rot of wood in storage.

There are three varieties recognized: variety *populinus* growing on living aspen poplar, variety *laevigatus* growing on dead birch and variety *igniarius* which attacks living birch, *Acer* and *Ostrya*. Of these varieties the one that infects living aspen poplar causes the most serious losses.

Most of the work with this fungus has been pathological and physiological in approach. Studies of basidiospore germination, growth of the mycelium in culture and rate of decay in living trees have been done by various workers. While many of these studies have provided significant information about the growth of the mycelium, the structure of the mycelium and the effect of various environmental factors on growth and development of this phase have received little attention.

Since this fungus is an important pathogen and since the vegetative mycelium is the cause of decay in the wood, the

present study was undertaken with the objective of providing more information about this stage in the life cycle. It was thought necessary to know whether the fungus behaves in a similar fashion in cultural environment and in the natural environment because it is only then that the study of the fungus under cultural conditions is of any practical significance in understanding its behaviour in natural environment. In this study the expression of both morphological and physiological characters of the mycelium under variable environmental conditions has been examined. However, the emphasis has been placed on the structure and development of the vegetative hyphae.

Since the advent of electron microscope as a tool for biological research, workers concerned with the developmental morphology of fungi have used this instrument to supplement light microscope observations. A perusal of the literature revealed that no work on the fine structure of the mycelium of *F. igniarius* has been reported. Therefore, electron microscope studies of the mycelium were carried out.

The importance of the study of mycelial characteristics becomes obvious when one looks at the lack of agreement among mycologists on the classification based on basidiocarp characters alone. Bondartseva (1961) in his review of the recent classification of the Polyporaceae concludes that, among the numerous systems of classification for the Polyporaceae, there is not one which could be regarded as generally accepted. Nobles (1948, 1958, 1965) pointed out

the significance of cultural characteristics in the classification of Polyporaceae. However, she did not make any attempt to segregate genera on this basis because the relationship between cultural characters of a species and those expressed in the natural environment was not established. It is my belief that a satisfactory system of classification for the Polyporaceae must include characteristics of all stages of the life history of the species. In this thesis information from comparative studies of the mycelium growing in different environments has been used to determine the stable characters which could be used in establishing a natural system of classification.

In this investigation of the mycelium of *F. igniarius* comparative studies of variety *populinus* and variety *laevigatus* were carried out. Three approaches were used: study of the mycelium in the natural environment, study of the mycelium under cultural conditions and an ultrastructure study of the different kinds of hyphae making up the mycelium in culture. The results from these studies have been brought together in the discussion to present a detailed account of the structure and development of the vegetative mycelium.

LITERATURE REVIEW

The fungi belonging to family Polyporaceae are one of the main causes of the destruction and deterioration of wood tissue. For example, Rcff (1964) reported that *Poria weirii* develops freely in wood and the growth of the mycelium is random and very much branched. Henningsson (1965) working with *Polyporus betulinus* reported that when attacking the wood, the hyphae penetrated all the cells and grew throughout the wood tissue.

According to Proctor (1941) the wood destroying fungi (Basidiomycetes) penetrate the lignified walls of the wood cells both by the enzymatic and the mechanical process. The penetrating hypha may secrete enzymes which cause complete, local dissolution of the wood cell wall resulting in the softening of the area around the penetrating hyphal tip. One of the most intriguing fundamental problems in the field of wood deterioration is presented by the striking differences between the two major types of wood decay - white rot and brown rot. They may be distinguished by differences in the color, solubility of alkali, strength, dimensional stability, pulping properties and chemical composition of the decayed wood (Cowling, 1961). Clearly, these distinguishing features are the result of differences in the enzymatic effects of the decay fungi. In a brown rot the cellulose and its associated pentosans are attacked while the lignin is left in a more or less unchanged condition; in a white rot all the components of the wood, including the lignin, are

decomposed (Cartwright and Findlay, 1958). The white rot fungus species in the group of Basidiomycetes studied by Cowling (1961) and Seifert (1966), are known to attack simultaneously cellulose and lignin in the wood without significantly changing their proportions.

Nobles (1948), in her cultural studies of 126 species of wood destroying fungi, found that host relationships and physiological and morphological characters were of diagnostic value. She (1958) suggested that cultural characteristics may serve as a guide to the taxonomy and phylogeny of family Polyporaceae. She found, after a careful study of 252 species, that this family is composed of (i) a primitive group of species that produce no extracellular oxidase, and, if heterothallic show a bipolar type of interfertility, and (ii) an advanced group of species that produce extracellular oxidase and, if heterothallic, show the tetrapolar type of interfertility. She did not make an attempt to segregate genera on the basis of cultural characteristics because relationships between cultural characters and those expressed in the natural environment have not been established.

Physiological studies of the mycelium of fungi could be used in developing a rational system of control of plant diseases. In a comparative nutritional study of the three wood-rot-fungi *Polyporus betulinus*, *Fomes pinicola* (= *P. marginatus*) and *P. versicolor*, La Fuze (1937) reported that among various sources of energy used, polysaccharides gave the best mycelial growth. Pentoses were not utilized as

extensively as hexoses.

Studies by Fritz (1923), Mounce (1929) and Hemmi and Kurata (1933) concerning growth, temperature relations, etc. for *Polyporus betulinus* and *P. marginatus* have revealed that mycelial growth occurs between 5° and 35°C; the optimum temperature being 27°-29°C. All the reported calculations of growth, including those of La Fuze, were based on measurement of radial growth on agar media.

Henningsson (1965) observed that the optimum temperature for growth of *Polyporus betulinus* was 20°C. He also reported that on malt-agar the mycelium survived at 35°C. The hydrogen ion concentration for growth was between pH 2.1 and pH 7 with a optimum of pH 5. He found that glucose was the best carbon source among the aldo-hexoses. Later, Henningsson (1967) reported that cellobiose, an extracellular end product in cellulose decomposition, is an excellent carbon source for the fungi attacking birch and aspen. Indeed, cellobiose was equal, and sometimes even superior, to its monomer glucose.

The wood-destroying fungi usually have a wide pH tolerance in the acid regions. In a detailed study, Wolpert (1924) reported that depending on the composition of the medium, the wood destroying fungi grew between pH 2.8 and pH 8. According to Birkinshaw *et al.* (1940) the decay fungi acidify the substrate by their production of acids. Brown rot fungi cause a greater acidification than do the white rot fungi.

Electron microscopic studies of fungal cells have revealed the similarity in their organization to that of the cells of other organisms. Studies of the cells of basidiomycete fungi have established that all these cells have a cell wall and protoplast. The protoplast consists of a plasma-membrane, nucleus or nuclei, mitochondria, endoplasmic reticulum, golgi bodies, ribosomes, vacuoles and certain, less well defined organelles. All these organelles are embedded in the cytoplasmic ground substance, or matrix (Wells, 1964b, 1965; Moore and McAlear, 1962b, 1963b; Girbardt, 1958; Lu, 1965; Willets and Calonge, 1969).

The many papers describing the techniques for preparation of fungal cells for the electron microscopic studies indicate that potassium permangnate (Moore and McAlear, 1963a; Wells, 1964b; Lu, 1965, Nair *et al.*, 1969), and osmium tetroxide (Shatkin and Tatum, 1959; Zalokar, 1961) are most commonly used as fixatives for the fungi. The former is superior for preserving the membrane system but it does not preserve the structures containing the nucleic acids, for example the nucleolus. On the other hand, material fixed with osmium tetroxide has been found to be reasonably well preserved except for the membrane system.

Willets and Calonge (1969) have reported the presence of only a single layered cell wall in *Sclerotinia fructicola*, *S. fructigena*, *S. laxa* and *S. laxa* forma *mali*. They also observed hyphal walls amongst these species with two distinct regions, a thin (0.04μ) electron dense outer layer and an

inner layer approximately 0.22μ thick. Berliner and Duff (1965) reported that the hyphal wall of *Armillaria mellea* was multilayered with the outermost wall layer often sloughed off.

Schmid and Liese (1968) observed a circular stratification of microfibrils in the transverse section of the fiber hyphae, but in the longitudinal section the microfibrils showed an axial orientation. In this study the thickness of the cell wall was found to be variable.

Fungal cells are characterized by the presence of lomasomes, a name first given by Moore and McAlear (1961a) to a "previously uncharacterized hyphal structure". Girbardt (1958, 1961) described such structures as 'rings' located in the matrix between cell wall and plasma membrane.

The origin or the mechanism of formation of lomasome is not very clear. Nair *et al.* (1969) reported the presence of concentric and parallel lamellar structures in the hyphal cell possessing dilated or club shaped ends. They believed that lomasomes originated by budding of concentric lamellae into tubular vesicles. Hyde and Walkinshaw (1966) observed that membranes appeared in several different forms and speculated that lomasomes, vesicular bodies, and concentric complex membranes are different manifestations of a single entity.

The role played by lomasomes in the physiological or morphological processes or in the cell-metabolism is not well understood. According to Wilsenach and Kessel (1965), the

lomasomes play a major role in cell wall formation. They observed that these peripheral bodies appear prior to the formation of cellular wall material in the matrix of the two membranes of the endoplasmic reticulum, and suggested that the vesicles of the fungal lomasomes coalesce and form the cell wall.

Girbardt (1958) suggested that vesicles seen in lomasomes may be plasmapores, or that they may be invaginations of the plasma membrane. He found that the 'rings' were larger in older cells than in younger ones and therefore, he concluded that the lomasomes may be related to the aging of the cells. However, Moore and McAlear (1961a) suggested that lomasomes may be involved in cell wall metabolism.

Marchant, Peat and Banbury (1967) reported that two vesicular systems are associated with wall synthesis. They believe that the endoplasmic reticulum produces a system of vesicles, which fuse with plasma membrane and, therefore, is responsible for 'Primary' wall synthesis. Furthermore, they suggested that endoplasmic reticulum also produces multivesicular bodies which finally form the lomasomes. These multivesicular bodies were thought to be associated with 'Secondary' wall synthesis and concerned with chitin deposition in the wall.

It has been established that the nucleus is enveloped by a double membrane in which there are many pores (Moore and McAlear, 1962a; Wells, 1964a; Hyde and Walkinshaw, 1966).

With a careful survey of 50 genera of fungi, Moore and McAlear (1963b) established that fungus mitochondria resemble those of higher organisms. These mitochondria are bounded by a double membrane, the inner one being folded to form numerous cristae. Fungal mitochondria are variable in shape and size and may be globose to ellipsoid to elongate, or even branched (Moore and McAlear, 1963b; Wells, 1965; Marchant, Peat and Banbury, 1967). The fungal cristae may be platelets or tubules and are randomly arranged (Moore and McAlear, 1963b, 1961b; Wells, 1964a, 1965).

Butler and Bracker (1965) found that the morphology and numbers of mitochondria varied in the cells of the senescence portion of the life cycle of *Rhizoctonia solani*.

The endoplasmic reticulum is a conspicuous feature in fungal cells. In thin sections, it may have the appearance of a system of circular or longitudinal cisternae (Wells, 1964a; Berliner and Duff, 1965; Moore and McAlear, 1962a). The endoplasmic reticulum is found to be continuous with the nuclear membrane (Moore and McAlear, 1962a; Wells, 1965), with the plasma membrane (McAlear and Edwards, 1959; Moore, 1963a) and with the mitochondrial membrane (Berliner and Duff, 1965; Moore, 1963b). It may fold and become lamellar (Wells, 1964a) or it may form a concentric complex (Berliner and Duff, 1965; Moore, 1963a). Vesicular endoplasmic reticulum has been reported also in *Coprinus lagopus* (Marchant, Peat and Banbury, 1967).

The appearance of membrane bound, unidentified dense

bodies in *Agaricus campestris* led Manocha (1965) to speculate that these bodies increase in size, become less dense in contents and coalesce to form vacuoles.

In the subclass Homobasidiomycetidae the septa have been observed to be of the complex pore type, referred to as 'Dolipore' by Moore and McAlear (1962c). This is not the case with some of the Heterobasidiomycetidae. In the dolipore septum the cross wall between the two adjacent cells is pierced in the centre by a pore. The edges of the cross wall bear a pronounced thickening (septal swelling). The material constituting this septal swelling is different from the wall material (Berliner and Duff, 1965; Bracker and Butler, 1963). The surface of the cross wall and the septal swelling are covered by an electron dense membrane, called the pore cap which has been termed the "Parenthesome" by Moore and McAlear (1962c).

The membrane of this pore cap has been observed to have pores in it (Wells, 1964a; Moore and McAlear, 1962c; Berliner and Duff, 1965; Giesy and Day, 1965; Wilsenach and Kessel, 1965; Nair *et al.*, 1969). Berliner and Duff (1965) found that the pore cap is multilayered and it is continuous with endoplasmic reticulum. Wilsenach and Kessel (1965) reported that pore cap pores in the Polyporaceae are at regular intervals of 400-800Å. They suggest that these pore caps act as a sieve and that the particles, smaller than the actual pore opening, migrate through the pore cap pores.

Bracker and Butler (1964) studied *Rhizoctonia solani*.

They consider that despite the complex structure, the septum is well adapted for protoplasmic continuity from one cell to another. This continuity is possible because the diameter of the septal pore increases during protoplasmic streaming.

Nair *et al.* (1969) observed organelles resembling mitochondria lying within the septal pore cap pores but they did not observe nuclei within the septal pore. Giesy and Day (1965) reported that during nuclear migration in *Coprinus lagopus*, the septal swelling and the pore caps are dissolved and the septum becomes a simple pore, like that of Ascomycetes. The septal pores are closed by a septal plug.

Fomes igniarius

1. Classification

Fomes igniarius (Fries) Kickx belongs to family Polyporaceae of the class Basidiomycetes and subclass Homobasidiomycetidae. This fungus, originally described by Linnaeus in 1753 as *Boletus igniarius*, has undergone many subsequent changes in nomenclature, e.g., *Polyporus igniarius* (L) Fries (1821), *Polyporus hyperboreus* Berk.(1841), *Polyporus novae-angliae* Berk. and Curt.(1872), *Fomes nigricans* (Fr) Gill (1878), *Phellinus igniarius* (L) Quel. (1886), *Pyropolyporus igniarius* (Fries) Murr. (1903), and *Fomes arctostaphyli* Long (1941). In North America, the name *Fomes igniarius* (Fries) Kickx. (1867) has been adopted (Overholts, 1953), but the designation *Phellinus igniarius* (L) Quel. is

preferred in Europe (Lyr and Heffe, 1959).

Verrall (1937) distinguished, on the basis of host range, three varieties of *F. igniarius*, *F. igniarius* var. *populinus* (Neuman) Campbell, which is most distinct and is differentiated by its small sporophores, slow growth in culture and occurrence on *Populus* species. *F. igniarius* var. *laevigatus* (Fries) Overholts, which occurs on dead birch and is distinguished by its applanate sporocarp and rapid growth in culture. *Fomes igniarius* var. *igniarius* distinguished by Wall (1962) has a large hoof shaped conk, shows fairly rapid growth in culture and occurs on birches, hornbeans, maples and other hardwoods. Verrall (1937) observed that the three varieties differ further in some microscopic characteristics, e.g., pore diameter, length of setae, or diameter of the hyphae of the context.

2. Distribution

F. igniarius occurs on many deciduous plants in temperate regions. In North America, it has been reported extensively on trembling aspen (*Populus tremuloides* Michx.), largetooth aspen (*P. grandidentata* Michx.), iron wood (*Ostrya virginiana* (Mill) K. Koch), yellow birch (*Betula alleghaniensis* Britton), paper birch (*Betula papyrifera* Marsh), red maple (*Acer rubrum* L), and sugar maple (*A. saccharum* Marsh). In addition to some other species of the genera mentioned above, it reportedly occurs on certain species of *Salix, Juglans, Carya, Carpinus, Umbellularia,*

Sassafrass, *Alnus*, *Fagus*, *Castanopsis*, *Quercus*, *Ulmus*, *Fraxinus*, *Sambucus* (Miller *et al.*, 1960), *Vitis* (Chiarrappa, 1959) and *Picea* (Shope, 1931). It is one of the most important agents of destructive heart-rot in aspen (Davidson *et al.*, 1959; Basham, 1960).

3. Basidiocarp structure

The basidiocarp structure has been useful for distinguishing *Fomes igniarius* from other closely related species. The sporocarp of *F. igniarius* is hard, woody, sessile, with grey-brown to dark brown pore surface. Annual growth of the tubes is about 2-5 mm. Young specimens and the growing margin of older specimens, are usually brown, becoming greyish-black or black and glabrous with age. The pileus surface is usually not incrusted but sometimes a slight crust, often furrowed, develops. The context is hard and woody, dark brown, 0.5 - 1 cm or rarely several centimeters thick. Setae occurs in the hymenium and are sometimes rare, sometimes rather abundant. They are sharp pointed and 12-18x4-6μ in size. *F. igniarius* is distinguished from other closely related species by the presence of (i) tubes which become stuffed with white hyphae when they are old and non-functional and (ii) globose to subglobose basidiospores, which are smooth, hyaline, and 5-6.5x4-5μ or 4.5-5.5μ in size (Overholts, 1953; Lowe, 1957).

Corner (1932) described the structure, development and biology of the fruit body of *Fomes levigatus*, a bracket

fungus, and several closely related species of *Fomes*. The fruit bodies were composed of aseptate, unbranched, thick walled, skeletal hyphae and septate, branched and thin walled generative hyphae. A hyphal system which consists of generative and skeletal hyphae or generative and binding hyphae was called dimitic. He further found that the basidiocarp of *Fomes igniarius* possessed a dimitic hyphal system of generative and skeletal hyphae. Cunningham (1954) also reported that the basidiocarp of *F. igniarius* is dimitic.

4. Basiodiospore studies

Although the basidiospores are discharged throughout the growing season (Riley, 1952), maximum spore discharge occurs during periods of rising humidity, shortly after rainfall (de Groot, 1960). A prolonged dry or cold period with a consequent decrease in hymenial development has been supposed to cause cessation of spore discharge (Mikalaikevicius, 1958).

Harrison (1942) reported that spores remained viable as long as 90 days in dry storage at laboratory temperatures. Good and Spanis (1958) noticed that certain spore collections of *F. igniarius* var. *populinus* would germinate even after two or three months, while in others the spores rapidly shrivel and loose their viability. Since many of these short-lived spores were collected when humidity was low, a correlation between longevity and moisture content during

maturation was indicated. They found that on suitable media and at an optimum temperature of 30°C, spore germination begins after 1 or 2 days, and then rapidly increases to a maximum after 3 or 4 days. However, good germination occurred over a range of 20° to 35°C. No germination of spores occurred in water or on water agar but a comparatively low percentage (10-50%) of spores germinated if the agar medium contained glucose or maltose and was buffered at pH 5 with citrate-phosphate. Germination was high (80-100%) on acidic media containing high concentration of malt extract or juices expressed from aspen sapwood. Interestingly enough, extracts of aged sapwood from wounds had greater stimulatory effect than those from fresh sapwood. This increased stimulus could not be explained as a result of nutrient content or pH, and was therefore thought to be due to unknown growth substances essential for rapid spore germination.

5. Pathology and Host resistance

Although this fungus will attack living sapwood after artificial inoculation (Riley, 1952) the natural decay due to *F. igniarius* is generally limited to the heart wood of the trunk and has been associated with stem cankers (Campbell and Davidson, 1941). The decay is yellowish in color with black zone lines and, in advance stages, the wood becomes spongy in texture (Basham, 1958). *F. igniarius* is believed to be an important cause of decay in living

trees but not of wood in storage (Cowling, 1957). Fritz (1954) found that the fungus survived in stored pulpwood but was not very active.

The association of decay pockets with various openings in the tree suggests the port of entry of *F. igniarius* into living hard woods. Most infections have been traced to dead branch stubs (Basham, 1958; Nordin, 1954), or to fire scars, mechanical injuries, frost cracks, insect tunnels and dead terminals (Meinecke, 1929).

The stained wood associated with branch knots, cankers, wounds, and insect tunnels of aspen has been found to have decay resistance and to contain soluble substances toxic to wood-destroying fungi (Hossfeld, Oberg and French, 1957).

The process of decay, following artificial inoculations was measured by Silverborg (1959). He observed that the decay caused by *F. igniarius* var. *populinus* in aspen spread about 21 inches annually, which is much more rapid than the decay caused by certain other heart-rotting species attacking hard woods. Sporophores appeared on the trees 4 or 5 years after inoculation (Hirt, 1949; Silverborg, 1959).

6. Community of fungi found with *Fomes igniarius*

Good and Nelson (1962) isolated 63 different fungi from living poplar trees, with decay typical of that caused by *F. igniarius* var. *populinus*. Most of these species were widely distributed in the tree and showed no consistent association with any stage of decay. Different zones of

decay yielded 19-36 fungi each; the zone of early incipient decay giving the largest number. Bacteria were isolated from every zone. Trees with stain but no decay yielded 24 fungus species, 15 of which were also found in the decayed trees. Many of these fungus species appeared to be precursors of *F. igniarius*. Several pockets of typical decay did not yield *F. igniarius*, suggesting that it had been suppressed by competition.

7. Morphology of the mycelium in wood

In the advanced stage of decay the hyphae of *F. igniarius* are abundant but are not found beyond the heavily discolored invasion zone. Initially the hyphae are stout but later they become very fine (about 1μ) and much branched. They penetrate the walls of the wood tissue freely causing large bore-holes. It is reported that zone lines are formed which consist of dark, thick-walled gnarled hyphae (Cartwright and Findlay, 1958). Clamp connections have not been observed in the mycelium in the wood.

8. Morphology of the mycelium in culture

The growth of the mycelium of *F. igniarius* in culture has been extensively studied (Fritz, 1923; Verrall, 1937; Campbell, 1938; Nobles, 1948, 1958, 1965; and Wall, 1962). Hopp (1936) reported much variation in the morphological and cultural characteristics of *F. igniarius* isolated from the same tree but he failed to cytologically distinguish these

variants. Campbell (1938) observed fruiting pads formed on an agar slant, which were usually fine wooly, warm buff to a ochraceous buff, and the rest of the mat was buckthorn brown to dresden brown, often with alternating light and dark bands. He distinguished *F. igniarius* from various closely related species on the basis of a more distinctly two-zoned nature and the mycelium peeling more readily from the agar leaving an attached whitish film.

Nobles (1948, 1958) reported that there are no clamp connections in the mycelium of *F. igniarius*, and no asexual spores are produced. The color of the mycelial mat is buff to brown. The culture of the growing fungus gives a positive reaction to gallic acid and tannic acid. She reported that the hyphae from the advancing zone were hyaline, frequently branched, with inconspicuous simple septa, whereas aerial mycelium from the older part of the colony was composed of (i) thick walled, brown-colored, aseptate, very rarely branched fiber hyphae, (ii) broad hyphae with slightly thickened cell walls, brown in color and (iii) cuticular cells arising as irregular swellings on hyphae. She distinguished *F. igniarius* var. *populinus* from all other species on the basis of a slow-growing thick, wooly mat over agar that remained unchanged in color or became dark only below isolated crustose areas. The wintergreen odor and the occurrence on *Populus* species were also distinctive features for identification.

In Verrall's cultural studies (1937) the variation in

the color and the texture of the mycelial mat of the three varieties was not clearly described. With few exceptions, variety *populinus* produced the methyl salicylate or wintergreen odor. Some cultures of *F. igniarius* var. *igniarius* also produced the same odor. Verrall reported that the variety *laevigatus* did not produce the methyl salicylate, although Nobles (1948) reported that variety *laevigatus* produced a strong wintergreen odor.

9. Physiological studies

Mycelial growth of *F. igniarius* has been studied both in liquid- and in agar media. Nobles (1948) reported that when grown in malt agar media at room temperature *F. igniarius* var. *laevigatus* was a comparatively fast-growing fungus and the plates were covered in two to three weeks while *F. igniarius* var. *populinus* grew 4.2 - 8.5 cm in six weeks. Verrall (1937) and Oshima (1953) reported an optimum temperature of about 27°C for the growth of all varieties. Noecker (1938) found that thiamine augmented the growth of *F. igniarius* on agar media. However, he did not demonstrate a complete thiamin deficiency, possibly because of the presence of this vitamin in agar (Robbins and Ma, 1941). Robbins and Hervey (1948) obtained poor growth of several hymenomycetes, including *F. igniarius*, on media containing all essential nutrients and vitamins plus amino acids and nitrogen bases. They could increase growth considerably by the addition of wood extract, tomato juice or malt. They

postulated the presence in the wood-extract of a water soluble, thermostable, charcoal-adsorbed, growth-promoting substance which was required for the rapid development of 'slow growing' wood-inhabiting fungi (Robbins and Hervey, 1955).

Most of the work on *F. igniarius* has been pathological and physiological in approach. Relatively little attention has been paid to the structure of the hyphae or effect of environmental factors on growth and development of the mycelium.

METHODS AND MATERIALS

The materials used in these experiments were obtained from the following sources.

Elk Island National Park - located 25 miles east of Edmonton. The main trees in this locality are poplar, birch, pine and spruce. *Fomes igniarius* var. *populinus* (Neuman) Campbell was quite common on the living aspen poplar, but *Fomes igniarius* var. *laevigatus* (Fries) Overholts was found only sporadically on the dead birch stumps. The sporocarps of both varieties were collected.

Botanical Garden, The University of Alberta - situated about 25 miles west of Edmonton. The naturally infected aspen poplar wood tissue was obtained from this locality. In addition, sound wood blocks from healthy poplar and birch trees, 10 years old, were obtained for inoculation.

Prince Albert, Saskatchewan - this area is 382 miles east of Edmonton city. The naturally infected wood of birch was obtained from this locality.

Cultures of *Fomes igniarius* were derived from the sporocarp context of one collection and were grown on a 2% malt-agar medium at 27°C. The identification of the two varieties of this fungus was made on the basis of host, sporocarp and cultural characteristics using the descriptions of Overholts (1953) and Nobles (1948). The stock cultures were stored at 5°C on the same medium in test tubes and were frequently transferred at about 25-35 day intervals.

A. Study of Wood Sections

Fomes igniarius variety *populinus* and variety *laevigatus* were grown on 2% malt-agar in 100x80 culture jars. For each variety ten cultures were prepared and incubated in the dark at 27°C to obtain maximum growth. Twelve days after inoculation, 1 inch^3 blocks of steam sterilized heartwood from living and sound poplar and birch trees were transferred to the jars. Aspen poplar blocks were placed in the jars inoculated with variety *populinus* and birch wood blocks in the jars inoculated with variety *laevigatus*. The blocks were put vertically in the centre of the culture jars on the mycelial mats and incubated at 27°C in the dark. Controls were established for both varieties by incubating sample blocks on malt-agar.

Two months after incubation the wood blocks were removed and the mycelial mats cleaned off with distilled water. Each block was cut in half with an electric saw and heated to just below the boiling point for 15-20 minutes in a 3:3:1 mixture of 95% alcohol, distilled water and glycerine (Boyce, 1918) so as to soften the wood.

Sections, 15-20μ in thickness, were cut with the sliding microtome. The staining procedure used by Cartwright (1929) was followed with some modifications. The sections were stained in 1% aqueous solution of safranine for 1½-2 minutes and thoroughly rinsed with distilled water. Then the sections, while still moist, were dipped in picro-

aniline blue prepared by mixing 25 ml of saturated aqueous aniline blue and 100 ml of saturated picric acid. The stained sections were heated to the point of simmering on a hot plate. They were again thoroughly washed with distilled water, dehydrated in an ascending series of 30% to 90% alcohol and finally twice in absolute alcohol. The timing, which varied from 1½-2 minutes, in the alcohol series, depended upon the thickness of the section and the intensity of the staining. Next, the sections were placed for 2-3 minutes in the clearing agent consisting of a mixture of 25 ml of xylene, 25 ml of alcohol and 50 ml of clove oil. This step was followed by washing in 1:1 alcohol and xylene for 1½-2 minutes, and finally in absolute xylene. The mounting media used was permount. Sections of naturally infected wood were prepared in the same manner.

B. Cultural Studies

1. Solid Media

a. Malt-agar media

The malt-agar medium, used by most of the workers for the study of this fungus [Verrall (1937), Nobles (1948), Wall (1962)] was used with slight variation in the amount of agar and malt extract. The medium contained: agar 20 g, malt extract 20 g, dextrose 20 g, peptone 1 g, and 1 liter distilled water. The medium was autoclaved at 15 lb. pressure for 20 minutes. The materials used for the medium were Difco 'Certified' products.

The effect of temperature on growth and mycelial development on this medium was studied. The inoculum of the fungus was grown on 2% malt-agar medium for 12 days in the dark at 27°C and was taken for inoculation from the growth at the periphery of the petriplates with a sterilized cork borer. Sixty, 9 cm petriplates were used in this experiment. Twenty-five ml of sterilized medium were poured into the sterilized petriplates. These plates were inoculated in the centre with 4 mm discs of the inoculum. After inoculation, the plates were incubated in the dark at 15°, 21°, 24°, 27°, 34°C and at room temperature (20-23°C). Five petriplates were used for each of the temperature x variety treatments. The colony diameter was radially measured every 5th day for 40 days starting 5 days after inoculation. Unless otherwise specified, the growing of the inoculum, and recording of the observations, was done in the manner described above. Five grams of gallic (or tannic) acid were added to malt-agar medium to test for the production of extracellular oxidase.

b. Wood Media

The wood medium was malt-agar medium with sterilized chips of Aspen poplar added to it. The medium was poured into the sterilized petriplates and a small autoclaved wood chip was added. This was done to simulate the natural substratum so that the variety *populinus*, which is a slow-growing fungus, might grow faster. This procedure was not necessary in case of fast-growing variety *laevigatus*.

2. Liquid Media

a. Chemically defined medium

A chemically defined medium was used to study the effect of various carbon sources on growth of the two varieties of *Fomes igniarius*. This was a modified Linderberg's medium (Sedlmayr *et al.*, 1961) and contained:

Major nutrients -	Dextrose	- 8.0 g
	NH_4-tartrate	- 5.0 g
	KH_2PO_4	- 1.0 g
	$MgSO_4 \cdot 7H_2O$	- 0.5 g
Minor nutrients -	$FeCl_3$	- 1 mg
	$ZnSO_4$	- 1 mg
	$MnCl_2$ (0.1M)	- 0.5 ml
	$CaCl_2$ (0.1M)	- 5.0 ml
Vitamins -	Thiamine-HCl	- 100 µg
	Biotin	- 5 µg
	Distilled water to 1 liter.	

The minor nutrients and vitamins were made up in stock solution and stored at 5°C. In addition to dextrose, three other carbon sources, maltose, carboxyl methyl cellulose and cellobiose were used. Each medium was adjusted to pH 5.0 with 1N KOH (Beckman Zeromatic SS-3 pH meter).

The inoculum for both varieties was grown on 2% malt-agar as described earlier (Page 25). The Erlenmeyer flasks containing 25 ml of sterilized synthetic medium were inoculated with 4 mm discs of inoculum. Care was taken to ensure

that the inoculum floated on the medium. Sterile, disposable foam plugs were used for closure, and these were covered with aluminium foil to reduce evaporation. Within one hour after inoculation the flasks were transferred to the dark growth chamber and maintained at 27°C. Twenty-six flasks were used for each of the variety x c-source treatment and for each observation three flasks were chosen at random. The experiment was repeated once.

The growth of the fungi was measured as mg dry weight of mycelium/flask. The dry weight of the inoculum was obtained by using preweighed filter papers No. 615 Eaton-Dickmon tared for separation of the mycelium. Each mycelial mat was washed with 100 ml of distilled water to remove the residue of the malt-agar media. It was then air dried and finally kept in an oven at 95-100°C for 2 hr. The dried filter papers with the mycelial mat were cooled in a desiccator and later weighed. This was repeated once. Likewise, the dry weight of the mycelium from various carbon sources was similarly measured, except that each mycelial mat was washed with 300 ml of distilled water to remove any residue of the media, and finally oven dried for 24 hr. The results reported for each harvest are the average of 6 replicate flasks.

b. Malt extract medium

The malt extract medium had the same composition as the malt-agar medium except that agar was omitted. This liquid medium was used to study the effect of temperature on growth, and the effect of shake and still culture on growth in the

two varieties of *F. igniarius*.

i) Effect of temperature on growth

A growth study was conducted at 15°, 21°, 24°, 27°, 34°C and at room temperature (20-23°C). The growth was measured as the dry weight of the mycelium/flask. Twenty-five ml of malt extract were poured into 125 ml Erlenmeyer flasks, and autoclaved for 20 minutes at 15 lb. pressure. The inocula were prepared in the manner described on page 25 . After inoculation, the flasks were transferred to growth chambers, which were dark and maintained at the desired temperatures. The observations were made as described earlier (Page 27).

ii) Effect of shake and still culture on growth

To determine the amount of mycelium produced by the two varieties of *Fomes igniarius* in shake culture, the method of Ward and Colotelo (1960) was followed. The varieties were grown in 250 ml wide mouth Erlenmeyer flasks. Each flask contained 50 ml of liquid malt extract medium and was inoculated with 1 ml aliquot of homogenized mycelium. The dry weight of 1 ml aliquot was approximately 2.0 mg. Fifteen-day-old still cultures growing on the malt extract liquid medium at room temperature were used as the source of inoculum. The culture medium was decanted and the mycelium, while still in the flasks, was thoroughly washed 3 times with 100 ml of sterilized distilled water. The flasks were then closed with sterile foam plugs. The washed mycelial mats were transferred to an alcohol-sterilized homogenizer and then blended at maximum speed for 90 seconds with 30 ml of steril-

ized distilled water. The homogenates were transferred into sterilized flasks and stored at 0°C. One ml sample of the homogeneous mixture was pipetted on tared and previously weighed filter papers, washed with distilled water, air dried, and transferred to an oven at 95-100°C for 2 hr. They were then cooled in a desiccator and weighed. The average dry weight of the mycelium/ml aliquot was determined. On this basis a calculated amount of sterilized distilled water was added to the homogeneous mixture stored at 0°C such that the dry weight of the mycelium was equivalent to 2.0 mg/ml. The flasks were then inoculated with 1 ml of aliquot. All these steps were conducted aseptically. Cultures were incubated at room temperature (20-23°C) on a Hoover Shaker having 260 oscillations per minute. The cultures were harvested every five days, starting ten days after inoculation, for a period of 40 days. To separate the mycelium, the culture medium was centrifuged at 32,000 x g for 4 minutes. The mycelial balls were washed three times by suspending them in cold deionized water. In each cycle the water was removed after centrifugation. The mycelial balls were transferred on previously dried and weighed filter papers. These were dried again at 95-100°C for 24 hours and weighed.

For still culture 50 ml of malt extract medium was poured into 250 ml wide mouth Erlenmeyer flasks, autoclaved, and then inoculated with 4 mm discs. The inoculum was grown at room temperature and the experiment was carried out at room temperature. The initial observations were made 10 days

after inoculation.

C. Microscopic Studies

1. Light Microscopy

For light microscope studies the fungi were grown on a 2% malt-agar medium at 10°, 15°, 21°, and 27°C and observed at 3-day intervals, starting five days after inoculation, up to 26 days. Aerial mycelium from the advancing zone, the centre of the colony and near the inoculum point was examined. The submerged mycelium was also studied. To observe the detail of the hyphae the mycelium was stained in 1% aqueous solution of phloxine-B and 3% potassium hydroxide. This experiment was repeated once and was conducted with both varieties. In addition, the mycelia growing in shake and still cultures, were observed under the light microscope, but not at definite intervals.

2. Electron Microscopy

The two varieties of *Fomes igniarius* were grown in malt extract liquid medium and 1% and 2% malt-agar medium at 27°C for ultrastructural studies. The mycelium for fixation was taken from the advancing zone and from the centre of the colony. Submerged mycelium was also fixed so that all the different types of hyphae would be present.

Two methods of fixation were used. The first method involved a double fixation by glutaraldehyde and osmium tetroxide. Glutaraldehyde was originally employed as a

fixative for electron microscopic studies by Sabatini, Bensch, and Barrnett (1962) and was found to be superior to several other aldehydes. In this study the fixation was carried out for 3 hours in 1:1, 3% glutaraldehyde suspended in 0.15 M Sorenson's phosphate buffer, pH 7.2 and formaldehyde. To prevent a reduction of osmium tetroxide by the glutaraldehyde, the mycelium was rinsed in several changes of buffer. It was then post fixed in 2% solution of osmium tetroxide in phosphate buffer (0.15M) for 2 hours at room temperature. The second method for fixation involved the use of potassium permanganate. Potassium permanganate was first used as a fixative by Luft (1956). Later, Mollenhauer (1959) observed that well preserved cell structure, particularly the membrane system, was obtained by using 2--5% unbuffered solution of potassium permanganate at room temperature. In this study, the mycelium was fixed in a freshly prepared 3% aqueous solution of unbuffered potassium permanganate for 1½ - 1¾ hours at room temperature.

The materials were dehydrated according to the following schedule:

30% ETOH - 15 minutes

50% ETOH - 15 minutes

70% ETOH - 15 minutes

85% ETOH - 15 minutes

95% ETOH - 15 minutes

Absolute ETOH - 2 x 15 minutes

Araldite, as described by Malhotra and Eakin (1967), was

used as the embedding medium. Following dehydration, the tissues were transferred to propylene oxide (two changes for 15 minutes each). A 1:1 mixture of fresh propylene oxide and araldite was poured over the material. The araldite material consisted of: 23 ml DDSA (Dodecyl succinic anhydride), 27 ml araldite 502 and 1 ml DMP30 (Dimethyl aminomethyl phenol).

The mycelium was allowed to remain for 16 hours in a dust free area in covered vials. The araldite-propylene oxide mixture remaining in the vials at the end of this period was carefully drained off and a fresh araldite mixture added. The mycelium was kept in this mixture for one hour. It was then transferred into gelatine capsules filled with a fresh mixture of araldite. The capsules were placed in an oven at 35°C for one hour to allow air bubbles to rise to the surface. At this stage, tissue orientation was also carried out wherever necessary. The capsules were then placed in an oven for 48 hours at 60°C.

After polymerization and curing, longitudinal, tangential and cross sections were cut with a glass knife at 600-1000Å on a Porter-Blum microtome. The sections were collected in a 10 percent solution of acetone in distilled water, expanded by chloroform vapors and picked up on formvar coated 200-mesh grids.

In order to intensify the contrast, sections were floated on droplets of stain placed on dental wax sheets with the section side down. The specimens were stained in a 2% solution of uranyl acetate pH 4.8 (Huxley and Zubay, 1961) for

two hours, followed by lead citrate for 3 to 5 minutes.

The $KMnO_4$ fixed sections were similarly dehydrated, embedded and stained.

A Philips EM 100B electron microscope with an objective aperture of 25μ was used. Electron micrographs were taken on 35 mm Kodak film P426 at an initial magnification of 4500-12,000X. These negatives were further enlarged photographically as desired.

OBSERVATIONS

A. The mycelium in the natural environment

Fomes igniarius var. *populinus*

The mycelium of *Fomes igniarius* var. *populinus*, whether observed in the naturally infected or inoculated aspen wood, has essentially the same features. The mycelium was concentrated mainly in the heartwood but will grow in the sapwood, at least in inoculated wood blocks. The dark brown zone lines similar to those formed in other white rot fungi were observed in aspen wood blocks. In naturally infected wood regular dark colored zone lines were observed between the sapwood and the heartwood. However, in the inoculated heartwood blocks circular and scattered zone lines developed. These zone lines consist mostly of cuticular cells* in compact form. These cells are irregular in shape and size, brown to dark brown in color, thick-walled, branched and irregularly septate. The cuticular cells are observed in all kinds of wood tissue - such as xylem rays, vessels and wood fibers (Figure 2, page 56).

Fiber hyphae, which are thick-walled, brown in color and occasionally branched, were sometimes observed in zone lines (Figure 5, page 57). Fiber hyphae are ocassionally seen scattered in vessels and wood fibers. The diameter of

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* Colony and hyphal terminology is that of Nobles (1958).

the fiber hyphae varies from 1.5 to 3.5 μ, with wall thickness of 0.65 to 1.3 μ.

Thin-walled hyphae observed in the naturally infected wood tissue were freely branched and ranged in diameter from 0.75 to 3.0 μ. The hyphae were present in all kinds of cells. They usually grow from one cell to another through bordered pits. However, hyphae penetrating only the border part of a pit were seen in some sections (Figure 4, page 57). General dissolution of bordered pits and the cell wall, presumably by the enzymes produced by the fungi, was observed (Figure 4, page 57). The hyphae were commonly seen in the bore-holes which they had made (Figure 7, page 58). The bore-holes were usually large enough for many hyphae to be present and still leave free space. The widening of the holes is probably due to the action of enzymes produced by the fungi acting on the walls of the wood cells. In the early stage of wall penetration the hyphae are very thin, but after penetration is complete they expanded to the usual size. Hyphae passing through ray cells and plugging the spring and summer wood were readily observed in transverse sections (Figure 6, page 58). However, the branching of the hyphae and their path through vessels and wood fibers are brought out more clearly in the longitudinal sections especially in radial sections.

In the inoculated wood of aspen poplar the hyphal characteristics and their growth, branching, etc., were essentially similar to that observed in naturally infected

wood tissue. However, the thin-walled hyphae were comparatively larger in size and their diameter ranged from 0.75 to 3.70 μ. They were not so profusely formed as in naturally infected wood tissue. The fiber hyphae were very rarely observed in inoculated wood.

Fomes igniarius var. *laevigatus*

The mycelium of *F. igniarius* var. *laevigatus* was observed in naturally infected and inoculated birch heartwood. The cuticular cells were found in abundance forming zig-zag zone lines (Figure 8, page 59) and, as was the case in variety *populinus*, were present in all kinds of cells. The cuticular cells found both in the naturally infected and inoculated wood tissue were larger in size than those found on malt-agar medium. These cells were oval to irregular in shape (Figure 9, page 59), brown to dark brown in color, irregularly septate and thick-walled. These cells develop in a very compact formation producing a characteristic dark area in the wood section. An individual cell appeared to be devoid of cell contents.

The thin-walled narrow hyphae, characteristic of this variety, were abundant in the wood. They were hyaline, very frequently branched, septate and did not possess clamp connections (Figure 3, page 56). The branches arose at acute angles in most cases but branching at right angles or even at obtuse angles was not uncommon. Narrow hyphae range in diameter from 0.75 to 2.6 μ. These hyphae were observed

passing through bordered pits, through the cell wall (Figure 3, page 56) and in bore-holes (Figure 10, page 60). Furthermore, these hyphae were observed in all kinds of cells (Figure 3, page 56).

Broad hyphae, which range in diameter from 2.5 to 4.5 μ, were occasionally seen in some sections. They were thin-walled, septate and branched. Fiber hyphae were observed very rarely. They range in diameter from 1.5 to 3.0 μ and are thick-walled with a narrow lumen (Figure 11, page 60).

Inoculated birch wood blocks had the same kinds of hyphae with similar features to those already described for naturally infected wood. In inoculated birch wood blocks, however, the diameter of the narrow hyphae ranged from 0.75 to 3.0 μ. They were comparatively fewer in number than in the naturally infected wood.

B. The mycelium in the cultural environment

Fomes igniarius var. *populinus*

1. Growth on solid media

a. Malt-agar medium

(1) Growth at 27°C.

The mycelium grows very slowly and the radius of the colony in six weeks was 8.1 - 8.8 cm. At first the advancing zone was even, but after three to four weeks it became bayed (Figure 13, page 61). In the dark, the hyphae were initially cream-colored, but later they became yellow, then buff, followed by light brown, clay color to tawny olive and finally

dark brown in color. The mycelium was raised, thick and wooly, frequently growing to the top of petriplates near the inoculum and sloping gradually towards the edge. The reverse (color changes in agar induced by growth of the mycelium) which was unchanged in color, showed conspicuous zones and radiating lines. The mat peeled from malt-agar easily leaving a white bloom. The mycelium gave a strong wintergreen odor when grown on malt-agar medium. On gallic and tannic acid agars the diffusion zones were weak to moderately strong and no growth was observed on either medium.

The hyphae of the advancing zone (marginal hyphae) were hyaline, thin-walled and full of protoplasm. They were frequently branched and possessed simple septa. Invaginations and constrictions in the thin-walled hyphae were occasionally noticed. Thin-walled hyphae ranged from 1.5 to 4.5 μ in diameter. The cell wall thickness varied from 0.2 - 0.5 μ. Sometimes pointed, hook-shaped hyaline structures were observed at the tips of the thin-walled hyphae. Branching of the thin-walled hyphae was in acropetal succession; the form of branching being monopodial. Branches usually developed singly, but occasionally branches were observed in pairs and whorls. The main, secondary, tertiary and other branches all continue to grow indefinitely (Figure 15, page 62). The marginal branches tended to grow away from other branches. Most of the branches originated at an acute angle from the main branch, although branches arising at right angles and obtuse angles were observed occasionally. In most

cases, the new branch was given out immediately behind the septum, but occasionally branches arose in other regions of the parent hypha. Furthermore, there were many septa without associated branches. Distance between the septa was variable. The septa near the hyphal base were located at distances of 8 μ to 45 μ, while those at the distal end were 50 μ to 75 μ or more apart.

Aerial mycelium (mycelium exclusive of the advancing zone) consisted of thin-walled hyphae, fiber hyphae and cuticular cells (Figure 14, page 62). The thin-walled hyphae, had the same features as those of the advancing zone. Hyphal anastomosis was commonly observed in the thin-walled hyphae. The branches in the centre were interwoven.

Fiber hyphae constituted the major portion of the aerial mycelium in older cultures. These hyphae originated from thin-walled hyphae and at the point of origin there was a simple septum (Figure 16, page 63). Fiber hyphae were brown to dark brown in color with a very thick cell wall and a narrow lumen. The young fiber hyphae had cytoplasm and relatively thin walls, while the older fiber hyphae lacked cell contents and possessed very thick walls. Fiber hyphae were not generally branched in variety *populinus*, but when the branches were present, they arose at acute to right angles. These hyphae varied in diameter from 1.5 to 4.5 μ and the cell wall varied in thickness from 0.5 to 1.5 μ. The tips of the fiber hyphae were comparatively wider (1.7 to 4.8 μ) than the rest of the hyphae. Pointed hook-shaped

hyaline structures were sometimes observed at the tips of the fiber hyphae similar to those seen in thin-walled hyphae. Expansion of the fiber hyphae were repeatedly observed in culture and the expanded portions were comparatively darker in color than the rest of the hyphae. After expansion, the hyphae usually continued their growth resuming normal shape but sometimes, they terminated with the expanded portion which was either circular or oblong. The length and width of the expanded part of the fiber hyphae ranged from 9.0 - 30.0 μ x 3.5 - 15.75 μ. In a single fiber hypha the cell wall thickness varied from 0.5 to 1.7μ and was uneven in appearance. Sometimes the walls were so thick that there was no lumen present. The fiber hyphae were rarely septate.

Cuticular cells originated from thin-walled hyphae as irregular swellings and were initially thin-walled, hyaline, branched and very frequently septate (Figure 17, page 63). At later stages of development they became thick-walled and brown to dark brown in color. They ranged in diameter from 1.5 to 7.5 μ. The older cells were devoid of protoplasm, but the young cells had protoplasm. Cuticular cells did not have any particular shape and varied from circular to oblong in outline. Usually these cells were scattered in the mycelium, but sometimes they grew in compact manner and formed a pseudoparenchyma.

The submerged mycelium was composed of the same kinds of cells as the aerial mycelium. However, cuticular cells and thin-walled hyphae were more common than fiber hyphae.

(2) Effect of variation in temperature

i. On growth of the mycelium

The mycelial growth at 15°, 21°, 24°, 27°, 34°C and at room temperature (20-23°C) was studied during a 40-day period by measurement of colony diameter (Figure 18, page 64). The growth was more rapid between the temperatures of 21 to 27°C. The changes in the color of the mycelium, already described for the cultures growing at 27°C, were seen to occur at all the temperatures. These changes in color were more rapid in the fungus growing at higher temperatures (21-34°C).

ii. On hyphal structure

The structure and development of mycelium was observed at 10°, 15°, 21° and 27°C. Seventeen days after inoculation very little growth of the thin-walled hyphae was observed at 10°C. After another three days cuticular cells were seen. Fiber hyphae were not observed at this temperature. Thin-walled hyphae and cuticular cells had the same characteristic features as already described for growth at 27°C. The septa in a thin-walled hypha occurred at intervals of 3.5 μ to 40 μ, but usually were at a distance of 12.5 to 21.0 μ. At 15°C, there was discernible growth of mycelium of thin-walled hyphae five days after inoculation, but cuticular cells and fiber hyphae were not observed before 17 days and 26 days after inoculation respectively. At 10° and 15°C, in cultures, 20 days old, over half the aerial and submerged hyphae were cuticular cells. At 21°C, measurable growth of thin-walled hyphae was observed 5 days after inoculation. The cuticular

cells and fiber hyphae were observed after 11 days of growth. In the aerial mycelium thin-walled hyphae were numerous in the early stages of growth, but after 23 days more fiber hyphae were observed. The branching, septation, hyphal anastomosis, hyphal constriction, etc., at the various temperatures were similar to that observed in growth at 27°C. Hyphal expansion in fiber hyphae was observed 23 days after inoculation only at 21° and 27°C. Expanded fiber hyphae had similar features to those described on page 40.

b. Growth on wood medium

The addition of slices of aspen heartwood to malt-agar plates resulted in more rapid mycelial growth of *F. igniarius* var. *populinus* than was obtained on malt-agar (Figure 19, page 65). In all the plates the fungus was growing over the wood slices and the mycelial growth was raised and more luxuriant than on malt-agar alone. However, the mycelial structure was similar to that described for growth on malt-agar at 27°C.

2. Growth in liquid media

(1) Malt extract medium

i. Effect of shake and still culture on growth

The growth of the mycelium in the shake and still cultures was recorded as dry weight in mg per flask as shown in Figure 20, page 66. In still culture the mycelial growth increased up to 30 days after inoculation and then showed a

decline in the dry weight. Likewise in shake culture, the mycelium increased during the first 35 days after inoculation and then showed a gradual decrease in weight. When the growth under the two methods (shake and still culture) of culture was compared it was found that there was significantly less growth in static culture. The difference in the dry weight of the mycelium grown under the two methods of culture increased with increase in time, reaching a maximum at 35 days after inoculation. There was a slight decrease in this difference in the next five days. This decrease in difference was mainly due to a greater decline in dry weight in the shake culture as compared to the decline in the static culture during this period. The lag phase in the static culture was prolonged (10 days) and was more marked than in the shake culture.

In shake culture the mycelium aggregated into small balls of generally uniform dimensions. Except for the absence of the expanded fiber hyphae, the mycelium grown in shake culture was similar to that already described under growth at 27°C (malt-agar). A characteristic feature of the mycelium in shake culture was the presence of more hyphae with wavy outlines.

ii. Effect of variation in temperature

The data on mycelial growth at 15°, 21°, 24°, 27° and 34°C, recorded in terms of dry weight (mg) per flask at various intervals of time (up to 40 days) are outlined in Figure 21, page 67. At all temperatures a lag phase in

growth was noticed in the first five-day-period after inoculation. Ten days after inoculation there was a slow increase in growth, followed by the more active growth period, referred to as the 'Exponential phase'. At this stage the effect of the various temperatures was noticeable. At 15° and 21°C there was a steady increase in growth up to 40 days, when the experiment was terminated. Under 24° and 27°C the growth increased up to 35 days, but 40 days after inoculation a decline in the dry weight content was noticed. This decline in dry weight at 34°C started 30 days after inoculation. Although 35 days after inoculation there was more growth at 27°C than at 24°C, yet 5 days later the growth at both temperatures was about equal. It appeared that growth during the first 35 days after inoculation and the decline in growth 5 days later are more rapid at 27°C than at 24°C.

No significant pH changes (Table 1, page 46) were recorded during the growth of the mycelium. There was, however, a slight decline in the pH under higher temperatures (24°, 27°, and 34°C) at later stages of growth (after 30 days of inoculation).

(2) Chemically defined media

In the chemically defined media carbon was supplied to the growing mycelium from cellobiose, carboxyl methyl cellulose, dextrose and maltose. The growing mycelium developed into a colony which had cream to light brown (1-2 weeks) to dark brown color (3-5 weeks). The mycelial growth was recorded in terms of mg dry weight per flask and is

reported in Figure 22, page 68. Maximum growth was obtained after 25 and 30 days with dextrose and cellobiose respectively. Using maltose or carboxyl methyl cellulose as the carbon source the mycelium continued to increase in weight up to 40 days at which time the experiment was terminated. When the four carbon sources are compared it can be seen that the greatest amount of growth was obtained with cellobiose 30 days after inoculation, followed by dextrose after 25 days and maltose and carboxyl methyl cellulose after 40 days. The growth with carboxyl methyl cellulose as the carbon source was significantly restricted. In all cultures the lag phase was less than 5 days. The mycelial characters were similar to that already described at 27°C on malt-agar medium.

TABLE 1

pH of the Malt Extract Medium at Various Stages of Growth of Variety *Populinus*

Days after inoculation	Temperature				
	15°C	21°C	24°C	27°C	34°C
0	5.1	5.1	5.1	5.1	5.1
5	5.2	5.1	5.2	5.1	5.2
10	5.2	5.2	5.3	5.2	5.3
15	5.2	5.3	5.2	5.1	5.2
20	5.3	5.3	5.1	5.1	5.2
25	5.3	5.2	5.0	5.0	5.0
30	5.2	5.2	4.9	5.0	4.8
35	5.2	5.3	4.9	4.9	4.8
40	5.2	5.2	4.9	4.8	4.7

Fomes igniarius var. *laevigatus*

1. Growth on solid media

a. Malt-agar medium

(1) Growth at 27°C

The growth of the mycelium was quite rapid and it completely covered the 9 cm - petriplate in 13-14 days. The advancing zone was even, hyaline, with the hyphae well separated from each other. The mycelial mat changed through cream color to yellow, cinnamon buff, clay or orange cinnamon color but the white border persisted. The mycelium was slightly raised, cottony in the inital growth then wooly to felty at later stages. Sometimes there were zones of aerial mycelium under which a white bloom formed over the medium. The mat peeled from the agar quite readily. The wintergreen odor was mild. Sometimes mycelial growth was interrupted by zones in which the aerial mycelium consisted of a thin white bloom over dark crustose areas. After a week the color of the reverse changed from tawny to cinnamon-brown and then to patches of dark brown (2-3 weeks). Finally, the whole medium was liver brown (4-6 weeks) in color. The diffusion zones were observed to be moderately strong on gallic and tannic acid agars. The mycelium grew on gallic acid agar with a colony diameter of 1.2-2.8 cm, but on tannic acid agar no growth was observed.

The hyphae of the advancing zone (marginal hyphae) were hyaline, thin-walled, narrow and possessed simple septa. They were much elongated and frequently branched. Narrow

hyphae ranged in diameter from 1.2 μ to 4.0 μ with a cell wall thickness of 0.2 - 0.4 μ. In the narrow hyphae monopodial type of branching was observed, the branches being produced in acropetal succession. Usually the branches developed singly, although the occurrence of the branches in pairs and whorls was also noticed. The marginal branches tended to grow away from the other branches. Usually the branches originated at acute angles from the main branch. The new branches usually formed immediately behind the septa, but sometimes, branches were seen to originate from other regions of the parent hyphae. There were many septa without the associated branches. The septa were at variable distances; those near the hyphal base were 12-45 μ apart whereas the septa at the distal end were 50-80 μ or more apart. Occasionally, the growing narrow hyphae formed flat coils or hyphal rings (Figure 23, page 69). These hyphae did not possess hyphal constriction or invagination of hyphal walls.

Aerial mycelium (mycelium exclusive of the advancing zone) consisted of thin-walled hyphae, fiber hyphae and cuticular cells. The thin-walled hyphae were of two types, namely, thin-walled, narrow hyphae and thin-walled, broad hyphae (Figure 24, page 69). The thin-walled, narrow hyphae had the same characteristic features as those found in the hyphae of the advancing zone. Hyphal anastomosis was observed in the narrow hyphae (Figure 25, page 70) and the hyphal branches near the centre of the colony were interwoven.

In early stages of growth the thin-walled, broad hyphae were hyaline, branched and possessed simple septa. These hyphae originated from thin-walled, narrow hyphae. The diameter of the broad hyphae ranged from 3.5 to 7.6 μ and the thickness of the cell wall ranged from 0.3 μ to 0.6 μ. At later stages of development the thin-walled, broad hyphae became slightly yellow to light brown in color.

Fiber hyphae originated from thin-walled, narrow hyphae with a septum at the point of origin (Figure 26, page 70). These hyphae are brown to dark brown in color, elongated and thick-walled with a narrow lumen. The fiber hyphae were occasionally branched and septate (Figure 27, page 71). They ranged in diameter from 1.5 to 4.0 μ with a wall thickness of 0.5 to 1.6 μ. The tips of the fiber hyphae were comparatively wider (1.7 to 4.3 μ) than the rest of the fiber hyphae (Figure 27, page 71). Expanded fiber hyphae were also observed in this variety.

Cuticular cells arose as irregular swellings from narrow, thin-walled hyphae (Figure 28, page 71). These cells were hyaline at first but became brown to dark brown at later stages of development. The cuticular cells were loosely arranged in groups or closely packed together to form a pseudoparenchyma. The shape of these cells was variable and they were irregularly branched and septate.

Submerged mycelium consisted of thin-walled, narrow and broad hyphae, fiber hyphae and cuticular cells. Cuticular cells were more plentiful near the inoculum point. The

narrow hyphae were frequently elongated and twisted.

(2) Effect of variation in temperature

i. On growth of the mycelium

The growth of the mycelium at 15°, 21°, 24°, 27°, 34°C and at room temperature (20-23°C) was studied for 40 days and the colony diameter was recorded (Figure 29, page 72). More growth was observed between temperatures of 21° to 27°C. The changes in color of the mycelium, as already described for the cultures growing at 27°C, were seen to occur at all temperatures. The change in color was more rapid at higher temperatures (21°-34°C).

ii. On hyphal structure

The structure and development of mycelium at various temperatures was observed in variety *laevigatus*. At 10°C the growth of thin-walled, narrow hyphae was discernible 5 days after inoculation. Broad hyphae and cuticular cells were observed 11 days after growth had begun. Fiber hyphae were not seen before the mycelium was 23 days old. The septa were located at a distance of 8.5 to 50 μ. A few thin-walled, narrow hyphae developed wrinkled, inflated terminal cells which measured 4.0 - 6.5 μ in diameter. After 20 days of growth there were more cuticular cells than narrow hyphae. At 15°C the growth of the mycelium was more extensive than at 10°C. Eight days after inoculation fiber hyphae and cuticular cells were seen. The broad hyphae were first observed after 11 days of growth in culture. The distance between the septa ranged from 14 μ to 60 μ in a narrow hypha.

In the broad hypha it ranged from 15 to 66 μ. Hyphal rings were seen after 14 days of growth. At 21°C the mycelial growth was plentiful and within 8 days all the kinds of hyphae were observed. Expanded fiber hyphae were observed after 17 days of growth. Narrow hyphae, broad hyphae, fiber hyphae and cuticular cells had the same characteristic features as described for growth at 27°C.

2. Growth in liquid media

(1) Malt extract medium

i. Effect of shake and still culture

The growth studies of variety *laevigatus* in shake and still culture were made in a manner similar to that for variety *populinus* and are shown in Figure 30, page 73.

In both kinds of culture a 10-day lag period was observed. It was at this stage that the effect of the two methods of the culture was noticed. The quantity of the mycelium was less in static culture than in the shake culture. However, these differences were not significant. The maximum growth under the two methods of culture was obtained 35 days after inoculation. Ball-shaped aggregates of mycelium were observed in shake culture and these balls showed little variation in size and shape. The expanded fiber hyphae were absent. The mycelium was characterized by the presence of numerous hyphae possessing wavy outlines.

ii. Effect of variation in temperature

The results of the growth measured as dry weight of the mycelium (mg) per flask are shown in Figure 31, page 74.

A lag phase of five days occurred under all temperatures. This phase was followed by a period of slow growth for the next five days. At all temperatures the active growth started 10 days after inoculation. At 15°, 21°, 24° and 27°C the growth in terms of dry weight of the mycelium continued to increase up to 40 days at which time the experiment was terminated. At 34°C the growth of the mycelium reached a maximum in 30 days. This peak growth was followed by a steady decline in the dry weight. The relative increase at 15°, 21° and 24°C at later stages of growth (after 35 days) was more than the increase at 27°C. At various temperatures, data obtained 40 days after inoculation indicated that the dry weight steadily increased with increase in temperature up to 27°C. This increase was more marked between 15° and 21°C and 21° and 24°C as compared to the increase between 24° and 27°C. At 34°C the dry weight of the mycelium was definitely less compared with growth at all other temperatures.

The data on pH value (Table 2, page 54) indicated a steady fall in pH at 24°, 27° and 34°C after 25, 25, and 20 days of growth respectively. Under 15° and 21°C the pH remained constant.

(2) Chemically defined media

The carbon sources used were cellobiose, carboxyl methyl cellulose, dextrose and maltose. The dry weight of the mycelium was recorded in terms of mg per flask and is reported in Figure 32, page 75. On all sources the mycelium formed a colony and the color of the mycelium varied from

cream to yellow, cinnamon buff, clay or orange-cinnamon. With each of the four carbon sources used the maximum dry weight was obtained 30 days after inoculation. The data recorded at the two subsequent 5-day intervals showed a decline in the quantity of dry weight when dextrose, maltose or cellobiose carbon sources were used. This decline was not noticed when carboxyl methyl cellulose was used; in this case the weight was constant up to the time the experiment was terminated. However, it was noticed that carboxyl methyl cellulose gave the minimum increase in dry weight over the growth period of 40 days as compared to the increase in weight with the other carbon sources. Maximum growth was obtained with cellobiose as the carbon source followed by maltose and dextrose. The lag phase was less than 5 days in all cases. All the different types of hyphae as described on malt-agar medium at 27°C were also observed in this medium.

TABLE 2

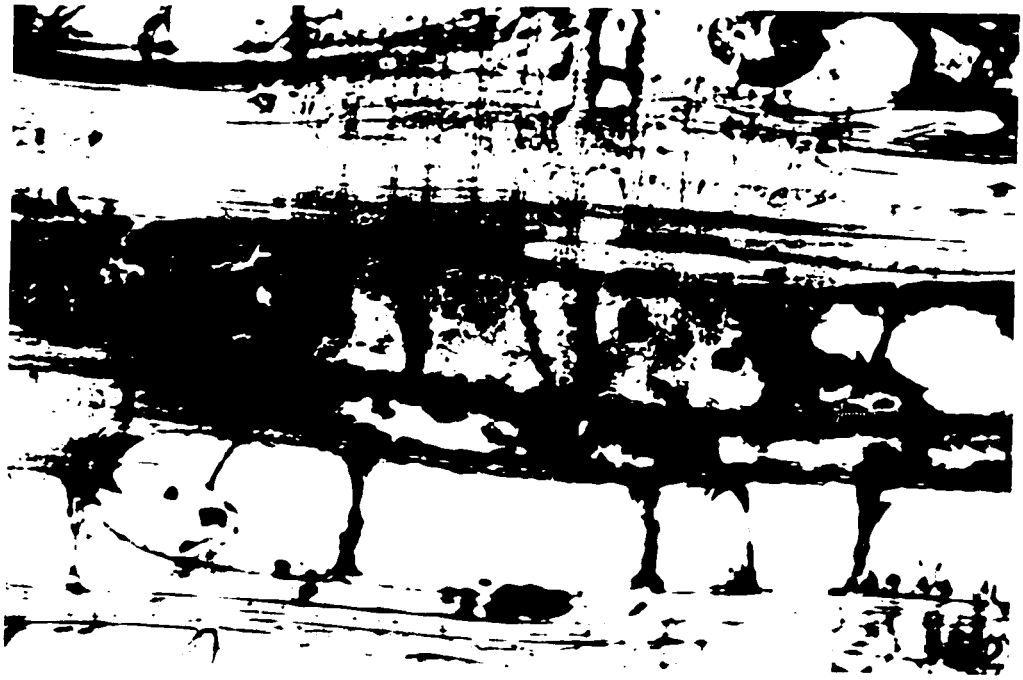
pH of the Malt Extract Medium at Various Stages of Growth of Variety *Laevigatus*

Days after inoculation	Temperature				
	15°C	21°C	24°C	27°C	34°C
0	5.1	5.1	5.1	5.1	5.1
5	5.1	5.1	5.1	5.1	5.2
10	5.1	5.2	5.2	5.2	5.1
15	5.2	5.1	5.2	5.0	5.2
20	5.2	5.2	5.1	5.0	5.0
25	5.2	5.1	4.9	4.9	5.0
30	5.2	5.2	4.9	4.9	4.7
35	5.2	5.2	4.8	4.8	4.7
40	5.2	5.2	4.8	4.7	4.6

FIGURE 1. Sporocarp of *Fomes igniarius* var. *populinus* on the trunk of a living aspen poplar tree.

FIGURE 2. Cuticular cells of variety *populinus* seen in zone lines formed in inoculated aspen poplar wood. X.310.

FIGURE 3. Branching of thin-walled, narrow hyphae in variety *laevigatus* and penetration of the cell walls of inoculated birch wood. X.195.



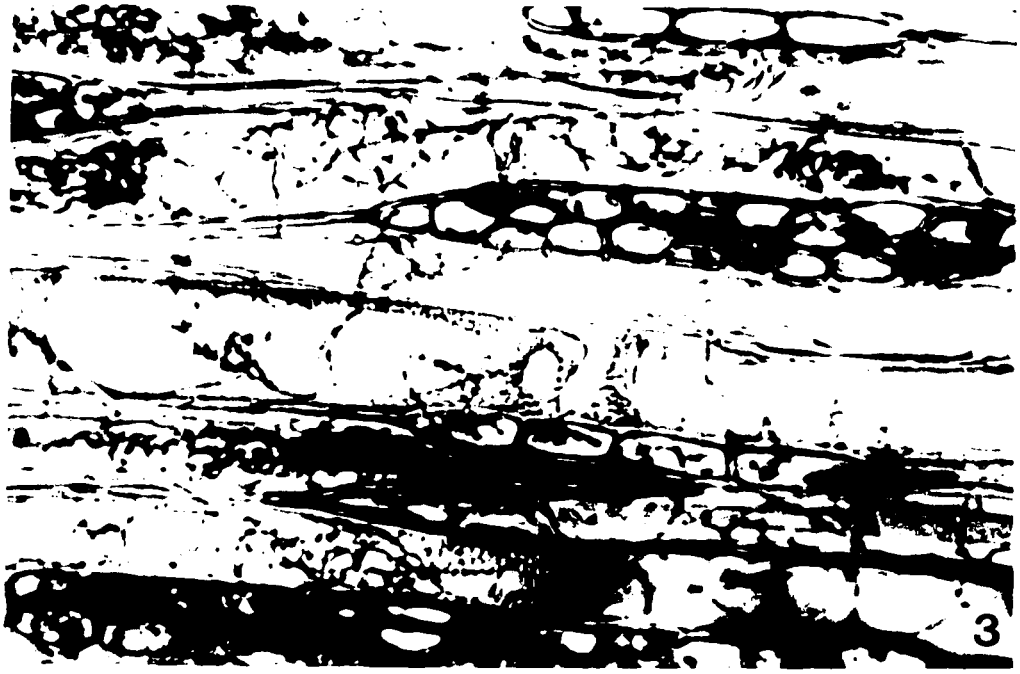

3

FIGURE 4. A thin-walled hypha of variety *populinus* penetrating the border part of a bordered pit in a cell of naturally infected aspen poplar. X.875.

FIGURE 5. Fiber hyphae of variety *populinus* in zone lines of inoculated aspen poplar. X.245.


4

FIGURE 6. Thin-walled hyphae of variety *populinus* present in various kinds of cells in inoculated aspen poplar. X.170.

FIGURE 7. A thin-walled hypha of variety *populinus* passing through a bore-hole in naturally infected aspen poplar. X.600.

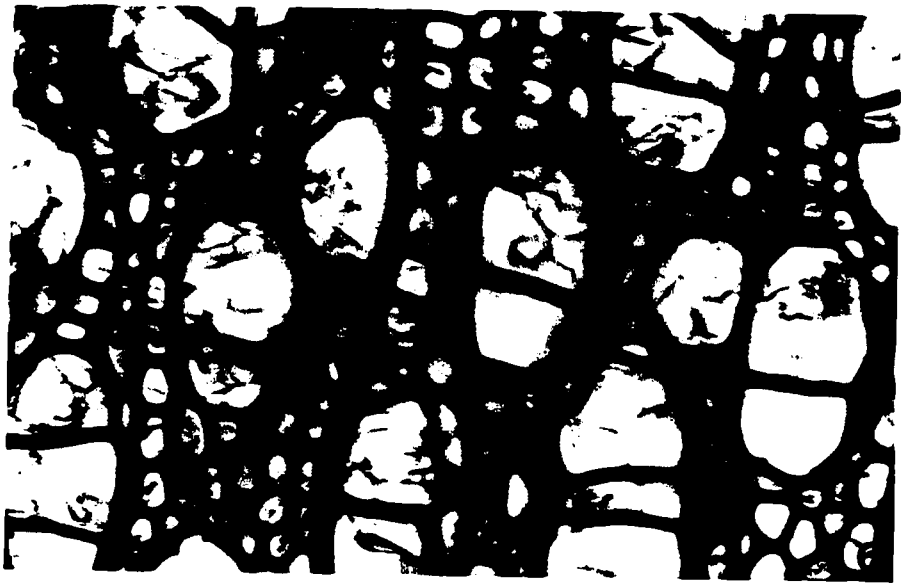


FIGURE 8. Zone lines formed in inoculated birch wood by the cuticular cells of variety *laevigatus*. X.195.

FIGURE 9. Oval cuticular cells of variety *laevigatus* in vessels and other cells of naturally infected birch. X.245.

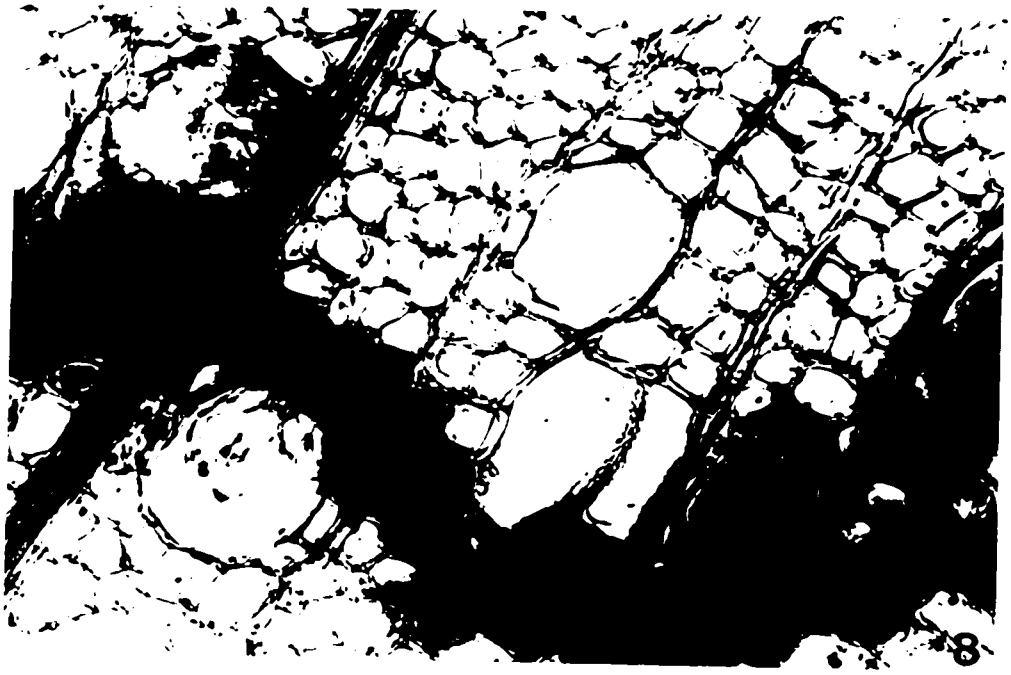
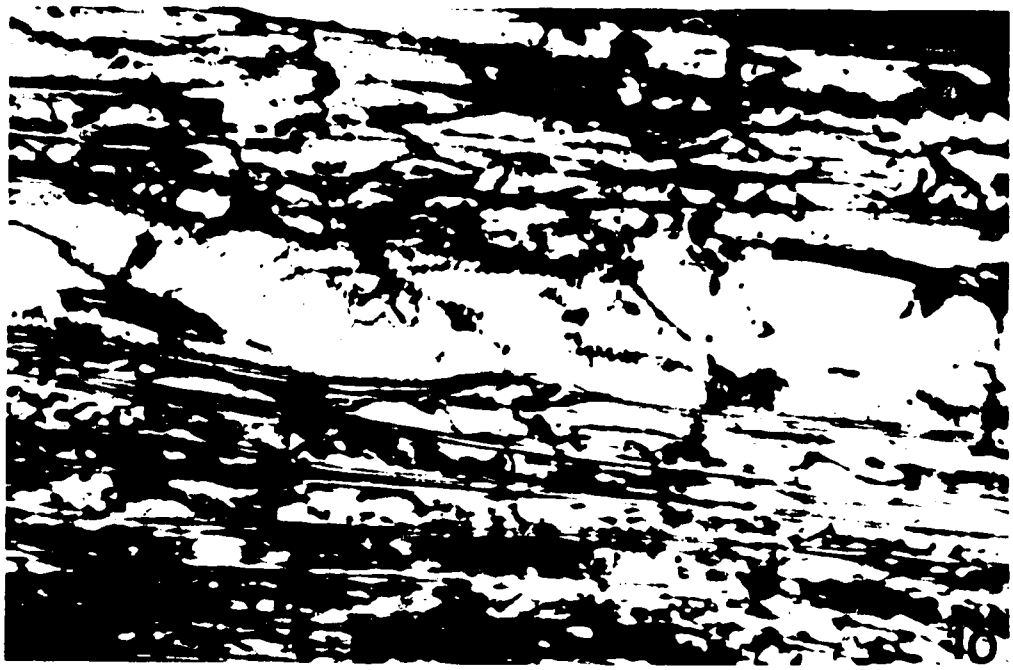

8

FIGURE 10. Section showing bore-holes and presence of thin-walled, narrow hyphae of variety *laevigatus* in several kinds of cells in inoculated birch wood. X.195.

FIGURE 11. Fiber hyphae of variety *laevigatus* in naturally infected birch wood. Thin-walled, narrow hyphae are also present. X.350.



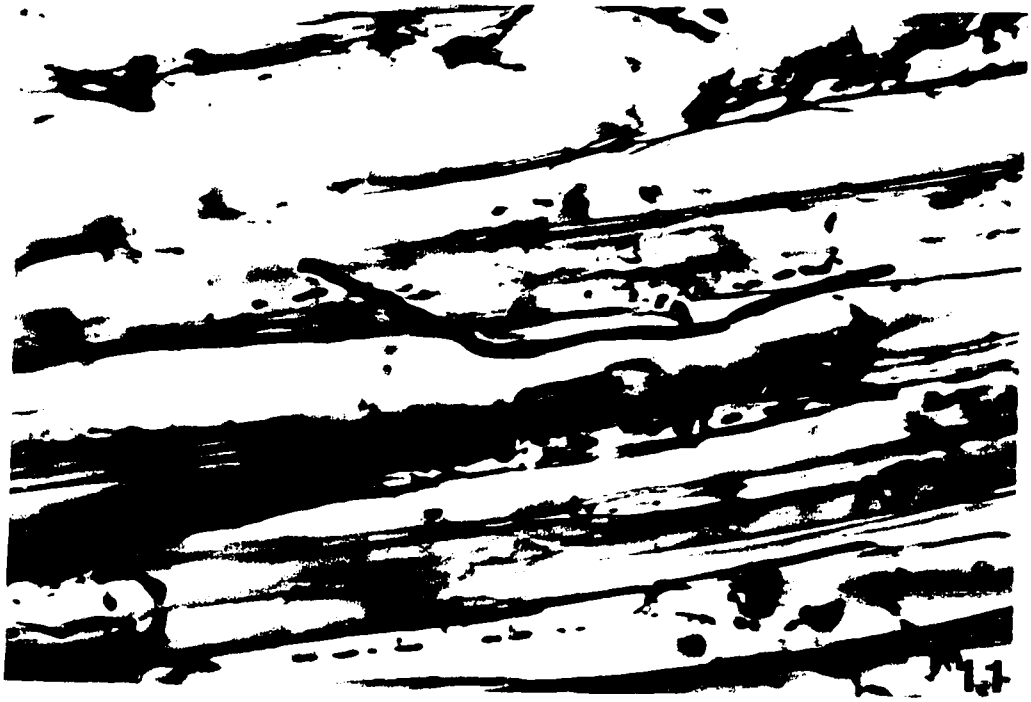
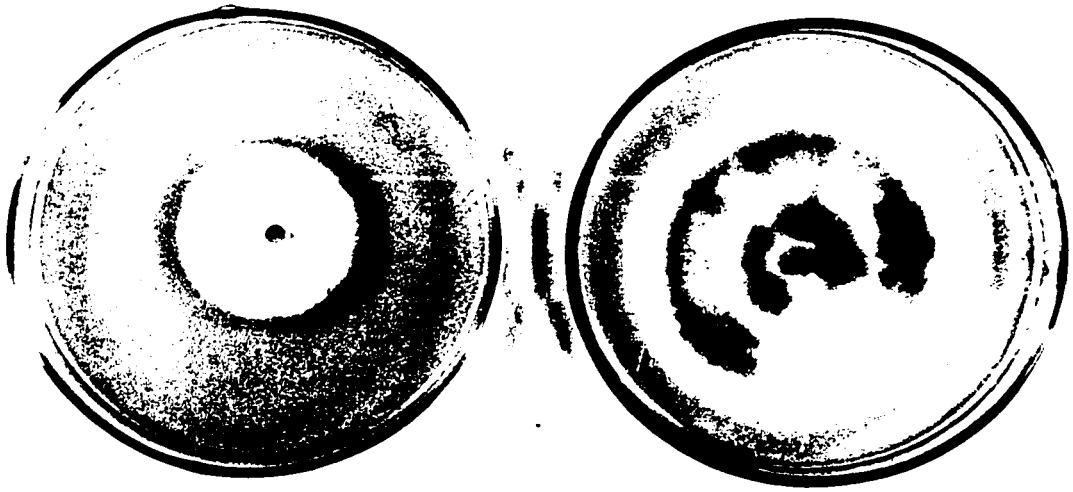
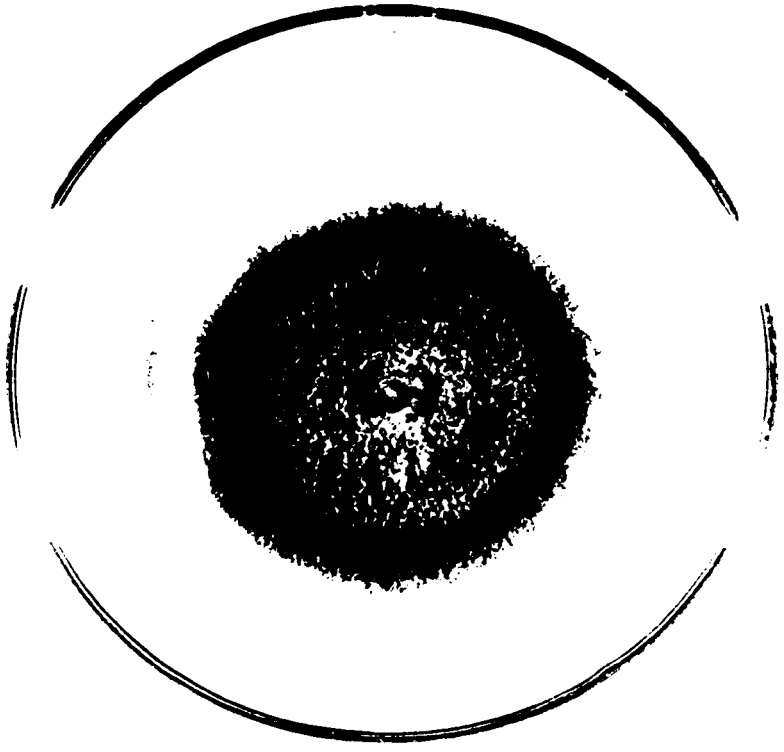


FIGURE 12. *Fomes igniarius* var. *populinus* (left) and variety *laevigatus* (right), 10 days after inoculation on malt-agar medium, at 27°C.

FIGURE 13. *Fomes igniarius* var. *populinus* 20 days after inoculation on malt-agar medium, at 27°C.



12



13

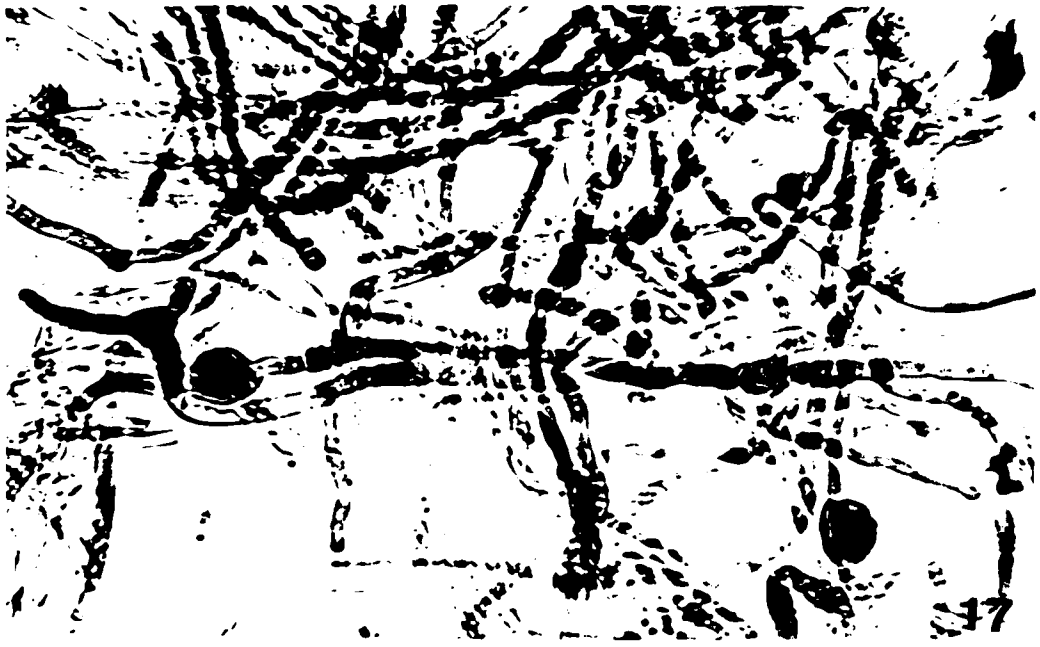
FIGURE 14. Thin-walled hyphae and fiber hyphae of variety *populinus* grown on malt-agar medium at 27°C. X.195.

FIGURE 15. Branching in thin-walled hyphae of variety *populinus* grown on malt-agar medium at 27°C. X.195.

FIGURE 16. Thick-walled fiber hypha originating from thin-walled hypha in variety *populinus* grown on malt-agar medium at 27°C. X.320.

FIGURE 17. Swollen, cuticular cells and thin-walled hyphae of variety *populinus* grown on malt-agar medium at 27°C. X.245.

16



17

FIGURE 18. Growth of *Fomes igniarius* var. *populinus* at various temperatures on malt-agar medium.

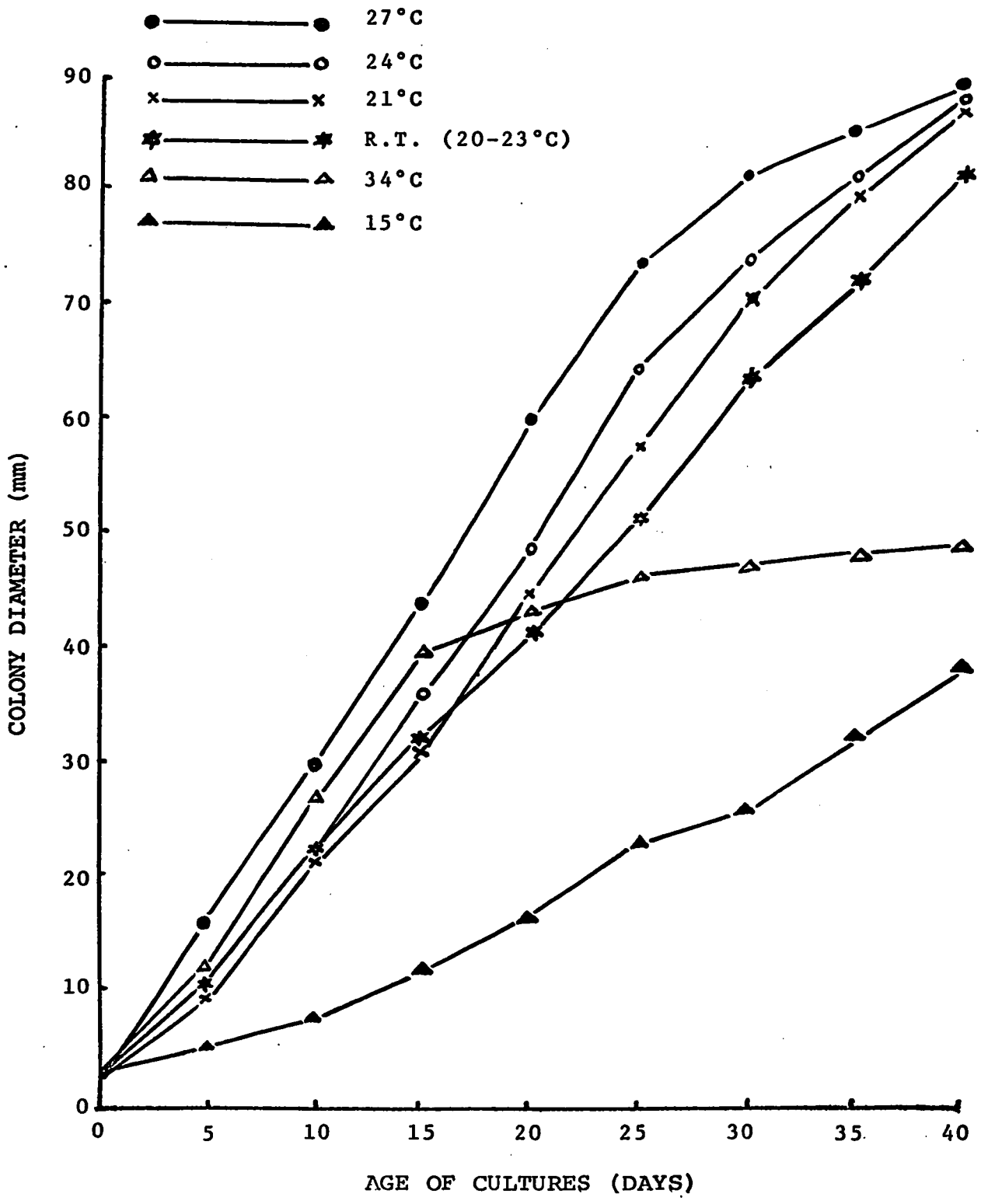

27°C
24°C
21°C
R.T. (20-23°C)
34°C
15°C
COLONY DIAMETER (mm)
0
10
20
30
40
50
60
70
80
90
0
5
10
15
20
25
30
35
40
AGE OF CULTURES (DAYS)

FIGURE 19. Comparative growth of *Fomes igniarius* var. *populinus* on wood and malt-agar media at 27°C.

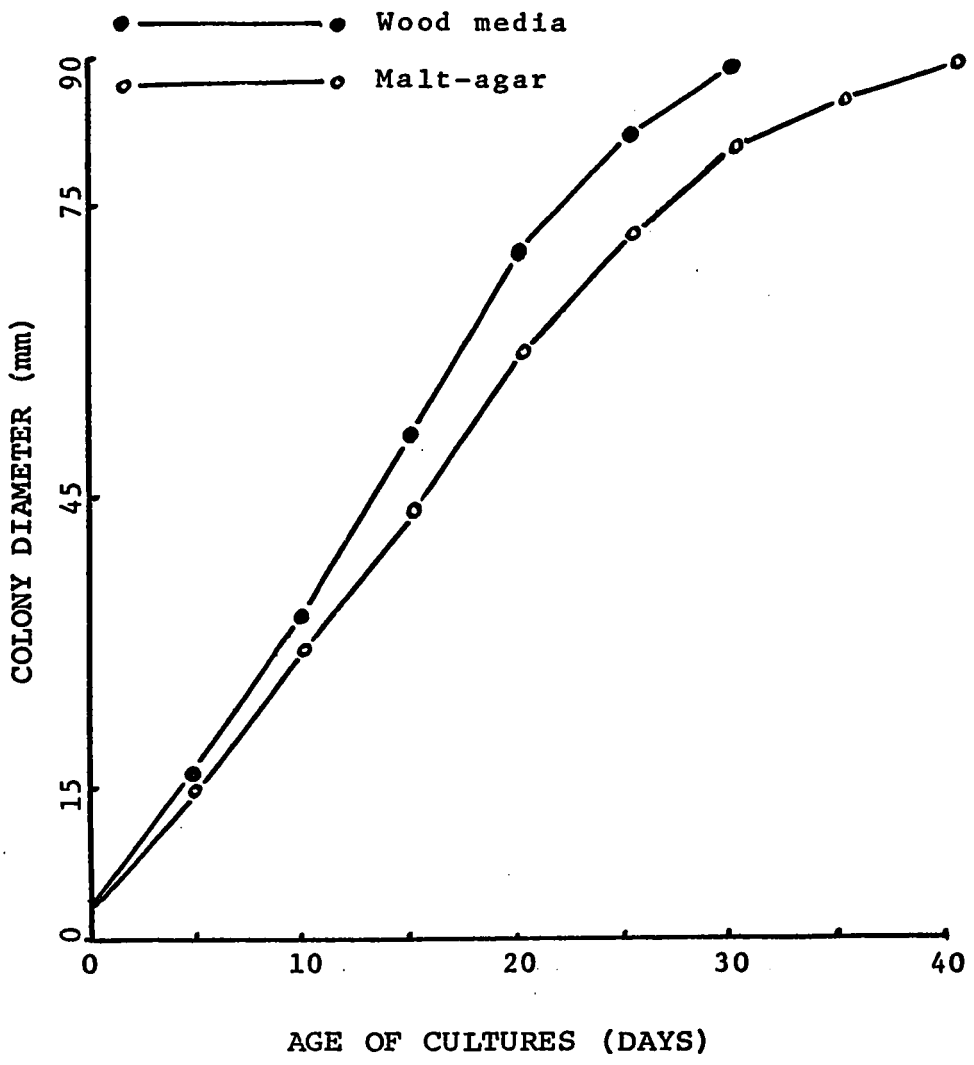

Wood media
Malt-agar
COLONY DIAMETER (mm)
0
15
45
75
90
0
10
20
30
40
AGE OF CULTURES (DAYS)

FIGURE 20. Yield of mycelium of *Fomes igniarius* var. *populinus* on malt extract medium in static and shake liquid cultures at room temperature (20-23°C).

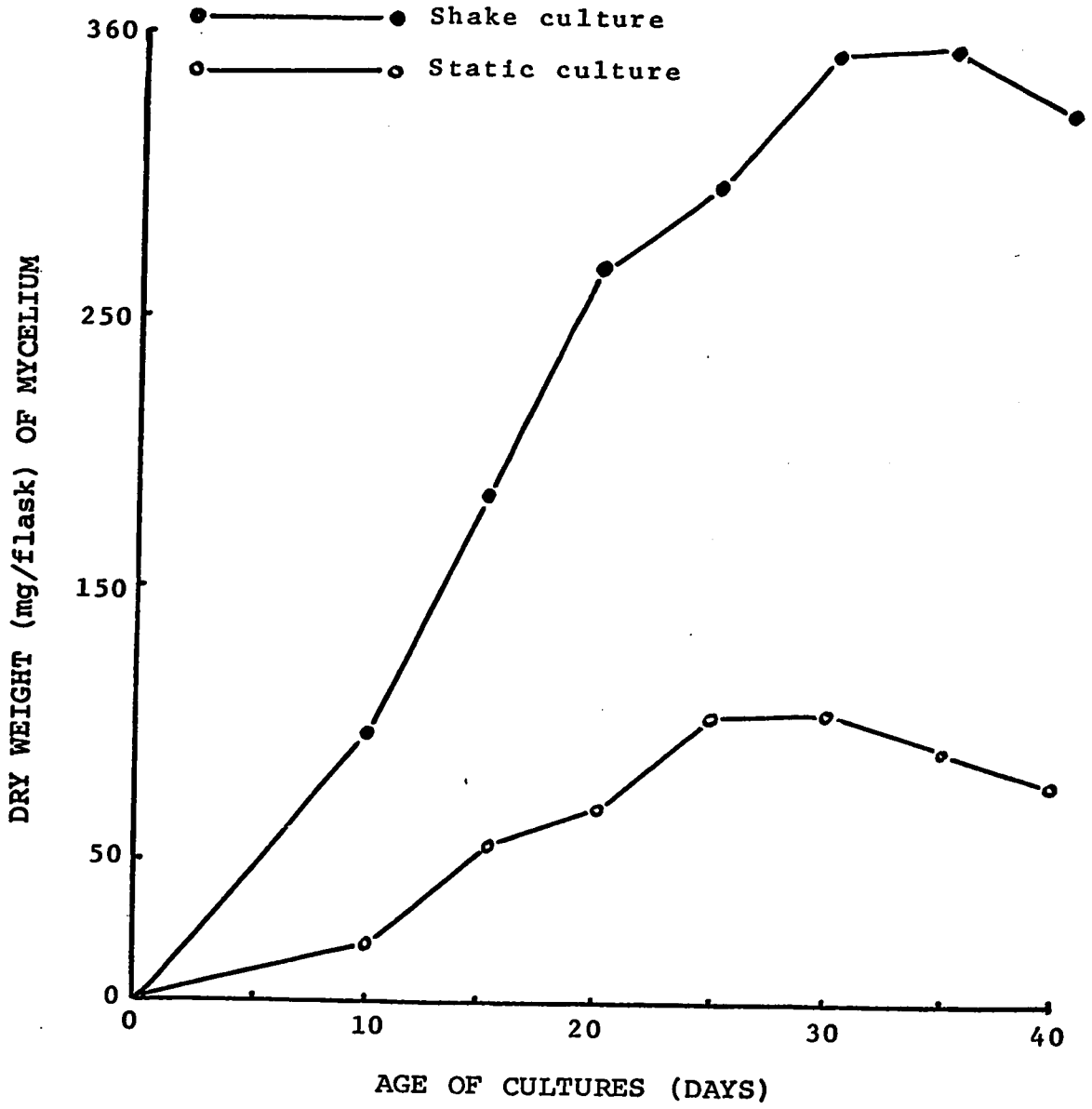

Shake culture
Static culture
360
250
150
50
0
0
10
20
30
40
DRY WEIGHT (mg/flask) OF MYCELIUM
AGE OF CULTURES (DAYS)

FIGURE 21. Yield of mycelium of *Fomes igniarius* var. *populinus* at various temperatures on malt extract medium.

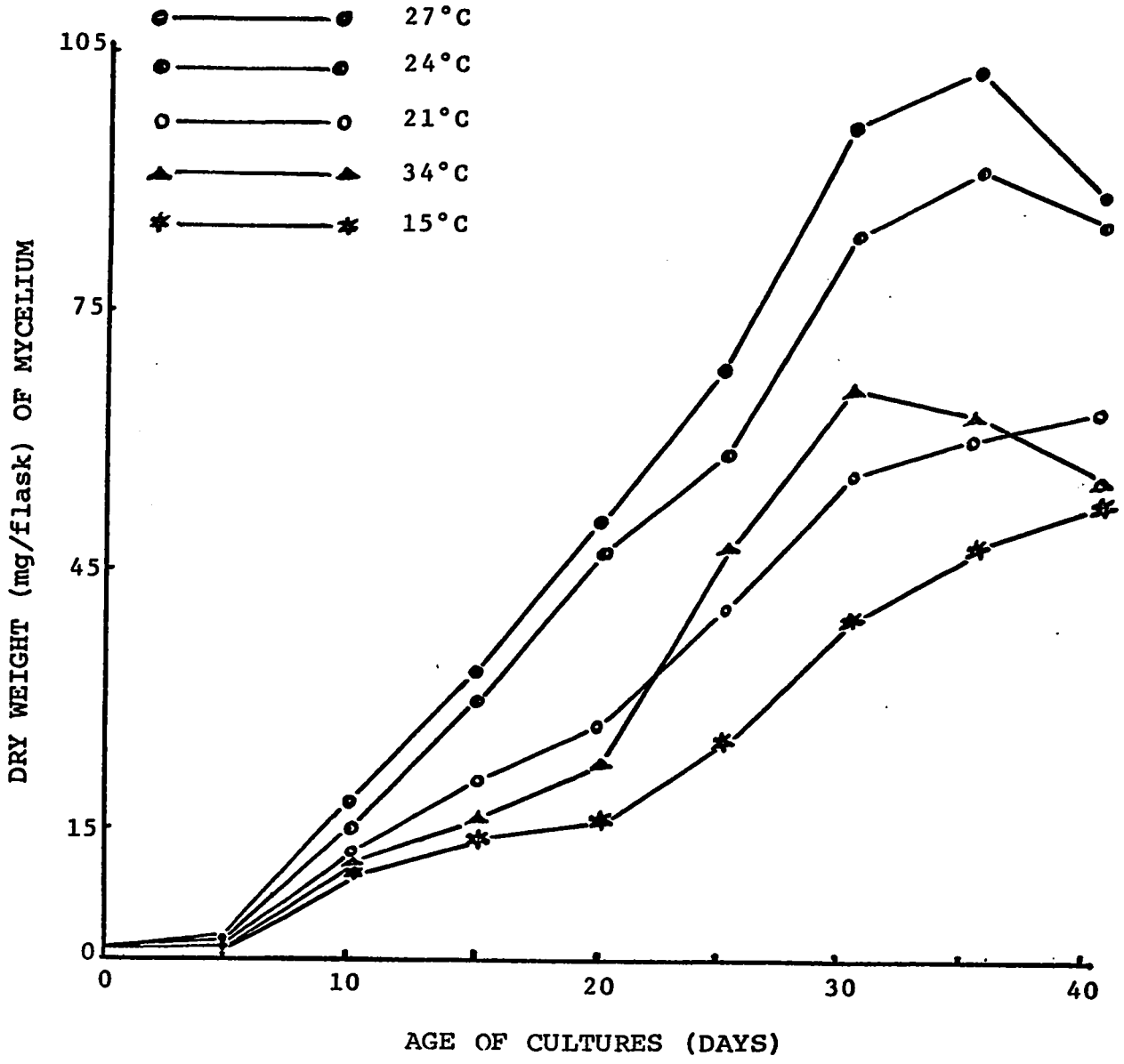

27°C
24°C
21°C
34°C
15°C
105
75
45
15
0
0
10
20
30
40
DRY WEIGHT (mg/flask) OF MYCELIUM
AGE OF CULTURES (DAYS)

FIGURE 22. Yield of mycelium of *Fomes igniarius* var. *populinus* on various carbon sources at 27°C.

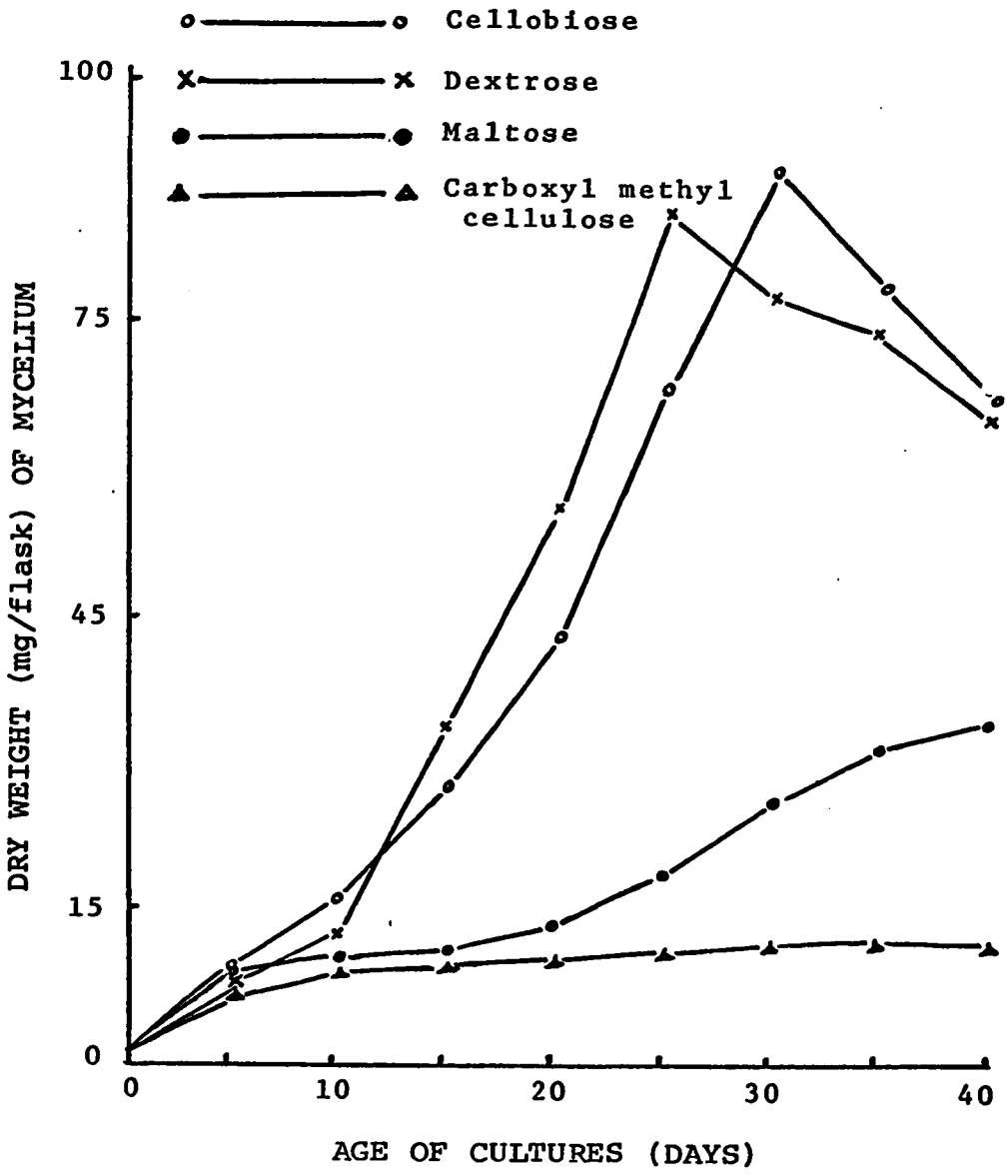
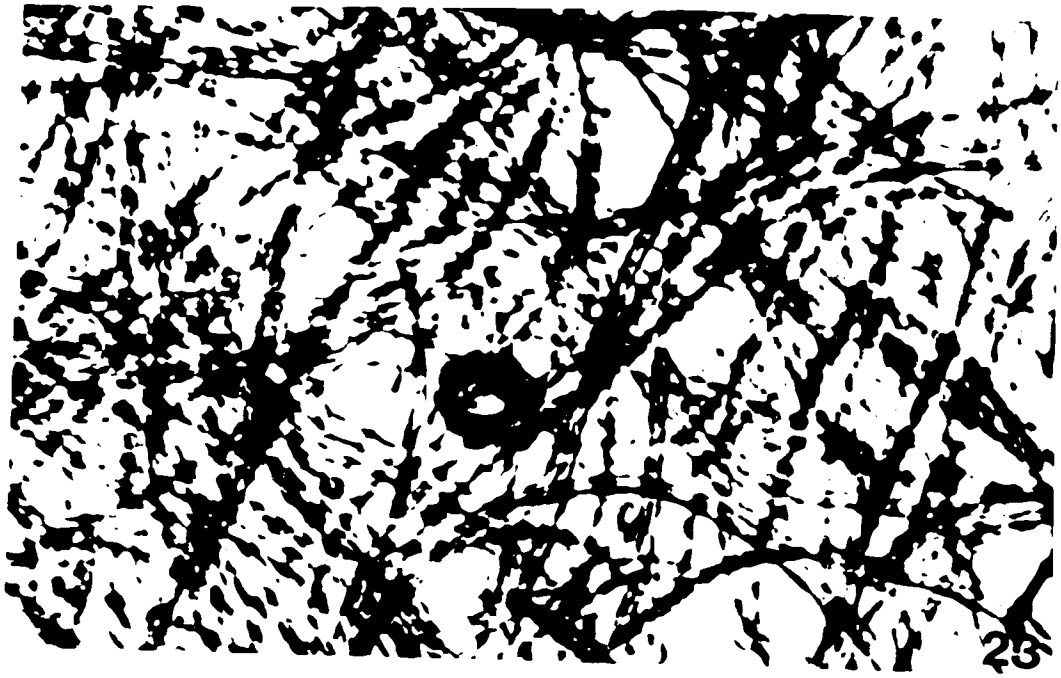

Cellobiose
Dextrose
Maltose
Carboxyl methyl cellulose
100
75
45
15
0
DRY WEIGHT (mg/flask) OF MYCELIUM
0
10
20
30
40
AGE OF CULTURES (DAYS)

FIGURE 23. Hyphal ring (flat coil) and thin-walled, narrow hyphae of variety *laevigatus* grown on malt-agar medium at 27°C. X.380.

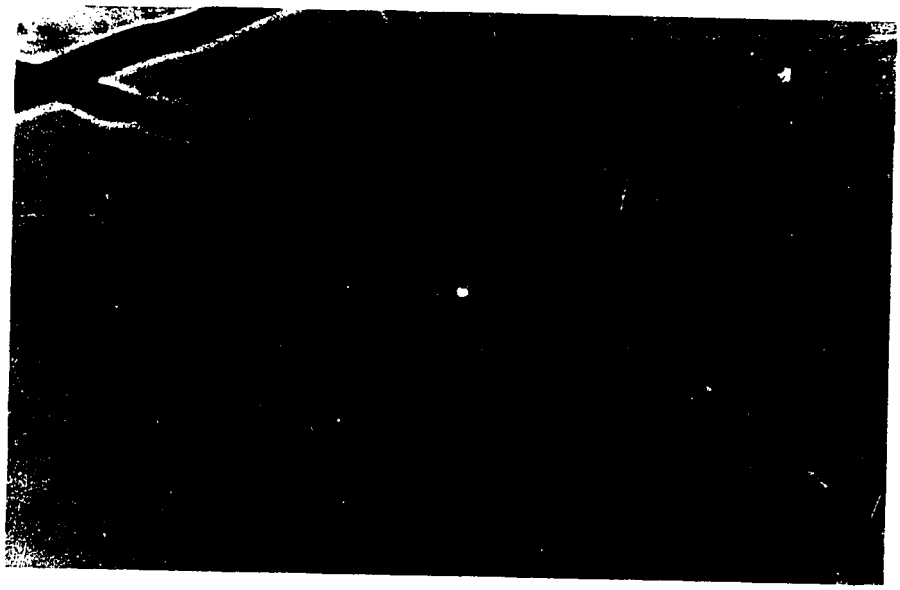
FIGURE 24. Thin-walled, narrow hyphae and thin-walled, broad hyphae of variety *laevigatus* grown on malt-agar medium at 27°C. X.220.


23


24

FIGURE 25. Hyphal anastomosis in thin-walled, narrow hyphae of variety *laevigatus* grown on malt-agar medium at 27°C. X.600.

FIGURE 26. Thick-walled, fiber hypha originating from thin-walled, narrow hypha in variety *laevigatus* grown on malt-agar medium at 27°C. X.600.



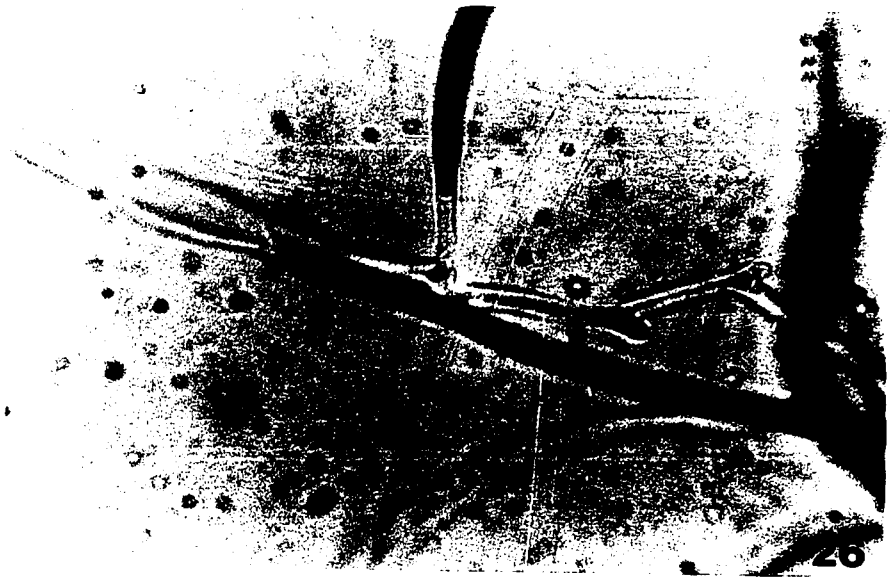


FIGURE 27. Branching in fiber hyphae of variety *laevigatus*, grown on malt-agar medium at 27°C. Thin-walled, narrow hyphae are also seen. X.600.

FIGURE 28. Cuticular cells of variety *laevigatus* grown on malt-agar medium at 27°C. X.600.

FIGURE 29. Growth of *Fomes igniarius* var. *laevigatus* on malt-agar medium at various temperatures.

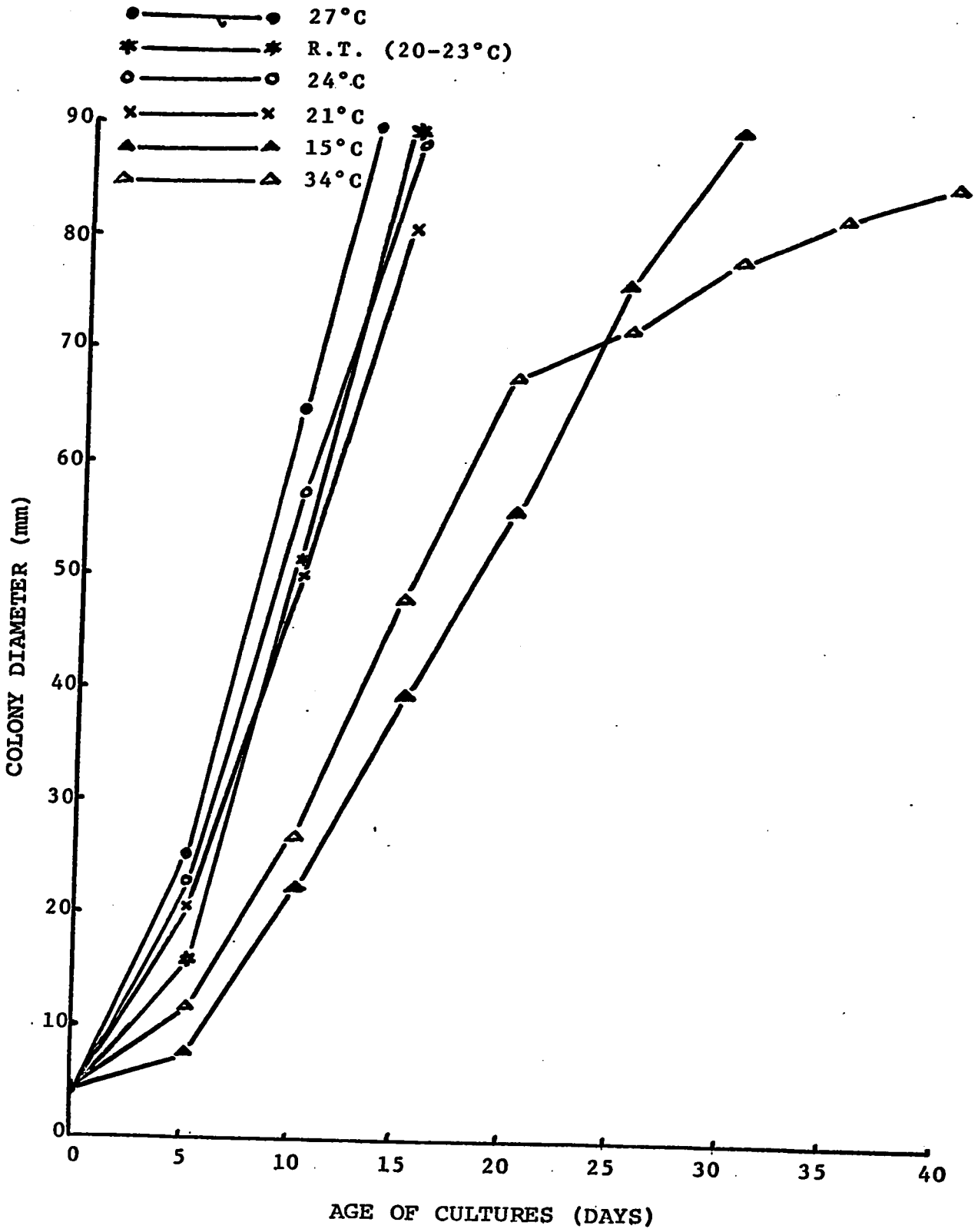

27°C
R.T. (20-23°C)
24°C
21°C
15°C
34°C
COLONY DIAMETER (mm)
0
10
20
30
40
50
60
70
80
90
0
5
10
15
20
25
30
35
40
AGE OF CULTURES (DAYS)

FIGURE 30. Yield of mycelium of *Fomes igniarius* var. *laevigatus* on malt extract medium in static and shake liquid cultures at room temperature (20-23°C).

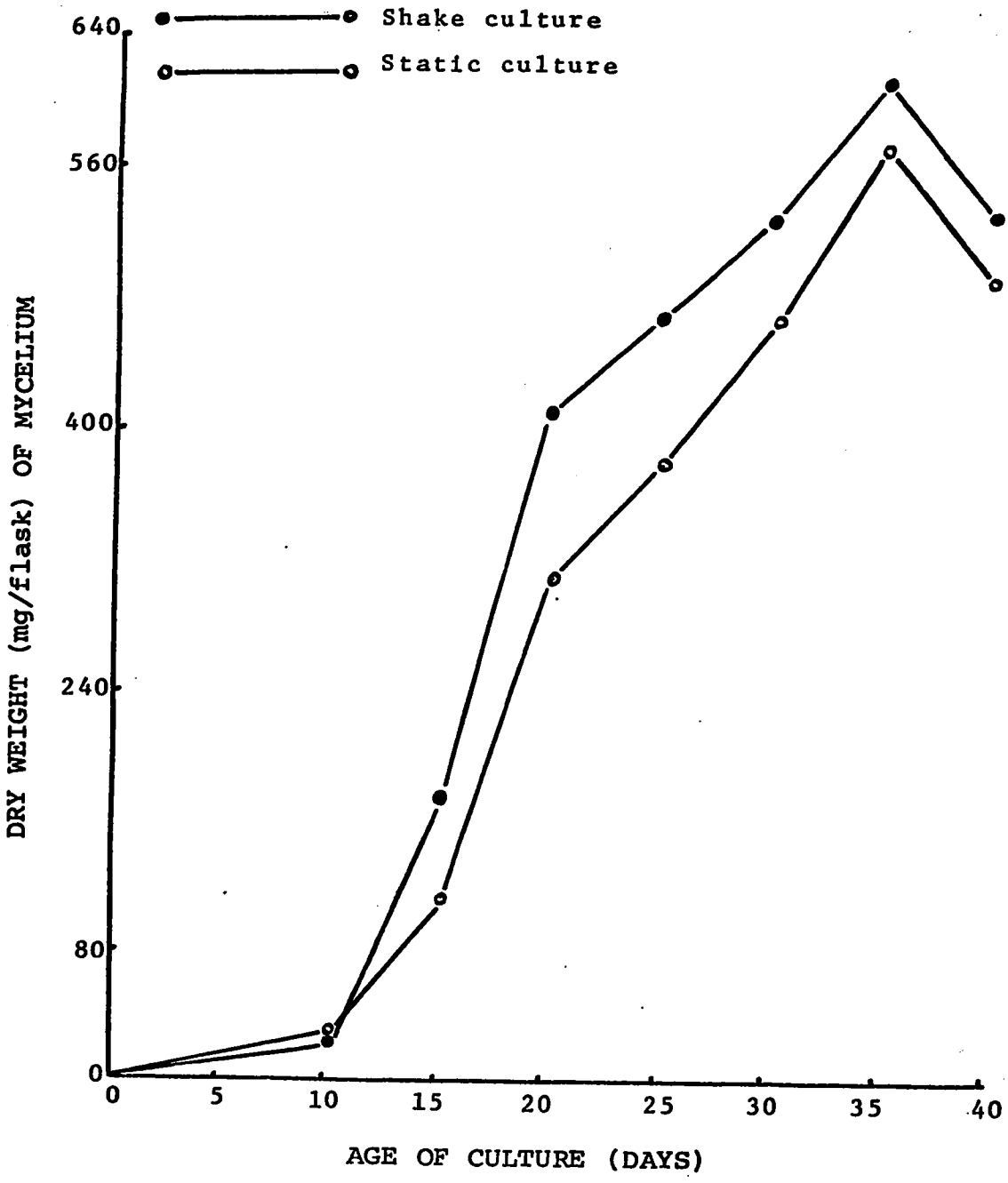

Shake culture
Static culture
640
560
400
240
80
0
DRY WEIGHT (mg/flask) OF MYCELIUM
0
5
10
15
20
25
30
35
40
AGE OF CULTURE (DAYS)

FIGURE 31. Yield of mycelium of *Fomes igniarius* var. *laevigatus* at various temperatures on malt extract medium.

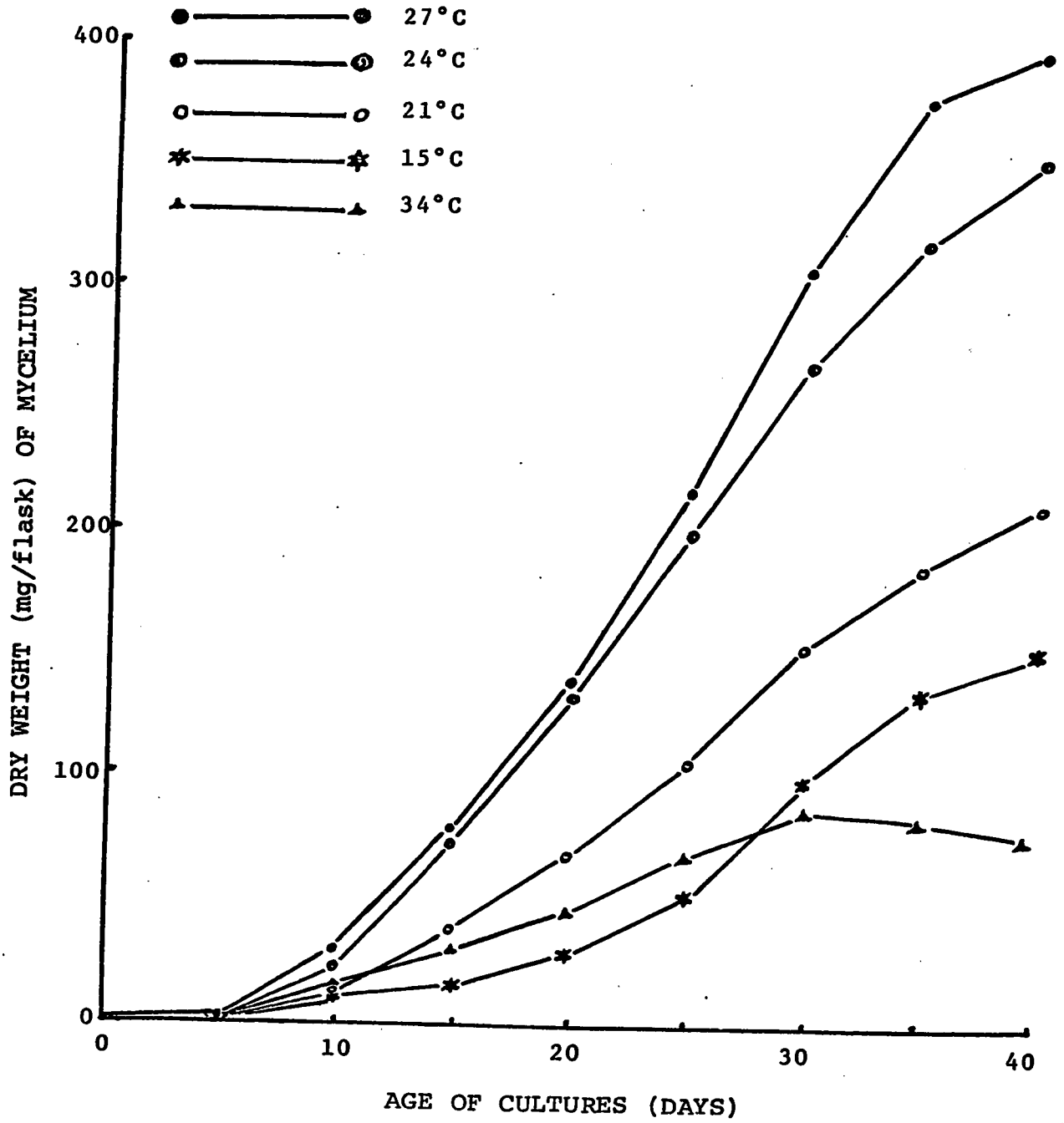
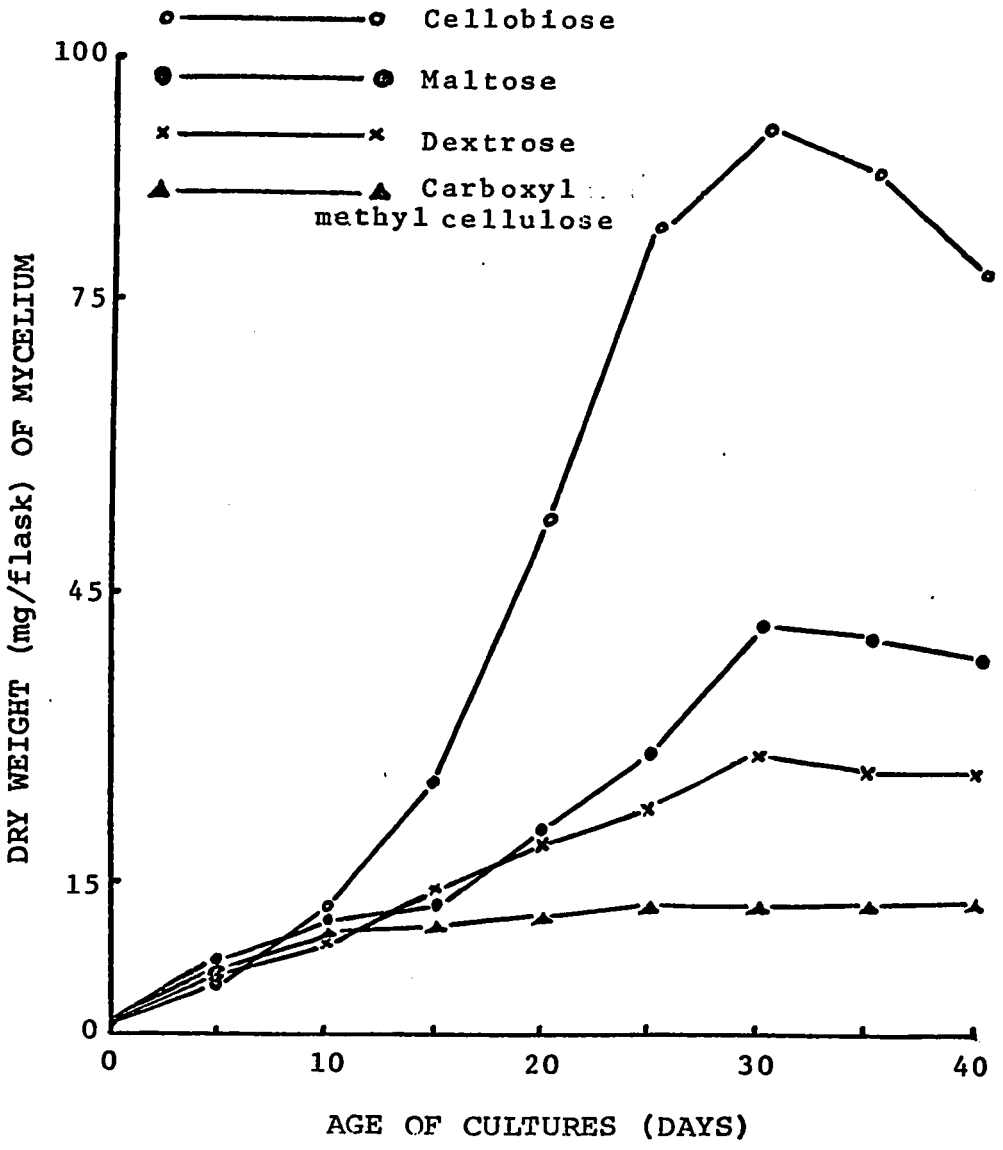

27°C
24°C
21°C
15°C
34°C
400
300
200
100
0
0
10
20
30
40
DRY WEIGHT (mg/flask) OF MYCELIUM
AGE OF CULTURES (DAYS)

FIGURE 32. Yield of mycelium of *Fomes igniarius* var. *laevigatus* on various carbon sources at 27°C.


Cellobiose
Maltose
Dextrose
Carboxyl methyl cellulose
100
75
45
15
0
0
10
20
30
40
DRY WEIGHT (mg/flask) OF MYCELIUM
AGE OF CULTURES (DAYS)

C. The ultrastructure of the mycelium

Three kinds of hyphae were observed in the varieties *populinus* and *laevigatus*, thin-walled hyphae, fiber hyphae and cuticular cells. In variety *laevigatus* the thin-walled hyphae were of two kinds; narrow hyphae and broad hyphae, while in variety *populinus* the thin-walled hyphae were of the narrow type only. In both varieties thin-walled, narrow hyphae were the first kind of mycelium observed in culture. Examination of the mycelium under the light microscope after a few days of growth showed broad, thin-walled hyphae, fiber hyphae and cuticular cells developing from the narrow, thin-walled hyphae. The fiber and cuticular cells had cytoplasm in their early stages of differentiation, but as they became older, the cell walls became thickened and the cells appeared empty. Fiber hyphae were brown in color and thick-walled at the time of differentiation. The cuticular cells were hyaline and thin-walled in the beginning, but at later stages of development they also became brown in color and thick-walled. Details about size, shape, branching, septation, etc., are described on pages 38-40 and 47-49.

1. Thin-walled hyphae

In most cases the walls of the thin-walled hyphae were triple layered. There was a thin electron-transparent surface layer of microfibrils, the outer part of which was usually sloughed off. Inside this layer was a thin electron-dense nonmicrofibrillar middle layer. The inner layer of

microfibrils lying adjacent to the protoplast was thick and more electron-dense than the outermost layer but more electron-transparent than the middle layer (Figures 33, 36, 41, 48, 52, pages 84, 86, 88, 92, 95). In some cells the wall appeared to have a single layer which is nonmicrofibrillar in appearance (Figure 34, page 84). Occasionally the cells appeared to show a thin outer and a broad inner dense layer on either side of the narrow central region (Figure 58, page 99). However, in certain cases the cell walls were multilayered (Figure 49, page 92).

The plasmalemma was continuous from one cell to another through the septal pore. It appeared smooth and continuous in many sections but was crenulate and discontinuous where vesicles were present in abundance (Figures 36, 41, pages 86 and 88).

The thin-walled hyphae contained numerous clearly-defined organelles. In some cases one large nucleus, which occupied a major portion of cross section of the hypha, was seen (Figure 33, page 84), in other sections two nuclei were observed (Figure 34, page 84). In one of the young growing hypha four nuclei were seen in a single cell (Figure 35, page 85). The nuclei were very distinct with a double nuclear membrane possessing nuclear pores. In the nucleoplasm there were a number of dark, electron-dense regions and light irregular regions of low electron-density.

The endoplasmic reticulum was smooth and was abundant in most of the cells and particularly so in younger cells (Figure 35, page 85). The continuity of endoplasmic

reticulum with the nuclear membrane (Figures 34, 35, pages 84, 85) and the mitochondria (Figure 37, page 86) was observed in many sections. In some cells vesicular endoplasmic reticulum was also seen (Figure 38, page 87).

The mitochondria were round (Figures 36, 37, page 86) or oblong in shape (Figures 40, 41, page 88) and had a double membrane and plate-like cristae. The mitochondria, accompanied by fragments of endoplasmic reticulum, were more frequent towards the growing point of the hypha.

The golgi bodies, observed in most plant and animal cells, appeared to be absent in the cells of this species. But there were present in most hyphal cells of *F. igniarius* structures with one to many concentric (Figure 43, page 89) or parallel (Figure 42, page 89) lamellae. These were smooth double membranes with a central electron-transparent layer surrounded by an electron-dense layer. The membrane system was two to many layered (Figures 42, 43, 45, pages 89, 90). The layers of concentric rings were continuous, although rings with discontinuous ends were also observed (Figure 46, page 91). In such cases the ends were recurved inwards. The lamellae possessed club-shaped ends which appeared to bud of tubular vesicles leading to the formation of lomasomes. The lomasomes consisted of a circular double membranous envelope enclosing small, tubular vesicles (Figures 44, 45, 46, 47, pages 90, 91).

Vesicles and multivesicular bodies were very commonly observed. Vesicles were found mainly in the regions of

active growth but they occurred occasionally in older cells. In older cells they are usually located near the cell wall (Figure 48, page 92). Occasionally, multivesicular bodies were observed aggregated in the hook-shaped terminal cells of the thin-walled hyphae (Figure 49, page 92).

Osmiophilic bodies and ribosomes were seen only in the material fixed in glutaraldehyde and osmium tetroxide. The osmiophilic bodies were in vacuoles and were associated with membranous elements (Figure 45, page 90). More vacuoles and lipid bodies were observed in comparatively older cells. The granular ribosomes were aggregated into clustures and were found free in the cytoplasm (Figures 44, 45, 46, pages 90, 91).

Hyphal anastomosis was frequently observed in the mycelium of *F. igniarius*. These openings were simple passages with cytoplasmic materials present in them (Figure 50, page 93). The hyphal branches form behind the septum of the parent hypha and a cross wall is laid down in the branch soon after it develops (Figure 51, page 94).

The septum observed in the thin-walled hyphae contained the complex pore referred to as 'Dolipore' by Moore and McAlear (1962c). The dome shaped pore cap with four lamellae was present on the two sides of the dolipore. In favourable preparations the pore caps appear continuous with the endoplasmic reticulum (Figure 38, page 87). The septal swelling, characteristic of dolipore septum, was always present and was surrounded by the plasmalemma. The pore cap pores were

present at irregular intervals in the pore cap (Figure 38, page 87). The septal pore appears to be well adapted for protoplasmic continuity between adjacent cells (Figures 51, 52, pages 94, 95). In older cells the pore plugs were present (Figure 39, page 87).

Membrane-bound dense bodies were seen in the thin-walled hyphae. These became larger in size and lighter in color in later stages of growth (Figures 33, 40, pages 87, 88).

The thin-walled, narrow hyphae of variety *populinus* and variety *laevigatus* had essentially the same fine structure features and the thin-walled, broad hyphae (Figure 52, page 95) found in variety *laevigatus*, resembled the narrow hyphae. The one difference between the two varieties was the presence of half septa (Figure 53, page 96) and invaginations of the cell walls of the thin-walled hyphae in the variety *populinus* (Figures 54, 55, page 96). Such structural variations were lacking in variety *laevigatus*.

2. Fiber hyphae

The fiber hyphae of the two varieties had similar characteristics. A dolipore septum was present at the point of differentiation where the fiber hypha originated from the thin-walled hypha (Figure 56, page 97). The cell wall structure was similar to that of the thin-walled hyphae and was triple layered, but in some cases (Figure 57, page 98) the arrangement of the wall material was different. There was an outer electron-dense layer, a middle electron-transparent layer and an innermost microfibrillar layer which

was very thick. The diameter of the mature fiber hypha was the same as that of the thin-walled hypha. The narrow central part (lumen) of the fiber hypha, which appeared electron-dense, was filled with granular material. A circular stratification of lamellae was seen in transverse sections of the fiber hyphae (Figure 60, page 100), but in longitudinal sections the microfibrils had an axial orientation (Figures 57, 58, 59, pages 98, 99, 100). The thickness of the cell wall was variable and in some cases no lumen was present at maturity (Figure 62, page 101). Constriction of the cell was seen quite often in fiber hyphae (Figures 59, 61, pages 100, 101).

3. Cuticular cells

The cuticular cells of the two varieties had similar features. The cell organelles present in the cuticular cells at early stages of development were essentially the same as those found in thin-walled hyphae (Figures 63, 64, pages 102, 103). The thickness of the cuticular cell wall in early stages was also the same but in later stages the wall became thickened like that of the fiber hyphae (Figure 66, page 104). The septa present in the formation of cuticular cells were dolipore septa (Figure 65, page 104).

ABBREVIATIONS USED IN FIGURES

ccm - complex concentric membrane

cpm - complex parallel membrane

cn - constriction

cw - cell wall

db - dense body

er - endoplasmic reticulum

ev - vesicular endoplasmic reticulum

hs - half septa

iv - invagination of plasmalemma

ivc - invagination of cell wall

l - electron transparent lamella in the center of the typical septum

lo - lomasomes

m - mitochondrion

mb - multivesicular body

n - nucleus

nm - nuclear membrane

np - nuclear pore

os - osmiophilic body

ow - outer wall

p - septal pore

pc - pore cap

pcp - pore cap pore

pl - plasmalemma

pp - pore plug

r - ribosome

s - septum

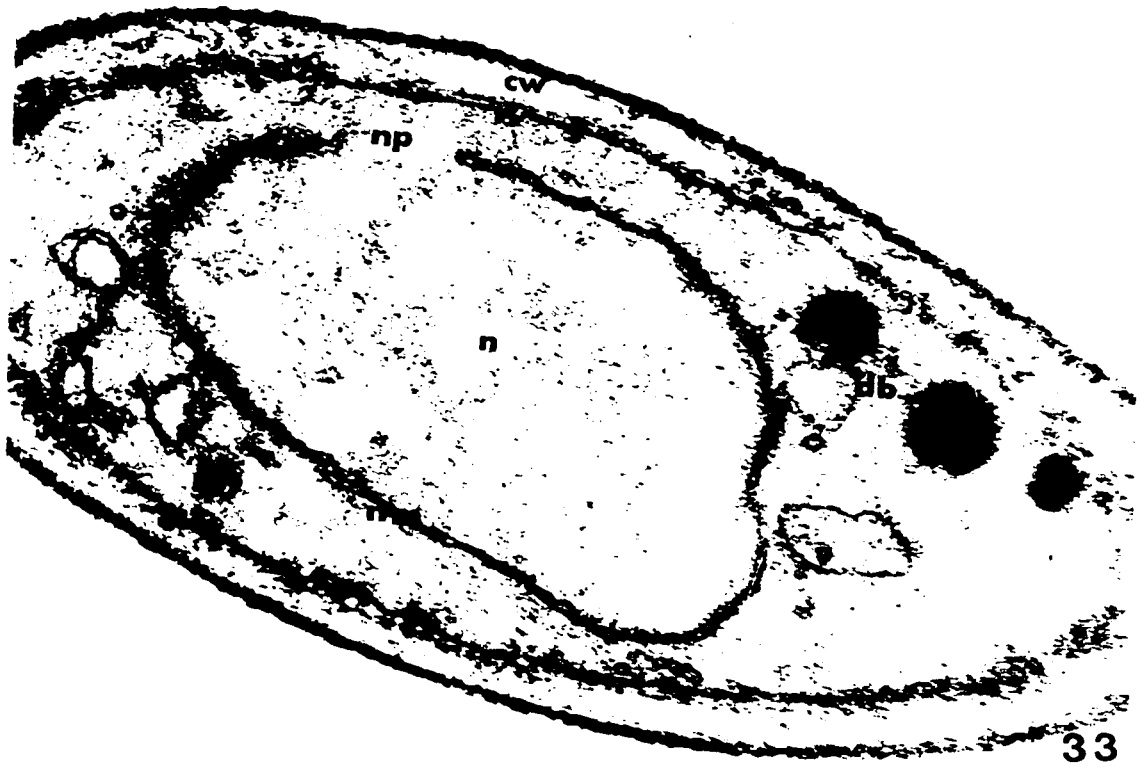
ss - septal swelling

v - vesicle

va - vacuole

FIGURE 33. Oblique, transverse section through a thin-walled hypha of variety *populinus* showing nucleus (n), dense bodies (db) and triple layered cell wall (cw). Fixation - $KMnO_4$. X.54,000.

FIGURE 34. Transverse section through a thin-walled, narrow hypha of variety *laevigatus* showing a single layered cell wall (cw), two nuclei (n) and the continuity of the endoplasmic reticulum (er) with nuclear membrane. Fixation - $KMnO_4$. X.22,000.



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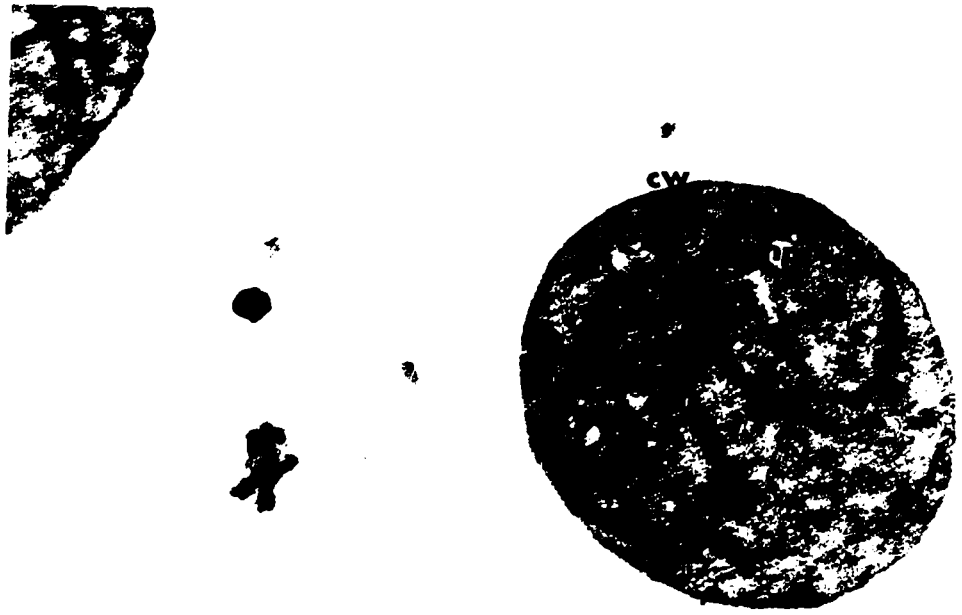

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FIGURE 35. Longitudinal section through a young, thin-walled hypha of variety *populinus* showing the smooth-surfaced endoplasmic reticulum (er), mitochondria (m), four nuclei (n) and a vacuole (va). Fixation - $KMnO_4$. X.21,600.


va

FIGURE 36. Longitudinal section through a thin-walled hypha of variety *populinus* showing mitochondria (m), dense bodies (db) and vesicles (v) near the triple layered cell wall (cw). Fixation - $KMnO_4$. X.42,000.

FIGURE 37. Transverse section through a thin-walled hypha showing continuity of endoplasmic reticulum (er) with a circular mitochondrion (m). Fixation - $KMnO_4$. X.63,800.

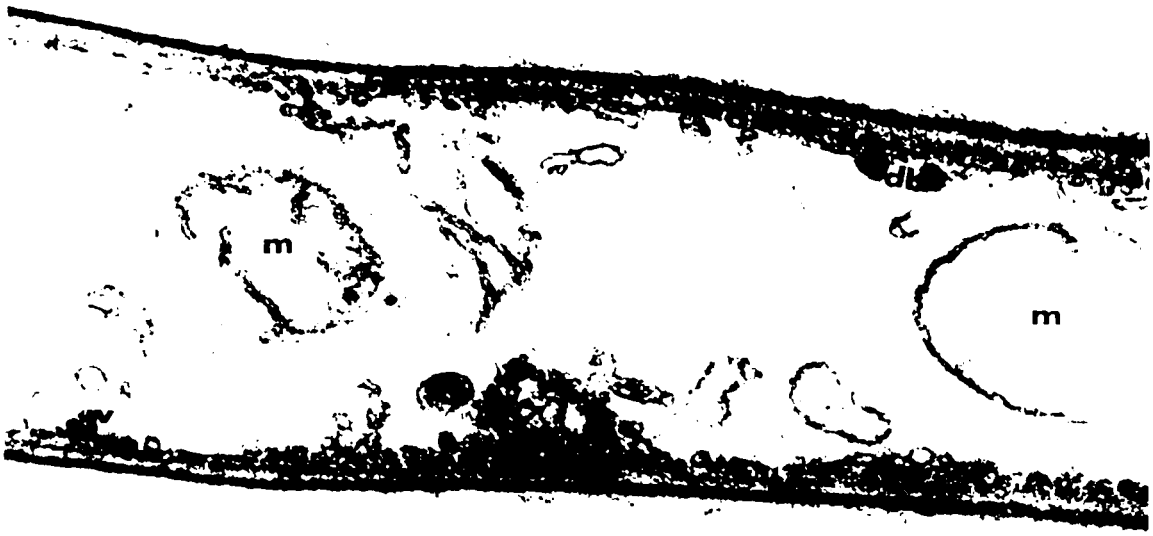

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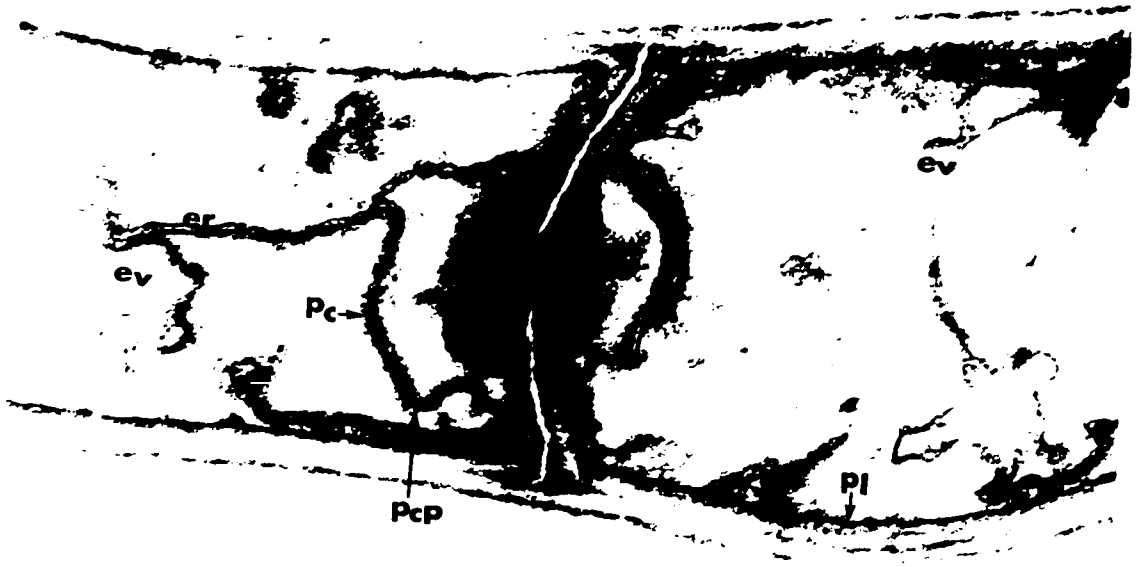
FIGURE 38. Longitudinal section through a thin-walled hypha of variety *populinus* showing multilamellate pore cap (pc), pore cap pore (pcp), continuity of pore cap with endoplasmic reticulum (er). Vesicular endoplasmic reticulum (ev). Fixation - $KMnO_4$. X.42,000.

FIGURE 39. Longitudinal section through a thin-walled hypha of variety *populinus* showing pore plug (pp), pore cap with multilamellae (pc) and electron transparent lamella in septum (l). Fixation - $KMnO_4$. X.45,000.



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39

FIGURE 40. Longitudinal section through a thin-walled hypha of variety *populinus* showing oblong mitochondria (m) and membrane bound unidentified dense bodies (db). Fixation - $KMnO_4$. X.39,000.

FIGURE 41. Transverse section through a thin-walled, narrow hypha of variety *laevigatus* showing a mitochondrion (m), endoplasmic reticulum (er) and numerous vesicles (v) near cell wall. Fixation - $KMnO_4$. X.39,000.

40

41

FIGURE 42. Longitudinal section through a thin-walled hypha of variety *populinus* showing complex parallel lamellae (cpm). Fixation - $KMnO_4$. X.67,500.

FIGURE 43. Longitudinal section through a thin-walled hypha of variety *laevigatus* showing complex concentric membrane (ccm). Fixation - $KMnO_4$. X.80,000.

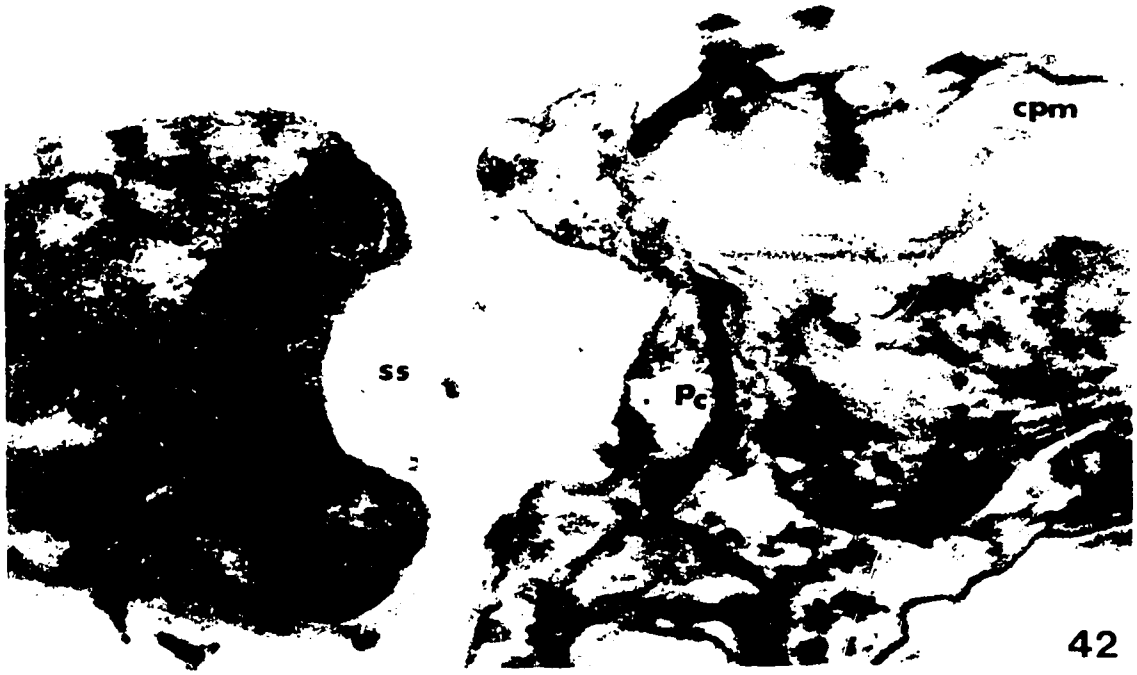

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FIGURE 44. Transverse section through a thin-walled hypha of variety *populinus* showing uneven thickness of cell wall (cw), invagination of plasmalemma (iv), lomasomes (lo) and free ribosomes (r).
Fixation - 1:1, Glutaraldehyde plus formaldehyde, and Osmium tetroxide.
X.51,600.

FIGURE 45. Longitudinal section through a thin-walled hypha of variety *populinus* showing complex concentric membrane (ccm), lomasomes (lo), osmiophilic body (os), vacuole (va), free ribosomes (r) and a nonmedian septum (s).
Fixation - 1:1, Glutaraldehyde plus formaldehyde, and Osmium tetroxide.
X.51,600.

44

45

FIGURE 46. Oblique, transverse section through a thin-walled hypha of variety *populinus* showing crenulate plasmalemma (pl), free ribosomes (r) and complex concentric membrane (ccm) with club-shaped ends leading into lomasomes (lo). Fixation - 1:1, Glutaraldehyde + formaldehyde, and Osmium tetroxide. X.51,600.

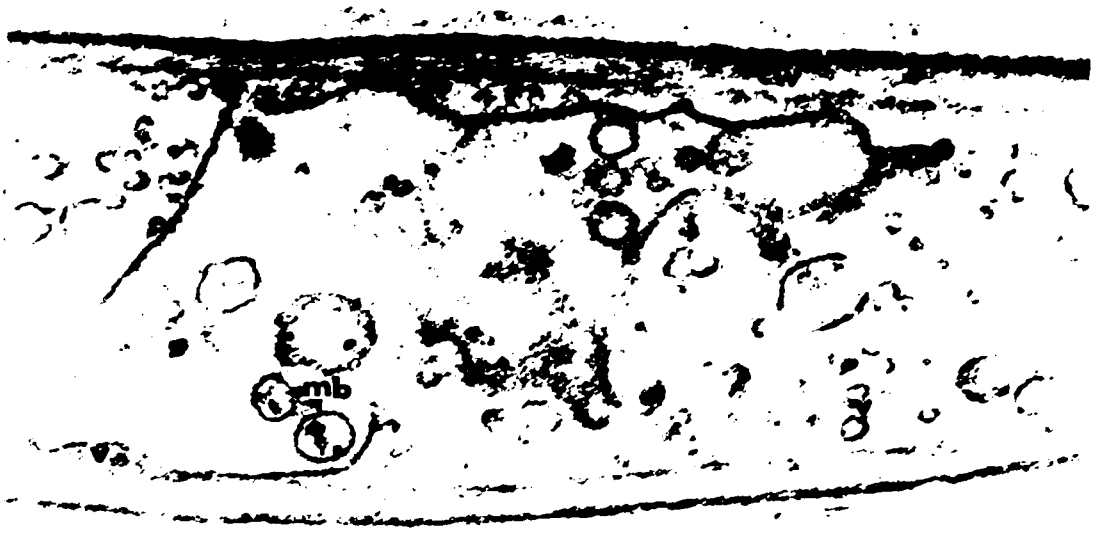
FIGURE 47. Longitudinal section through a thin-walled hypha of variety *populinus* showing lomasomes (lo) and circular mitochondria (m). Fixation - $KMnO_4$. X.28,600.

46

47

FIGURE 48. Longitudinal section through a thin-walled hypha of variety *populinus* showing triple layered cell wall (cw), vesicles (v) and multivesicular bodies (mb) near the cell wall. Fixation - $KMnO_4$. X.51,600.

FIGURE 49. Longitudinal section through a thin-walled hypha of variety *populinus* showing hook-shaped apex with multivesicular bodies (mb), a nonmedian septum (s) and multilayered cell wall (cw). Fixation - $KMnO_4$. X.51,600.



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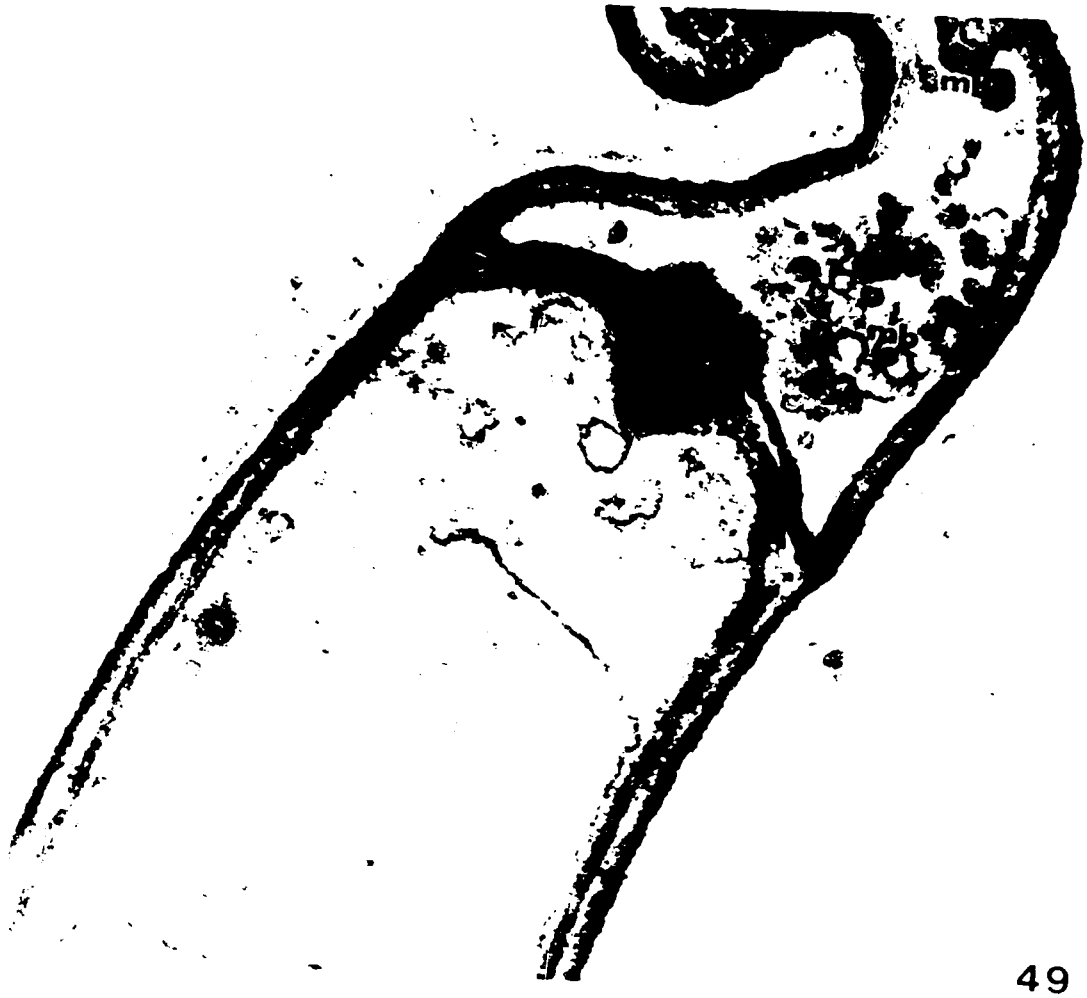


49

FIGURE 50. Longitudinal section showing hyphal anastomosis in thin-walled hyphae of variety *populinus*. Mitochondria (m), multivesicular bodies (mb), vesicles (v) and vacuoles (va) are seen. Fixation - $KMnO_4$. X.47,300.

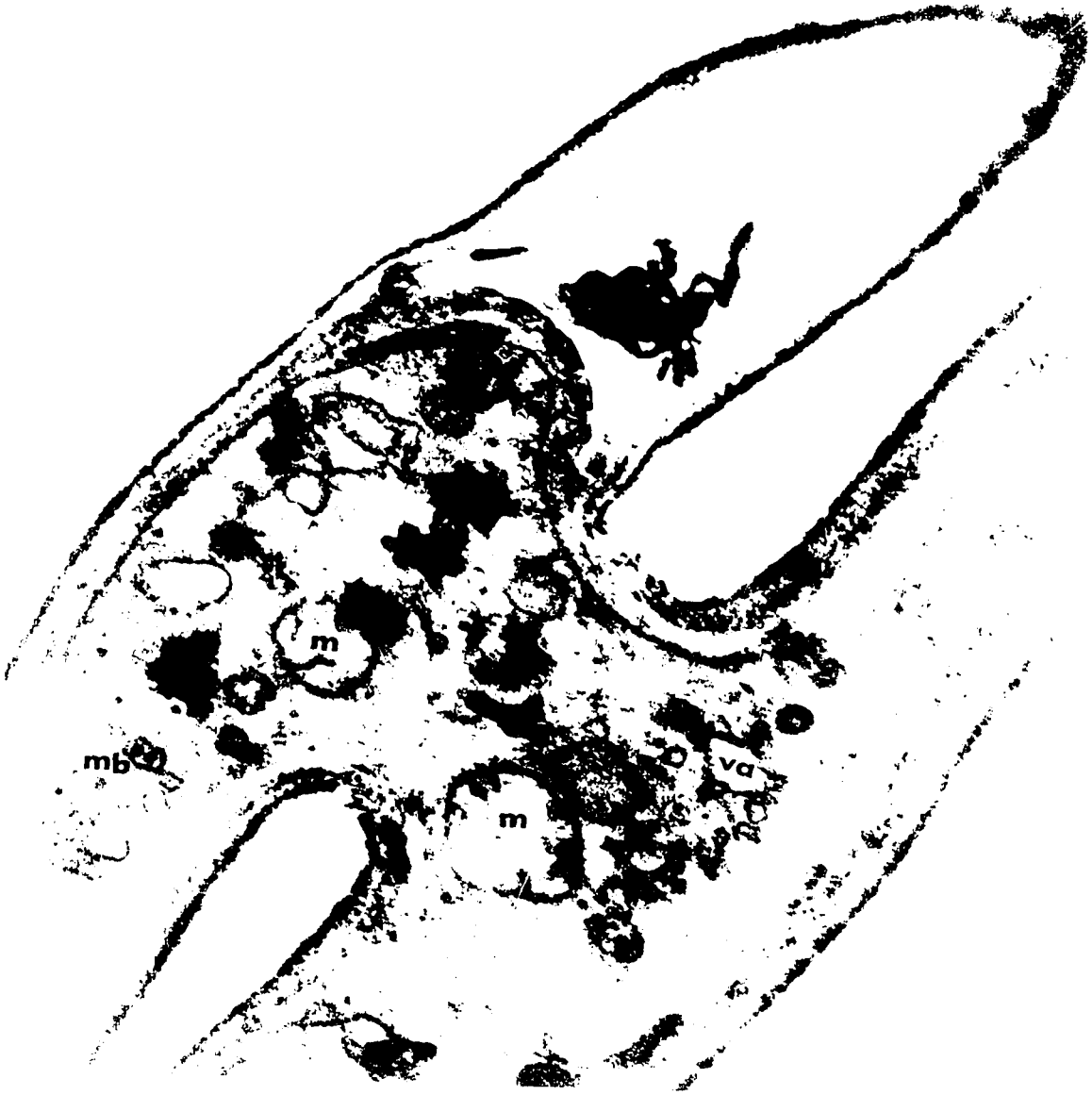

m
mb
m
va

FIGURE 51. Longitudinal section through a thin-walled hypha of variety *populinus* showing dolipore septum and branching at an acute angle with a nonmedian septum (s) near the end of branch. Fixation - $KMnO_4$. X.51,600.

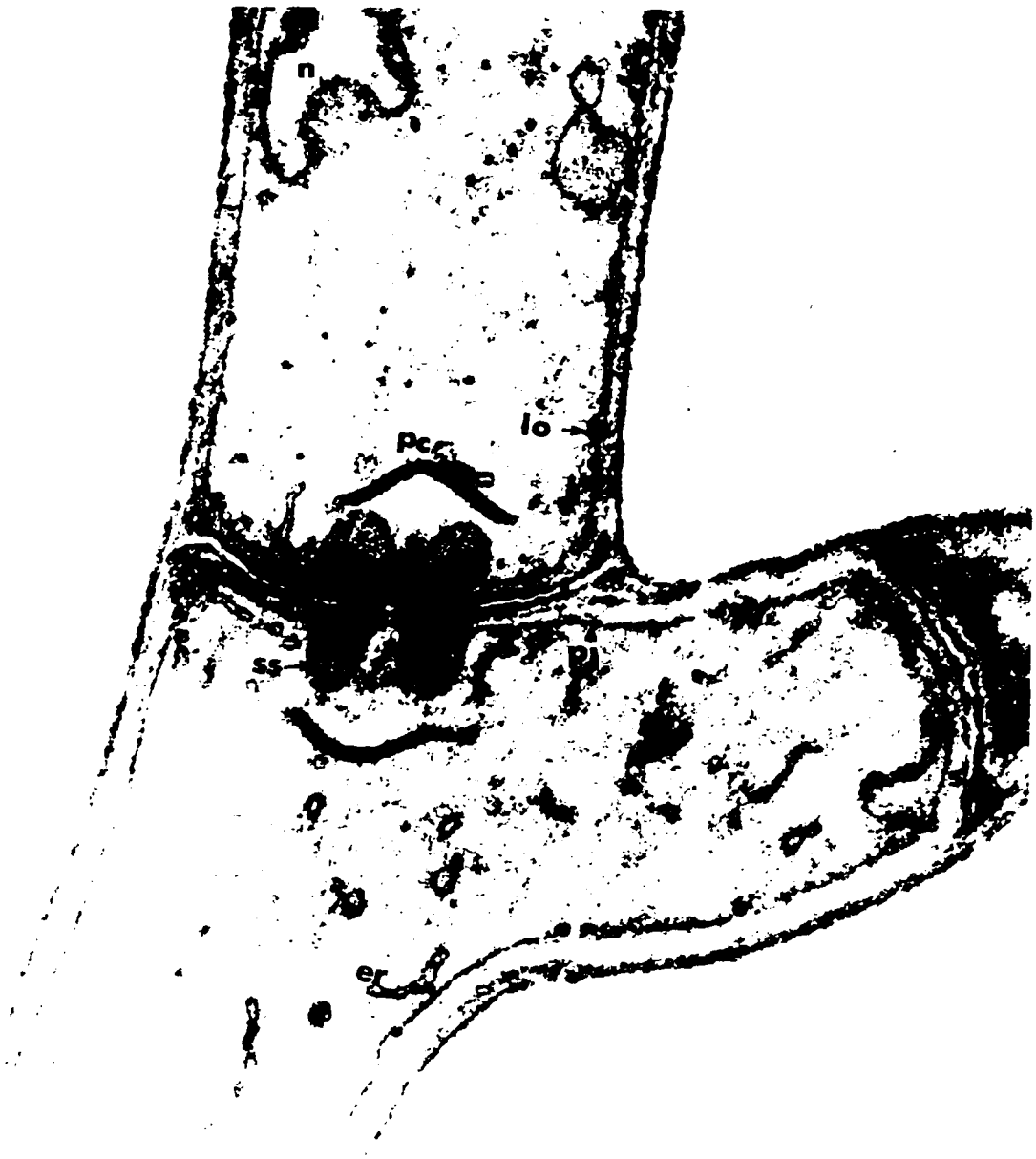

n
lo
Pc
ss
Pl
er

FIGURE 52. Longitudinal section through a thin-walled broad hypha of variety *laevigatus* showing protoplasmic continuity through dolipore septum, electron transparent lamella (l), continuity of plasmalemma (pl) between cells, multi-lamellate pore cap (pc) and endoplasmic reticulum (er). Fixation - $KMnO_4$. X.43,000.

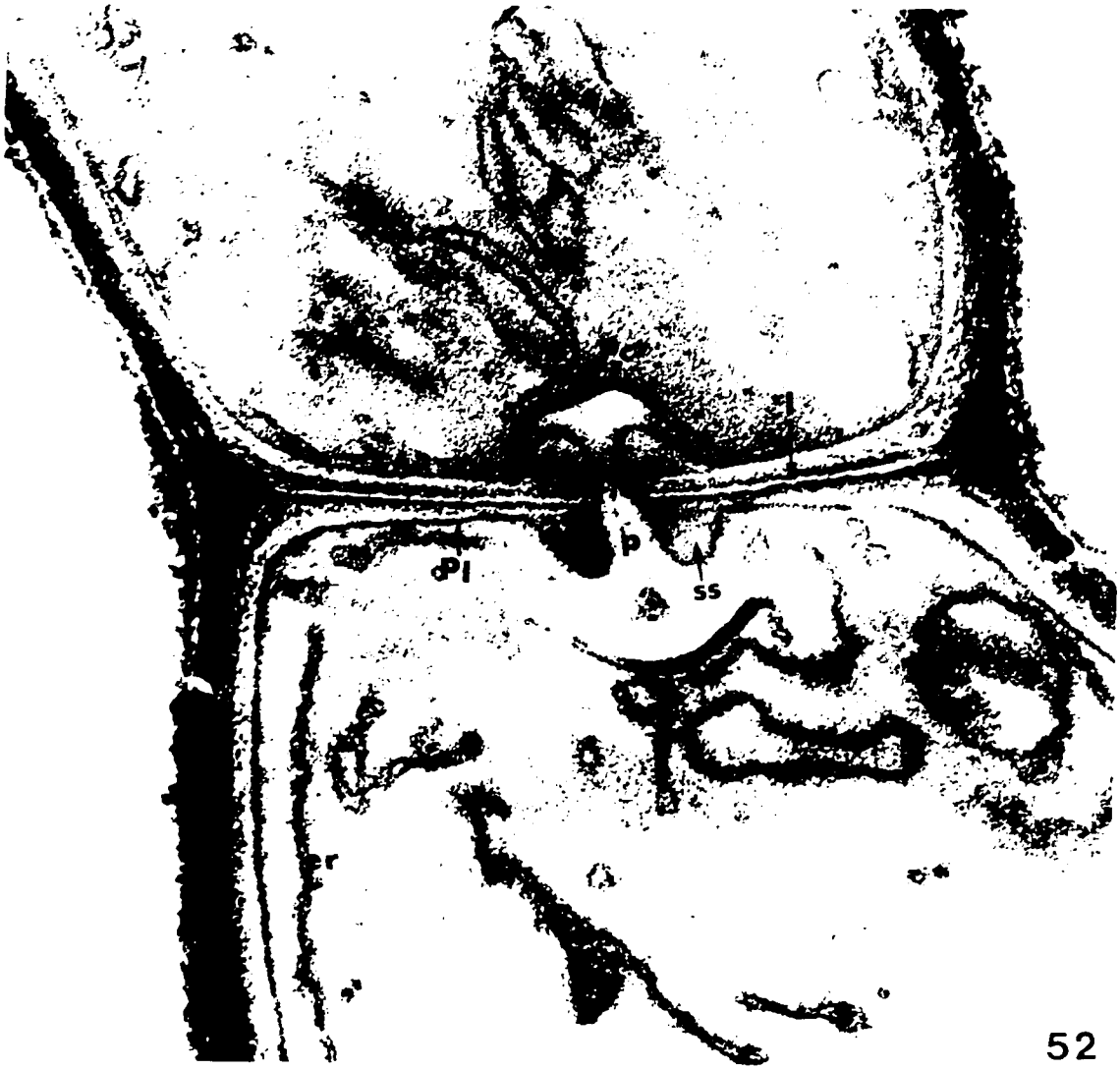
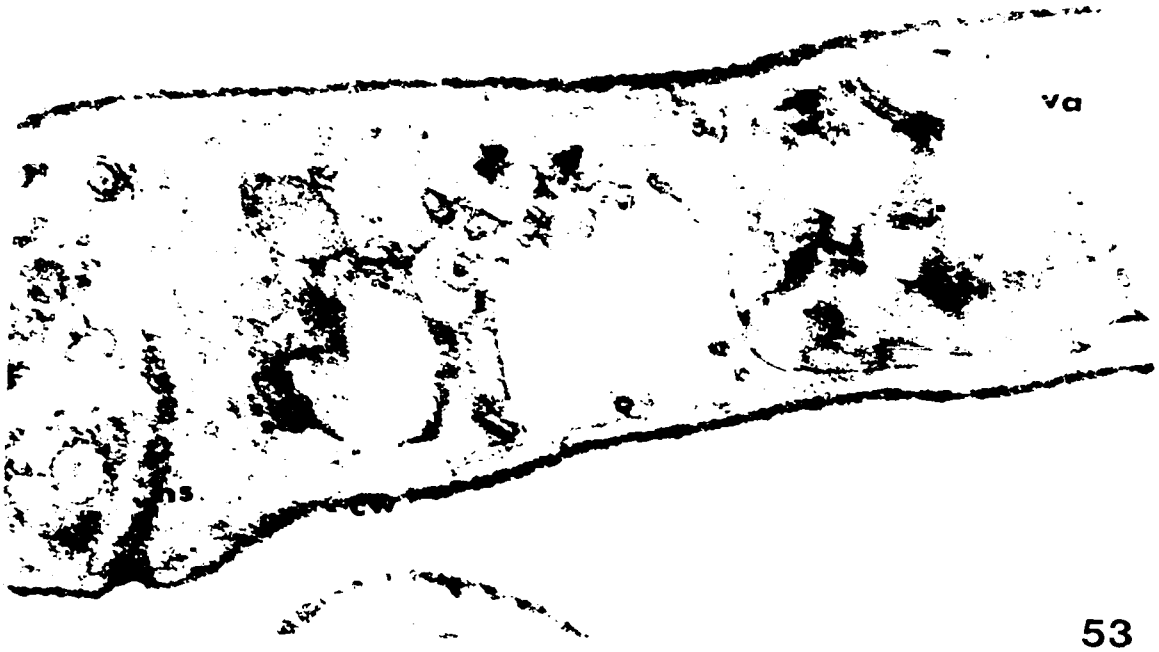

pl
p
ss

FIGURE 53. Longitudinal section through a thin-walled hypha of variety *populinus* showing half septum (hs). Fixation - $KMnO_4$. X.56,250.

FIGURE 54. Longitudinal section through a thin-walled hypha of variety *populinus* showing cell wall invagination (ivc) from both sides of the wall. Fixation - $KMnO_4$. X.42,000.

FIGURE 55. Longitudinal section through a thin-walled hypha of variety *populinus* showing bifercated cell wall invagination (ivc), complex concentric membrane (ccm) and vacuole (va). Fixation - $KMnO_4$. X.38,500.



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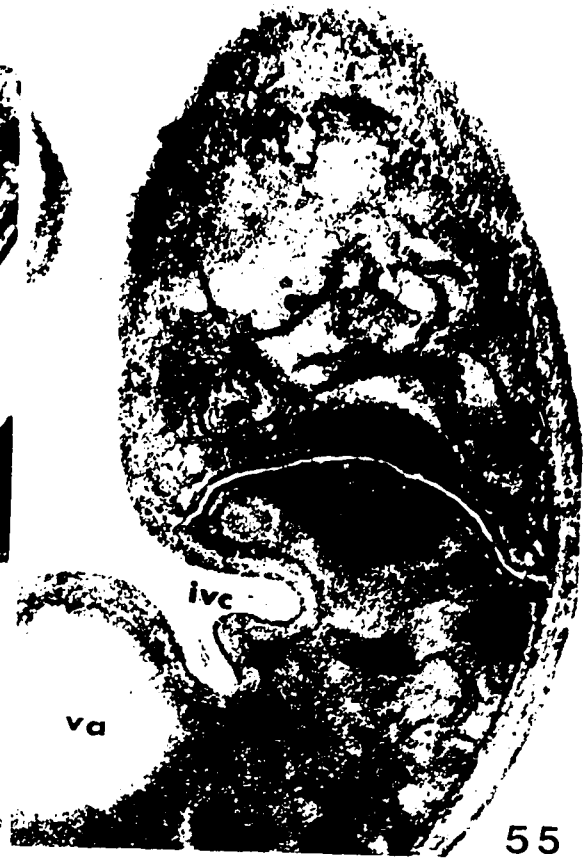

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FIGURE 56. Longitudinal section through the differentiation point showing origin of fiber hypha from thin-walled hypha. A nonmedian dolipore septum (s) is seen at differentiation point.
Fixation - $KMnO_4$. X.51,600.

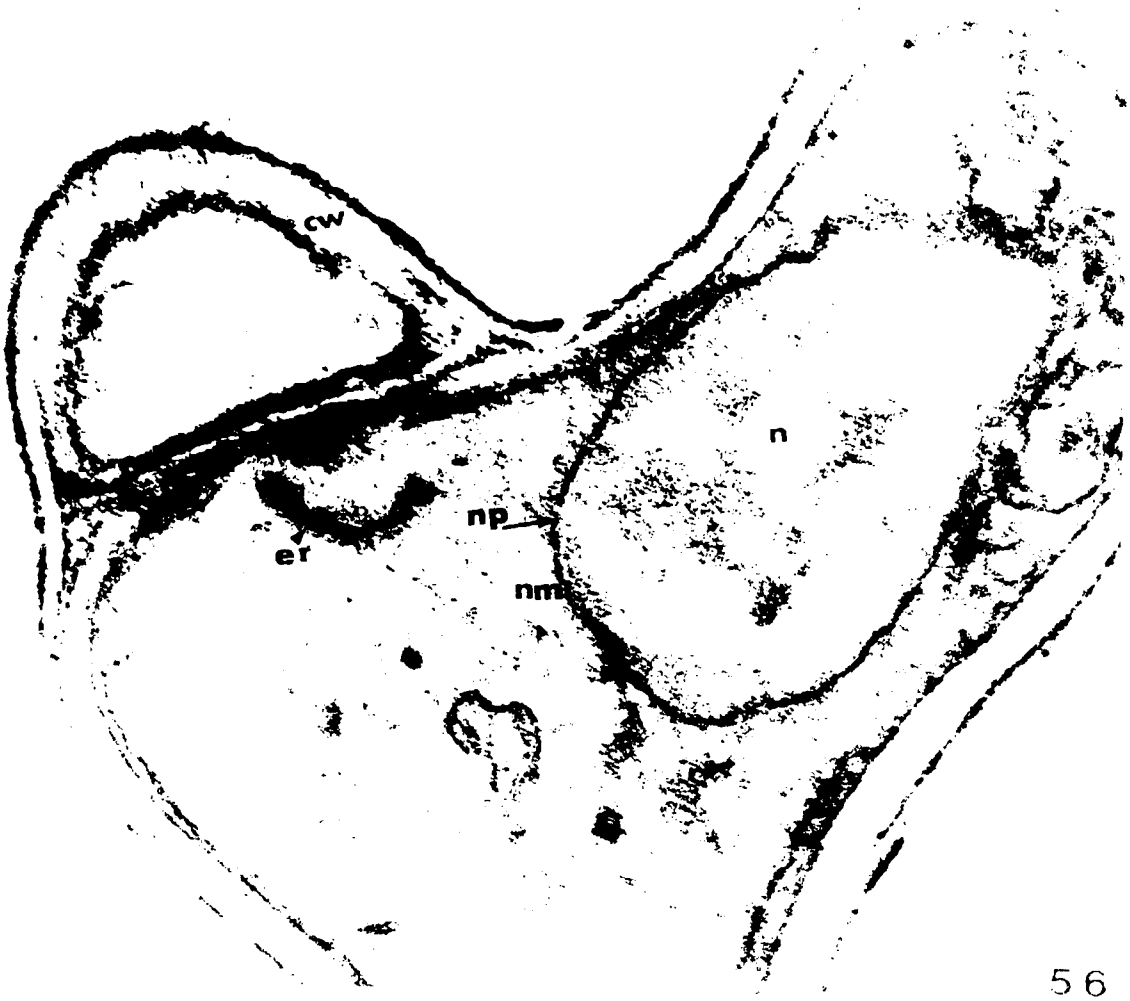

cw
n
np
er
nm

FIGURE 57. Longitudinal section through a fiber hypha of variety *populinus* showing a dolipore septum and dense bodies (db). Fixation - $KMnO_4$. X.45,000.

57

FIGURE 58. Longitudinal section through a fiber hypha of variety *laevigatus* showing double layered cell wall (cw) and dense cytoplasm in central region. Fixation - $KMnO_4$. X.60,000.

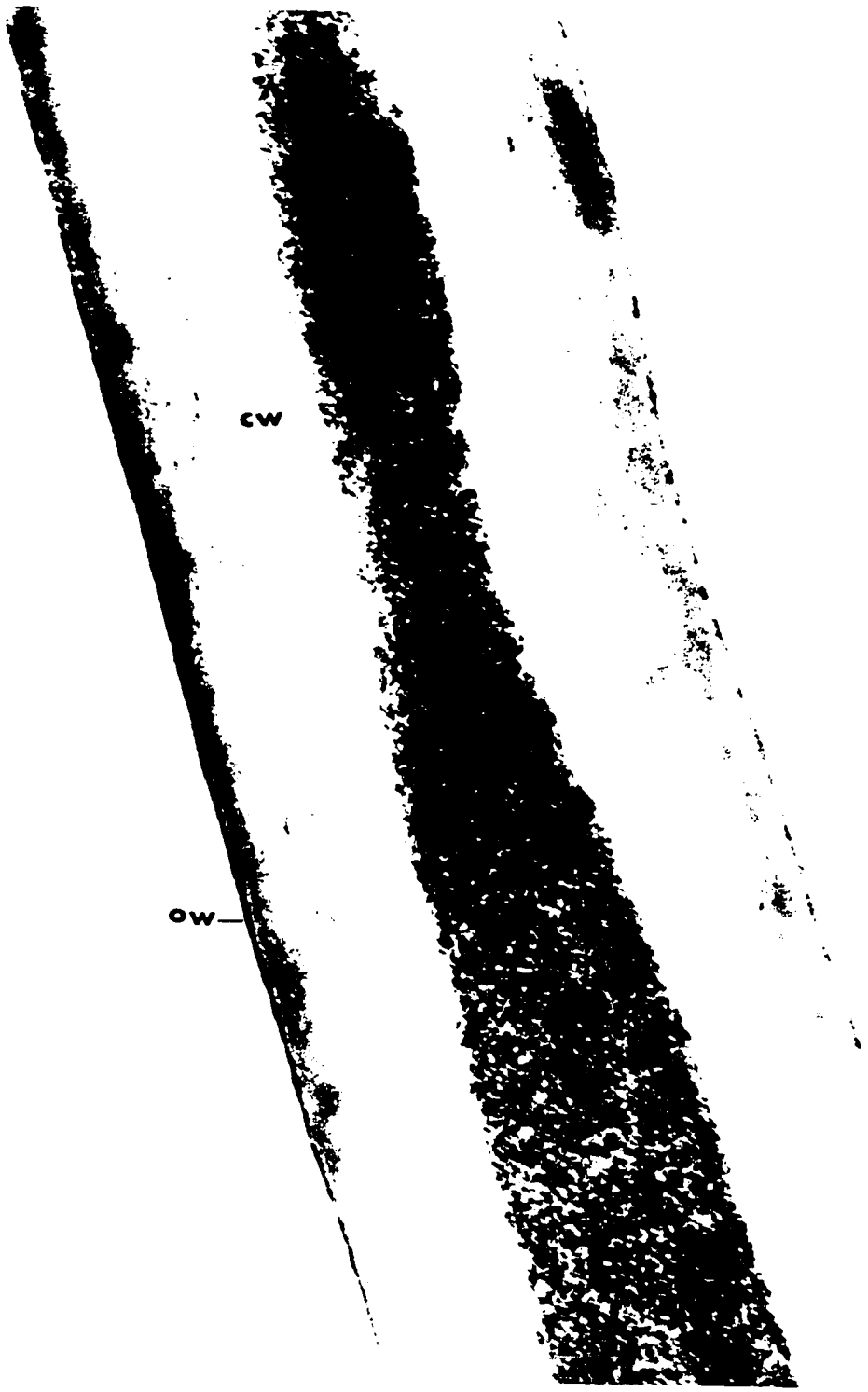
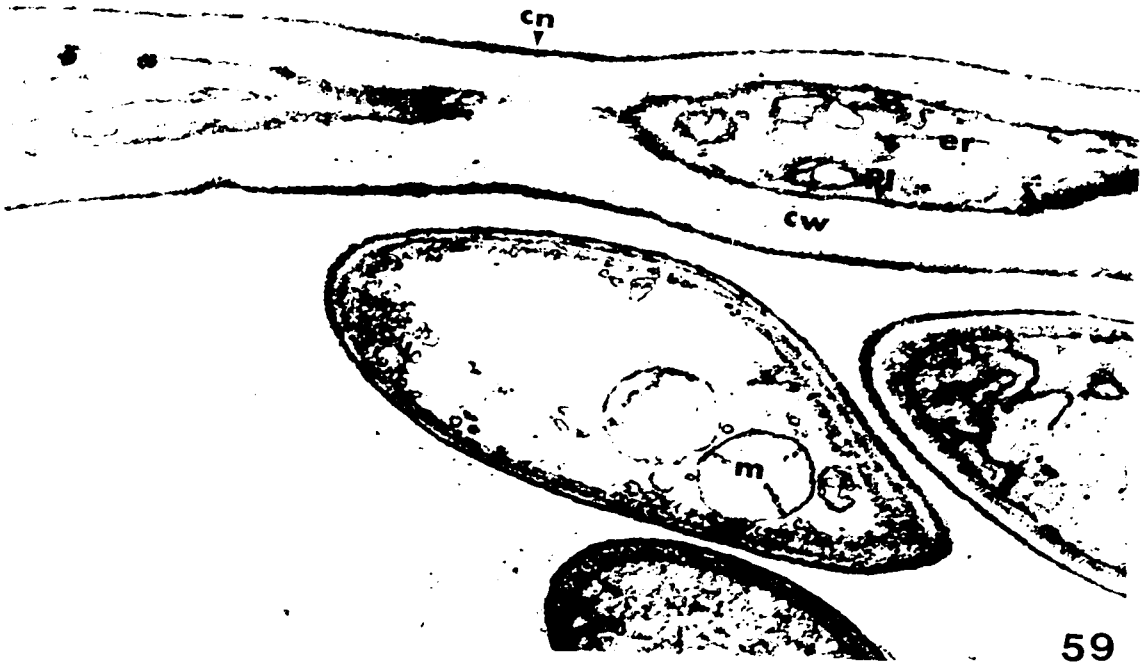

cw
ow

FIGURE 59. Longitudinal section through a fiber hypha and oblique, transverse section through a thin-walled hypha of variety *populinus*. The former showing a constriction (cn) of the cell wall (cw) and axial orientation of microfibrils. The latter showing mitochondria (m) and vesicles (v) near the cell wall. Fixation - $KMnO_4$. X.21,600.

FIGURE 60. Transverse section through a young fiber hypha of variety *populinus* showing cell wall (cw) with circular stratification of microfibrils and diffused cytoplasm with endoplasmic reticulum. Fixation - $KMnO_4$. X.54,000.



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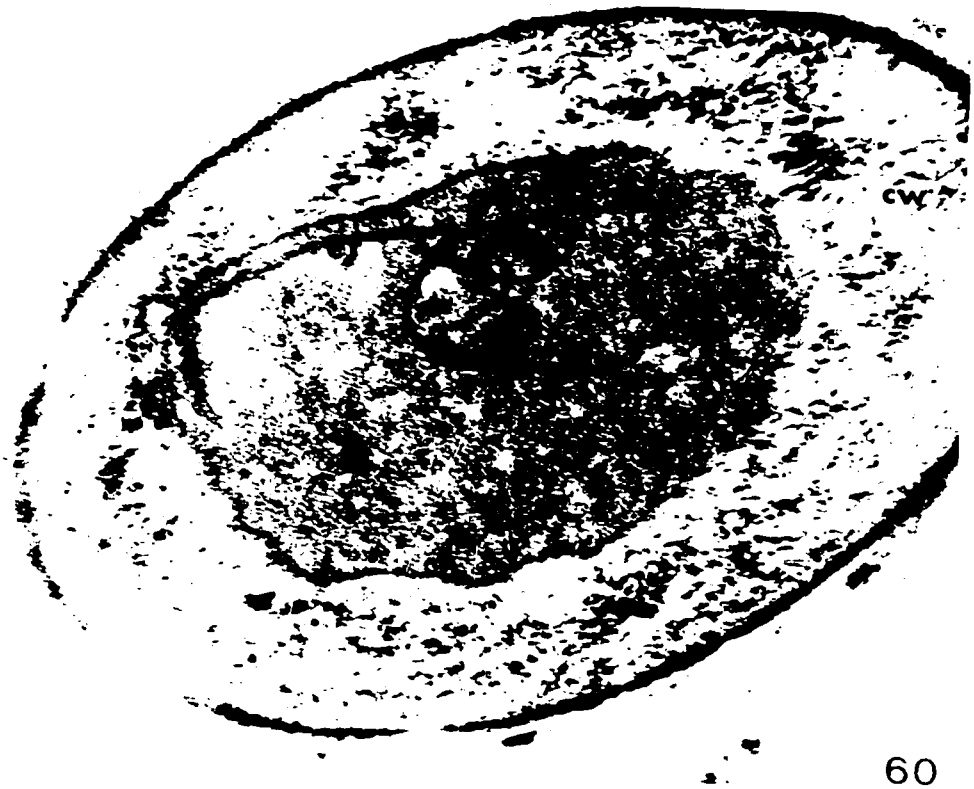

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FIGURE 61. Longitudinal section through a fiber hypha of variety *populinus* showing diffused cytoplasm, a constriction (cn) in cell wall and axial orientation of microfibrils in triple layered cell wall (cw). Fixation - $KMnO_4$. X.51,600.

FIGURE 62. Longitudinal section through a mature fiber hypha of variety *populinus* with thickened cell wall (cw) and apparently no lumen. Fixation - $KMnO_4$. X.42,000.

61

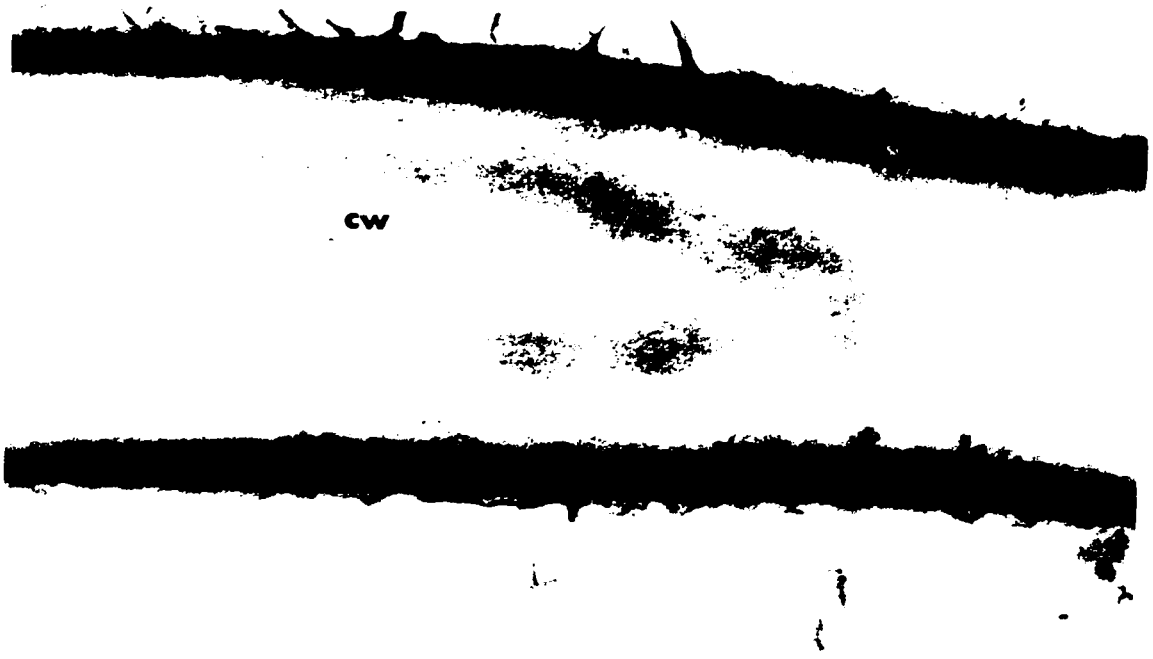

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FIGURE 63. Young cuticular cells of variety *populinus* showing a new branch coming out with plenty of vesicles (v), endoplasmic reticulum (er) and mitochondria (m). Continuity of endoplasmic reticulum (er) with nucleus (n) in upper cell can be seen. Fixation - $KMnO_4$. X.32,400.

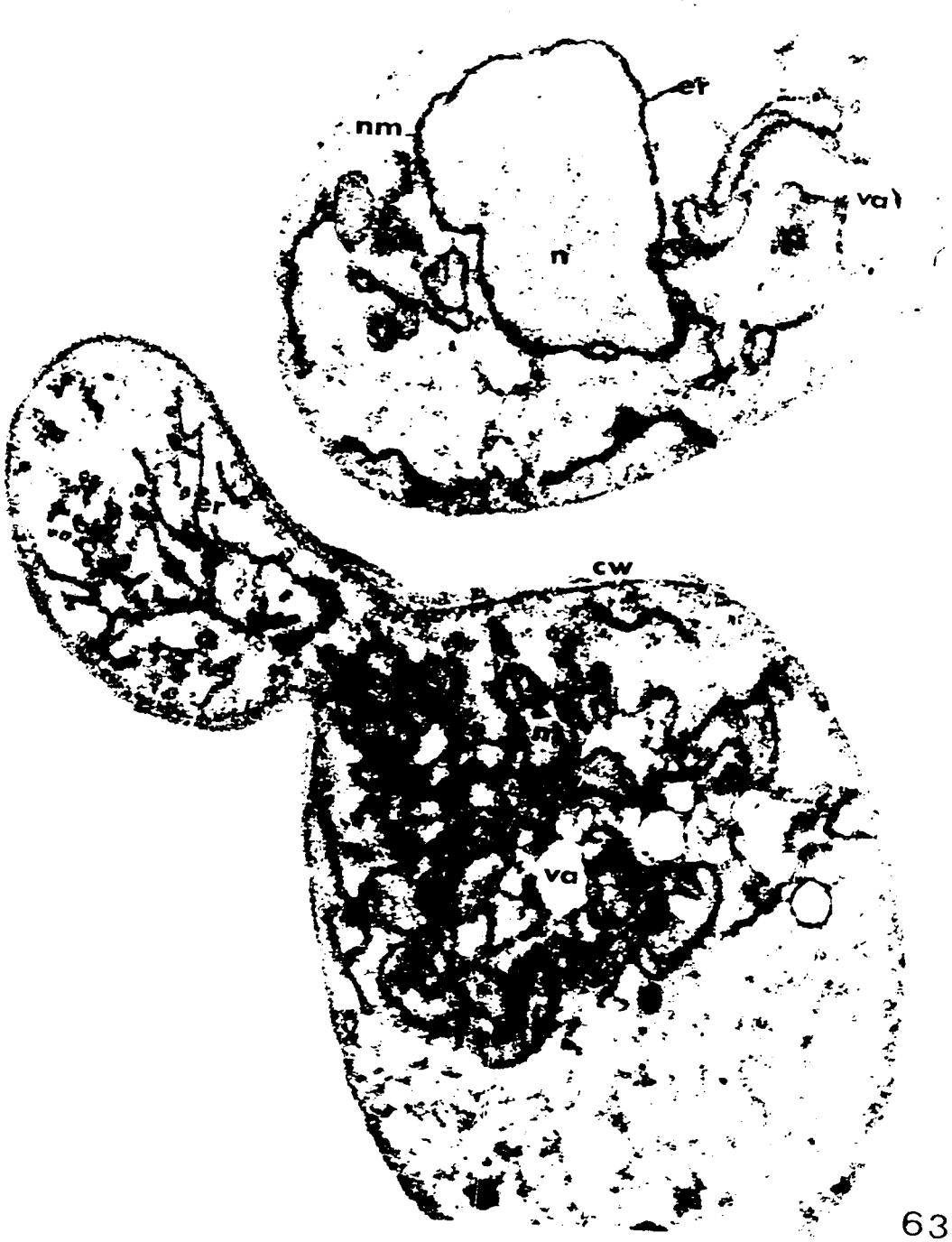

er
nm
n
val
cw
n
va

FIGURE 64. Young cuticular cells of variety *laevigatus* showing nuclei (n), lomasomes (lo), vacuoles (va), endoplasmic reticulum (er) and nonmedian septa (s). Fixation - $KMnO_4$. X.21,600.

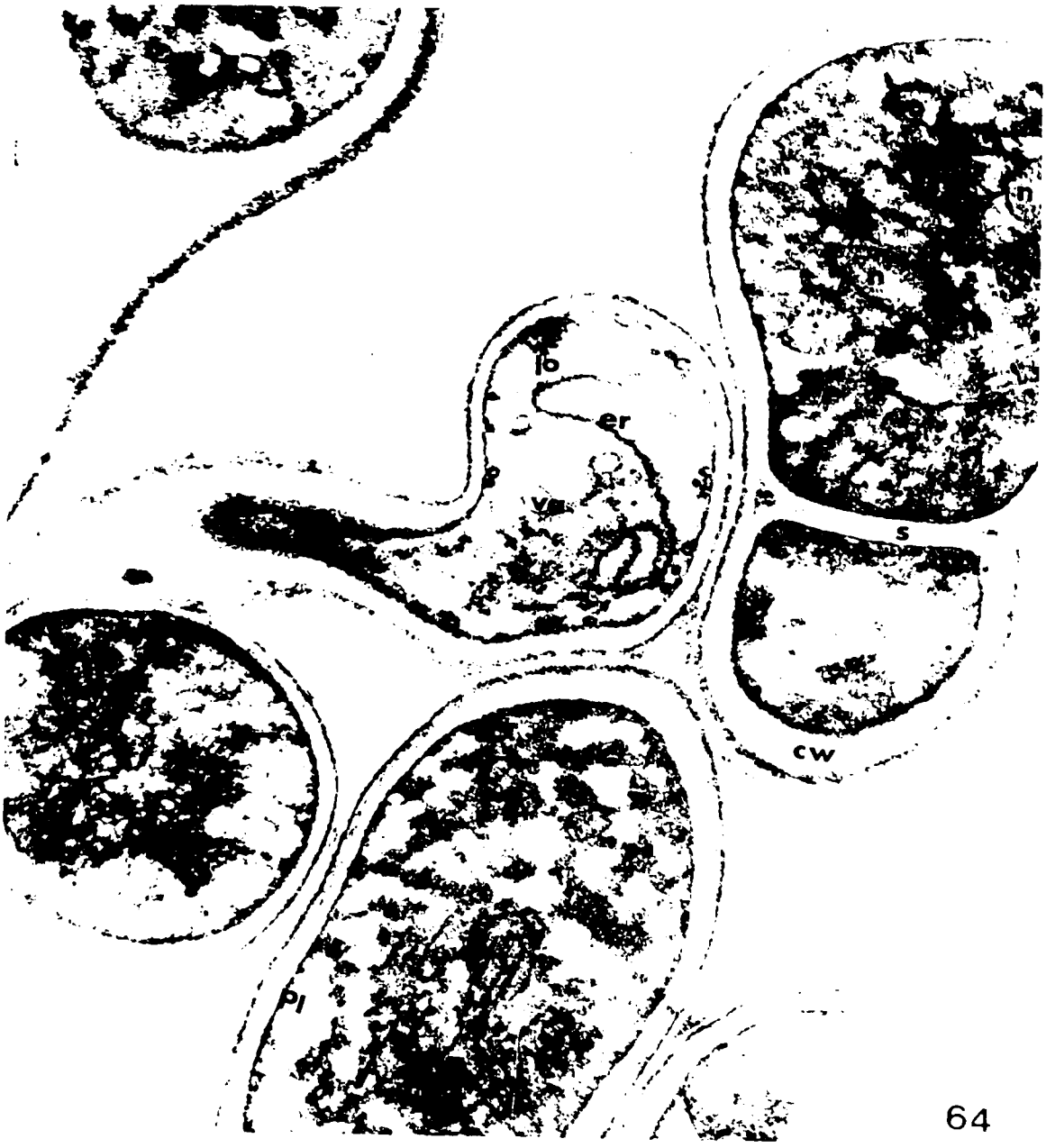
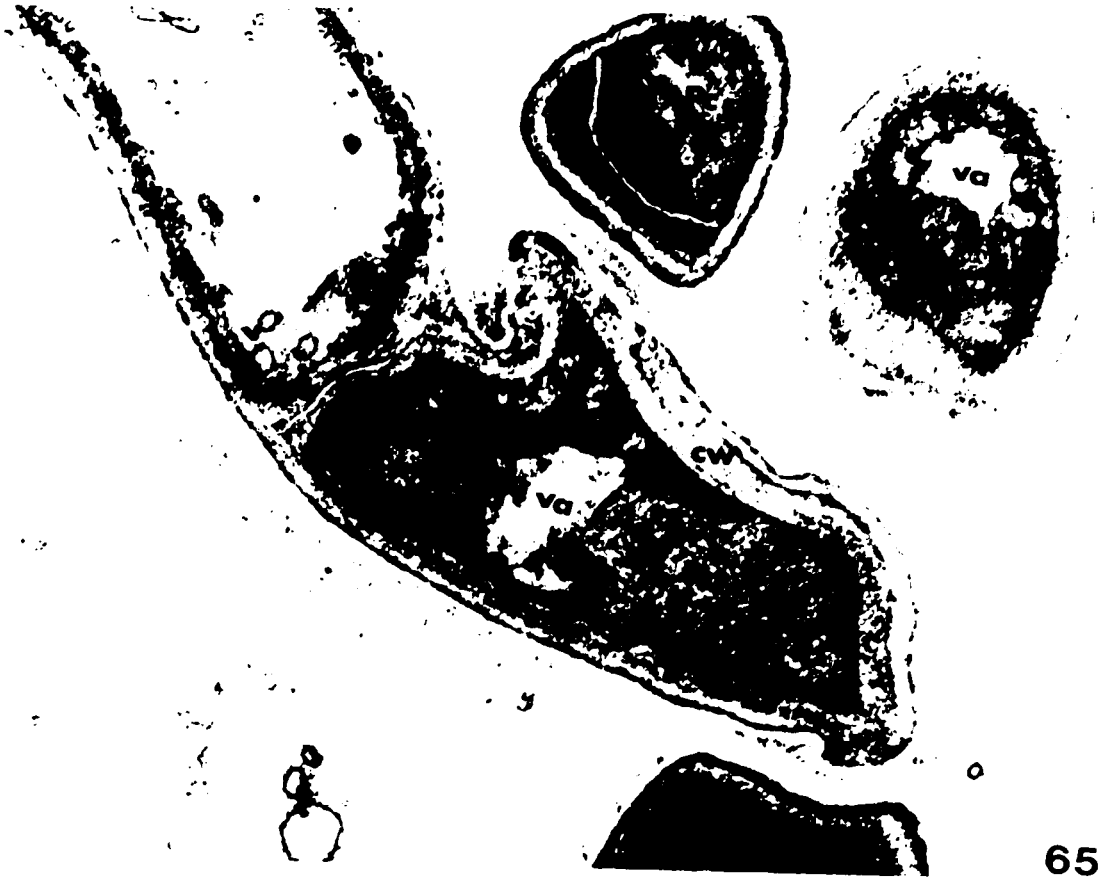

lb
er
vs
s
cw
pl

FIGURE 65. Young cuticular cells of variety *populinus* showing triple layered cell wall (cw), vesicles (v), vacuoles (va) and pore cap (pc) with a nonmedian septum (s). Fixation - $KMnO_4$. X.25,200.

FIGURE 66. Mature cuticular cells of variety *populinus* showing thick cell wall (cw) and diffused cytoplasm in the centre. Fixation - $KMnO_4$. X.21,600.



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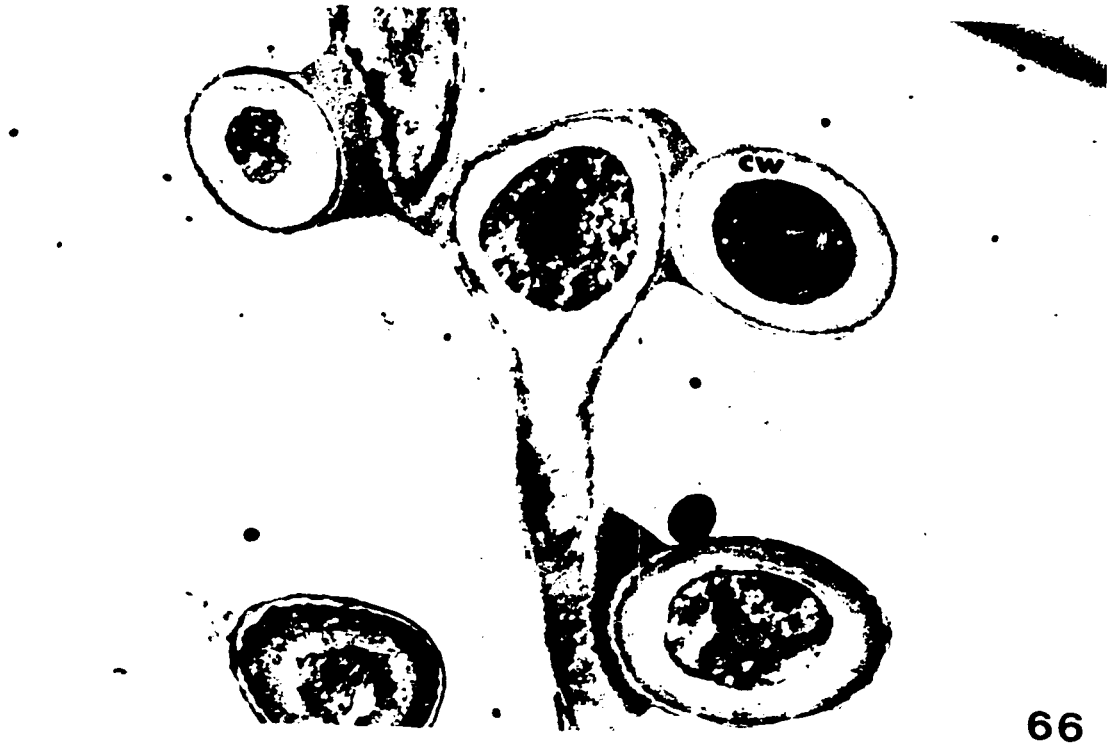

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DISCUSSION

"Largely invisible, little studied, the vegetative mycelium of fungi provides an almost endless series of problems whose investigation is long overdue." (Burnett, 1968).

Macroscopic and microscopic characters of the mature basidiocarp have played the major role in the classification of the Polyporaceae. However, the sporophores of pore fungi do not develop regularly and often appear many years after the mycelium is established in the wood. To solve the practical problem of identification of species causing various wood decays, investigators began culturing the mycelium of various species isolated from wood and describing the cultural features. This type of classification is separate from that based on the structure of the mature basidiocarp, although the identity of the cultures has to be established by comparison with cultures from named basidiocarps. Nobles has carried out comparative morphological studies of the mycelia of a large number of species and from the results developed a system of classification for the Polyporaceae based on morphological and physiological characters of the mycelium grown on malt-agar medium. Since it was necessary to employ standard conditions for such work, the development of the mycelium under diverse environmental conditions was not investigated.

In my view, the characteristics of the vegetative

mycelium which remain constant under variable environmental conditions must be determined and used in the classification of species of the Polyporaceae if a natural system is to be developed. The incorporation of such characters into species descriptions will also be of practical value in Forest Pathology. It should also be pointed out that there is a need for morphogenic studies of the vegetative hyphae in these fungi in order that the growth and differentiation of the mycelium and the way it responds to the environment may be understood. The present study has been concerned with the growth of the mycelium of *Fomes igniarius* and I believe that the results provide a more comprehensive understanding of the structure and development of the mycelium of this species and its varieties in the natural and cultural environment.

A. The Mycelium in the Natural Environment

In both variety *populinus* and variety *laevigatus* the thin-walled, narrow hyphae were observed in all kinds of wood tissue. These hyphae were very much branched. Henningsson (1965) working with *Polyporus betulinus* reported the presence of hyphae in all kinds of wood cells in infected birch. Cartwright and Findlay (1958) reported that the two kinds of hyphae, present in *Fomes fomentarius*, were mainly in the vascular rays. These workers found that in *F. ulmarius* the hyphae occurred in the vessels and vascular rays. They noted that the hyphal branches, coming off at right angles from the main hypha, penetrated the cell wall directly. They

also found that in *F. igniarius* hyphae were plentiful in advance stages of rot. They observed stout hyphae in the initial stages which became very fine and much branched at later stages of growth.

The mycelium of variety *laevigatus* growing in birch wood tissue also possessed thin-walled, broad hyphae. Fiber hyphae were present in wood tissue in both varieties. Cuticular cells were also observed in the wood tissue but these cells were mainly restricted to the zone lines. Cartwright and Findlay did not report the presence of fiber hyphae or cuticular cells in the mycelium of *Fomes igniarius* growing in the wood tissue. However, they have reported the presence of thick-walled, gnarled hyphae in the zone lines. These gnarled hyphae were probably the cuticular cells observed in the present study. Thick-walled, fibrous hyphae produced by *Fomes fomentarius* in wood tissue have been reported by Cartwright and Findlay. Fisher (1934), working with *F. pomaceus*, reported the presence of a weft of fine, thick-walled, rather fibrous, honey colored hyphae which filled the large vessels. He further reported that in the zone of incipient decay, where the wood appeared dark purplish brown, a copious deposit of brown gum could be seen filling the vessels.

The presence of the three kinds of hyphae in both the varieties suggests that such mycelial characters can be used as a basis for the identification of *F. igniarius* and other species of this family. The occurrence of two kinds of thin-

walled hyphae, thin-walled broad and thin-walled narrow hyphae in the variety *laevigatus* serves to distinguish varieties of this species.

There was some difference in the diameter of the hyphae growing in naturally infected wood tissue compared with that of hyphae growing in inoculated wood. In variety *populinus* the diameter of thin-walled, narrow hyphae ranged from 0.75 to 3.0μ in naturally infected aspen poplar wood tissue, whereas in inoculated wood tissue the hyphal diameter varied from 0.75 to 3.7μ. In variety *laevigatus* the diameter of the thin-walled, narrow hyphae ranged from 0.75 to 2.6μ in naturally infected birchwood tissue, while in the inoculated wood the diameter of the hyphae ranged from 0.75 to 3.0μ. The greater diameter of the hyphae in inoculated wood could be attributed to more nutrients in culture.

In both varieties the hyphae were observed passing through the bordered pits and the border part of a pit. The dissolution of the bordered pit was also observed. Proctor (1941) has reported general dissolution of the bordered pit, penetration of the border part and growth of hyphae through the bordered pit by *Fomes annosus* growing on eastern white pine. The penetration of the cell walls by fungal hyphae is a problem which has attracted the attention of a number of workers. In the present study the thin-walled hyphae in the early stages of penetration were very narrow but they regained their usual diameter after penetration. The penetration would seem to be effected by hyphal enzymes,

since it appears difficult for so fine a thread with no appressorium to penetrate a thick, lignified cell wall by mechanical means. Nutman (1929) suggested a similar mechanism of penetration in *Polyporus hispidus*.

Bore-holes in the wood cells were frequently observed where the mycelium of this species was growing. In size these holes were several times the diameter of the hyphae which produced them. Probably bore-holes were also due to enzymatic activity by the fungal hyphae. In reports on *Polyporus hispidus* (Nutman, 1929), *Poria monticola* (reported as *Tramates serialis*, Cartwright, 1930), *Fomes annosus* (Proctor, 1941) it is assumed that the bore-holes are formed by enzymatic activity of the hyphae.

Nutman working with *P. hispidus* and Cartwright working with *Poria monticola* observed that on approaching or on contact with the cell wall, the contents of the hyphae become concentrated at the tip. From this tip a fine projection emerged which developed into a fine thread that penetrated the cell wall. In the present study such a projection was not observed. Perhaps the rapid penetration of the cell wall by the hypha prevented observation of the projection, or it may not be formed in this species. Proctor has reported that the penetration of the walls of the wood cells is accompanied by secretion of enzymes at the tip of the penetrating hypha and that there is total, local dissolution of the cell wall by enzymatic activity in advance of the actual passage of the hypha through the wall. He postulated

that penetration is accomplished through a preformed passage without contact between the hypha and the cell wall, except perhaps at the very first point of penetration.

B. The Mycelium in the Cultural Environment

Cultural work provides a basis for evaluating the stability of various characters associated with the development of the mycelium. Information concerning the stability of characters under different environmental conditions is of fundamental importance in working out the taxonomy of a group. The present study revealed the stability of various mycelial characters of *F. igniarius* and therefore, these characters could be employed in identification of this species. The lack of stability of certain characters can be explained on the basis of changes in environmental factors.

Fomes igniarius var. *populinus* was found to be a slow growing fungus. On gallic and tannic acid agars the diffusion zones were weak to moderately strong suggesting the presence of extracellular oxidase (Nobles, 1948). No growth was observed on either medium.

The variety *laevigatus* was found to be a fast growing fungus. The diffusion zones were observed to be moderately strong on gallic and tannic acid agars. It grew on gallic acid agar with a colony diameter of 1.2 - 2.8 cm, but on tannic acid agar no growth was observed. Nobles (1948) reported no growth to 2.0 cm diameter on tannic acid agar for this variety.

Both varieties produce extracellular oxidase as indicated by the formation of diffusion zones, but the different intensities of these zones indicate that there exists a quantitative variation between and within the varieties. Therefore, this criterion cannot be used to distinguish the different varieties within this species.

In both varieties the color of the mycelium changed with age. In variety *populinus* it ranged from cream to dark brown color, whereas in variety *laevigatus* it was cream to orange-cinnamon color. These observations were in agreement with those of Nobles (1948, 1958, 1965). Since each variety has a distinctive coloration, pigment analysis could provide a useful tool in classification.

The varieties *laevigatus* and *populinus* were found to produce a mild and strong wintergreen odor respectively. Since Nobles (1948) and others have made similar observations, this character is another feature of taxonomic importance in classifying at the varietal level.

In liquid, malt extract medium the dry weight of the mycelium in variety *laevigatus* steadily increased up to the termination of the experiment - under all temperatures except 34°C, where a decrease in dry weight was noticed after 30 days of growth. This decrease in dry weight could have been due to the production of toxic substances or the process of autolysis or both. At low temperatures (15°, 21° and 24°C) the mycelial growth showed a linear pattern. This linear pattern of growth was observed at 27°C as well up to 35 days

of growth. The rate of growth then decreased giving the growth curve a curvilinear appearance. At 27°C the growth is more rapid and the nutrients are consumed in a shorter time. This decline in nutrients could be a reason for the decrease in the rate of the growth of mycelium at this temperature during later stages.

In variety *populinus* there was a decline in growth at temperatures of 24°, 27° and 34°C. This decline in growth was not noticed at low temperatures (15° and 21°C), in which case the dry weight of the mycelium continued to increase up to the termination of the experiment. This difference in the growth rate could be due to the same processes suggested under variety *laevigatus*.

The optimum temperature for the growth of both varieties of this fungus in solid and in liquid medium was found to be 27°C. Verrall (1937), Oshima (1953), Cartwright and Findlay (1958), Wall (1962) also obtained optimum growth at around 27°C. Hubert (1931) gives the optimum temperature for *F. igniarius* as 30°C but does not mention the nature of the medium used.

Source of carbon is another important factor affecting the rate of growth of fungi. In the present study, of the carbon sources used, cellobiose was found to be the best in terms of growth rate of mycelium. The growth obtained was even better than on its monomer dextrose. Henningsson (1965) working with *Polyporus betulinus* sometimes obtained better growth with cellobiose than with dextrose. In experiments

where cellobiose and dextrose were the carbon source, a decline in the dry weight of the mycelium started after a period of steady increase (about 30 days) in growth. The decline in weight of mycelium could be due to initiation of the process of autolysis. Cessation of growth is considered to occur because of the lack of nutrients in the medium or the production of toxic substances at later stages. However, when maltose was the carbon source the decrease in growth was noticed only in variety *laevigatus*, the dry weight of the mycelium in the other variety continued to increase until the termination of the experiment. These results with maltose could be explained by the difference in the growth of the two varieties. Variety *laevigatus* grew more rapidly in this medium than variety *populinus*. With carboxymethyl cellulose as the carbon source decrease in growth was not observed in either variety. Probably the explanation here is the very slow growth of this fungus in the medium having carboxy-methyl cellulose as the carbon source.

The results of the experiments in which the mycelium was grown in still and shake culture represent the yield of mycelium more accurately than experiments with growth on solid media. The reason is that most of the gelatinous material covering the mycelium can be washed off before determining the dry weight. Both the varieties produced more mycelium in shake culture than in still culture. Shaking of the medium provides the best availability of dissolved oxygen - which is the main reason for better growth

of the fungi in shake culture. Soon after the maximum growth was obtained, a decrease in dry weight was observed in both varieties.

During the growth period in liquid medium, there was a fall in pH of the medium at higher temperatures (24° and 27°C). The pH change could have been due to the production of acid substances or to the preferential utilization of certain salts or both, at different stages of growth. The pH change was greater with the culture of the variety *laevigatus* than with the variety *populinus* and may have been due to the faster growth of the mycelium of variety *laevigatus*.

The growth of variety *populinus* was enhanced when a wood chip was added to the malt-agar medium. According to Wall (1962) some substances which act as a stimulant for growth may be present in the wood tissue.

Under all cultural conditions provided both varieties of *F. igniarius* possessed thin-walled narrow hyphae, fiber hyphae and cuticular cells. In addition to these structures, variety *laevigatus* produced thin-walled, broad hyphae. Nobles described similar kinds of hyphae in her cultural work with this species. Ring formation (flat coils) by variety *laevigatus* was very frequently observed. Such hyphal rings have been reported by Mounce (1929) in *Fomes pinicola*. States observed them in *Gleophyllum saepiarium* (1969).

In variety *populinus* the hyphae frequently show half septa and constrictions and invaginations of cell walls were also seen. Nobles did not report these characteristic

features of variety *populinus*. Both varieties produced expanded fiber hyphae. Nobles (1965) reported such expanded fiber hyphae in *Fomes pini* but not in *Fomes igniarius*.

Broad hyphae, fiber hyphae and cuticular cells originated from the thin-walled narrow hyphae, which made up the first kind of mycelium observed in culture.

The branching pattern reported for the mycelium of *F. igniarius* in the present study is in agreement with the branching pattern observed in other Basidiomycetes by Langeron, Butler, Groove, Park (cited by Butler, 1966). Hyphal anastomosis was not observed in juvenile marginal hyphae in spite of their frequent close proximity, but, it was common in older parts of the colony in *F. igniarius*. This feature was observed by Buller (1933) in Basidiomycetes, Ascomycetes and in many Deuteromycetes.

All the expected kinds of hyphae were observed in both varieties under variable temperatures and on various media which provides evidence of the stability of these characters. The expanded fiber hyphae did not develop at low temperatures, e.g. 10°C, where the mycelial growth was very slow.

In the foregoing discussion a number of characters of *F. igniarius* which are stable under variable environmental conditions have been described. The more important of these characters are the invariable presence of the three kinds of hyphae and absence of clamp connections. At the varietal level differentiation on the basis of stable characters can be made in accordance with the color of the mycelium,

intensity of odor and the rate of growth. In addition, variety *populinus* differs from the other variety in the presence of half septa, hyphal constrictions and invaginations of the cell walls. The variety *laevigatus* is characterized by ring formation in the hyphae and the presence of thin-walled, broad hyphae.

C. Ultrastructure of the Mycelium

Cultural studies provided evidence in support of the proposition that the mycelium can provide characters of taxonomic significance. A greater understanding of the structure of the mycelium of the two varieties of *Fomes igniarius* required a study of its fine structure, which had not been investigated. To my knowledge, there are no reports of ultrastructural work with any *Fomes* species.

The development of electron microscopy since 1950 has provided clear evidence of the occurrence of microfibrillar, structural elements in the walls of fungal cells. Willets and Calonge (1969) have reported that there is usually a single layered cell wall in brown rot fungi (Ascomycetes), although in some cases a double layered wall has also been observed. Girbardt (1958) for *Polystictus versicolor* and Giesy and Day (1965) for *Coprinus lagopus* reported the presence of a double layered cell wall. Berliner and Duff (1965) working with *Armillaria mellea* described a multilayered cell wall in which the outer wall was usually sloughed off. In *Fomes igniarius* mycelium, the hyphal cell

wall was found to be single layered or multilayered and was usually triple layered. In the region bounded by the cell wall are present all the organelles such as endoplasmic reticulum, mitochondria, nuclei, etc., which have been found in other Basidiomycetes. However, a characteristic feature of the cell of this fungus is the presence of complex concentric membranes. Similar structures have been reported in *Lenzites saepiaria* by Hyde and Walkinshaw (1966) and in *Sclerotium rolfsii* by Nair *et al.* (1969). The latter believe these structures to be involved in the formation of lomasomes. In my study the lomasomes were seen to bud off from the complex concentric membranes an observation which gives support to this belief.

A second characteristic feature of the hyphae is the presence of dense bodies similar to those reported by Manocha in *Agaricus campestris* (1965). He believes that these bodies increase in size and that their contents later become less dense and then coalesce to form vacuoles. In *F. igniarius* as well, these unidentified dense bodies are present and become lighter in color as they enlarged in size with increasing age (Figure 33). These bodies are seen to congregate together but no clear evidence of their fusion was obtained.

The usual location of vesicles and multivesicular bodies near the cell wall lends support to the argument put forth by Marchant, Peat and Banbury (1967) that these bodies are associated with the deposition of the microfibrils of the cell wall. The vesicles and multivesicular bodies are

presumed to originate from endoplasmic reticulum. These vesicles appear to fuse with plasmalemma and perhaps help in the thickening of the cell wall. The crenulate appearance and discontinuity of the plasmalemma is further evidence in favour of this argument.

The dolipore septum found in the hyphae of *F. igniarius* conforms in a general way to the one described for other Basidiomycetes by a number of investigators especially Girbardt (1958), Moore and McAlear (1962c), Wells (1964), Wilsenach and Kessel (1965), Bracker and Butler (1963), Giesy and Day (1965). Giesy and Day (1965) report a two-lamellar pore cap in the dolipore septum of *Coprinus lagopus*, but in *F. igniarius* there is a four-lamellar pore cap which is similar to the pore cap found in *Rhizoctonia solani* (Bracker and Butler, 1963).

Wilsenach and Kessel (1965) have drawn the general conclusion that, in the Polyporaceae, pore cap pores occur at regular intervals of 400-800Å. I did not find this to be the case in *F. igniarius*. In this species pore cap pores occur at irregular intervals and are of varying size. However, the hypothesis put forward by them that the nuclei, mitochondria and endoplasmic reticulum cannot pass through the pore cap pores seems justified. In view of the observation that the pore cap pore is much smaller than these cell organelles, the passage of the organelles through the pore cap pore does not seem to be possible in spite of their elasticity.

A dolipore septum is present at the differentiation point where the fiber hypha originates from a thin-walled hypha and it is thought to help in maintaining protoplasmic continuity between the adjacent cells in early stages of fiber development. During the thickening of the cell wall in these fiber hyphae the cell organelles gradually disintegrate which results in a diffuse appearance of the cytoplasm. At the same time the cell wall becomes thick by deposition of additional layers. These observations are in agreement with the observations made by Schmid and Liese (1968) on fiber formation in *Armillaria mellea*. A constriction of the fiber hypha is often seen in *F. igniarius* var. *populinus* and similar constrictions have been reported by Willets and Calonge (1969) in the hyphae of the stroma of brown rot fungi.

The presence of cuticular cells in the mycelium of this fungus is another characteristic feature. Like the fiber hyphae these too show a diffuse appearance of the cytoplasm in intermediate stages of development and are thick-walled at later stages. In these cells remnants of the cytoplasm remain at maturity.

In contrast to the thin-walled hyphae where the cell organelles such as mitochondria, endoplasmic reticulum, vesicles, etc., are present in abundance, in the fiber hyphae these organelles are not so numerous and, when the cell wall thickens during the maturation of the fiber, the organelles and cytoplasm disintegrate leaving a narrow lumen. It is presumed that most of the cytoplasmic material is

utilized in cell wall thickening. The cuticular cells undergo similar changes during later stages of development.

Electron microscopy contributed valuable information regarding the detailed structure of the various kinds of hyphae found in *Fomes igniarius*. Future ultrastructure studies of the vegetative mycelium in other species of the Polyporaceae and various *Fomes* species, in particular, would help in establishing relationships between various taxa of these fungi. Such studies would also lead to an understanding of the development of hyphae modified for particular functions.

D. The Developmental Morophology of the Mycelium of *Fomes igniarius*

The following description of the mycelium of *F. igniarius* and its varieties will serve to sum up the features of this stage which are thought to be of taxonomic significance. It includes only characteristics which have been found to remain constant under changing environmental conditions and are therefore considered to be of taxonomic value.

Characters of the Mycelium in Wood

In both varieties, *populinus* and *laevigatus*, thin-walled, narrow hyphae occur in all kinds of wood tissue. The fiber hyphae and cuticular cells are also present, the latter being confined to zone lines. In addition, thin-walled, broad hyphae, characteristic of variety *laevigatus*, occur in wood tissue. The thin-walled hyphae are plentiful and are

very much branched and septate. They penetrate the cell wall freely causing bore-holes. Clamp connections do not form on the mycelium in wood.

Characters of the Mycelium in Culture

Macroscopic and Physiological Characters

In *Fomes igniarius* the maximum mycelial growth takes place at 27°C in both solid and liquid media. In the early stages of growth the advancing zone is even and mycelium is cream colored. The mycelium peels from the agar readily. The fungus produces a wintergreen odor on malt-agar medium. Diffusion zones are produced by *F. igniarius* on gallic and tannic acid agars.

F. igniarius var. *populinus* is a slow growing fungus. The advancing zone becomes bayed after three to four weeks on malt-agar medium. The color of the mycelium becomes dark brown at later stages (4-5 weeks) of growth. The mycelium is raised, thick and wooly, frequently growing to the top of petriplates near the inoculum plug and sloping gradually towards the edge. The reverse is unchanged in color and shows conspicuous zones and radiating lines. No growth takes place on gallic and tannic acid agars. The fungus produces a strong wintergreen odor on malt-agar medium.

The variety *laevigatus* produces a faster growing mycelium than the variety *populinus*. The advancing zone is hyaline with the hyphae well separated from each other on

malt-agar medium. The color of the mycelium becomes orange-cinnamon at later stages of growth but the white border persists. The mycelium is slightly raised, cottony in the initial growth, then wooly to felty at later stages. Zones of the aerial mycelium are also observed. The color of the reverse is changed and finally the whole medium is liver brown in color. The wintergreen odor is mild in this variety. This fungus grows on gallic acid agar but there is no growth on tannic acid agar.

Microscopic Characters

The mycelium of *Fomes igniarius* consists of three kinds of hyphae - thin-walled hyphae, fiber hyphae and cuticular cells. The thin-walled hyphae are frequently branched and lack clamp connections. The type of branching is monopodial and branches form in acropetal succession. The branches arise mostly at acute angles, but branching at right angles and in whorls occurs occasionally. Hyphal anastomosis is frequent in thin-walled hyphae. Fiber hyphae and cuticular cells originate from the thin-walled hyphae with a septum at the differentiation point. The fiber hyphae and cuticular cells are dark brown in color when fully developed.

The distinctive characters of the variety *laevigatus* are the presence of thin-walled, broad hyphae and the development of hyphal rings (flat coils). In variety *populinus* the presence of half septa, cell wall constrictions and invaginations are the distinguishing features.

Ultrastructure Characters of the Mycelium

The hyphal cell wall in *F. igniarius* may be single or multilayered. The dolipore septum is found in all hyphae and there is a dolipore septum at the differentiation point where a fiber hypha and cuticular cell originate from a thin-walled hypha. Vesicles and multivesicular bodies, occur near the cell wall. Cell organelles such as endoplasmic reticulum, mitochondria, nuclei, vesicles, etc., are abundant in young hyphae. Complex concentric membranes, which give rise to lomasomes, are present in the cytoplasm. The mature fiber hyphae and older cuticular cells have thick micro-fibrillar cell walls and diffused cytoplasm.

It can be seen from the above description that thin-walled hyphae, both narrow and broad, fiber hyphae and cuticular cells which develop under cultural conditions are also characteristic of the mycelium growing in the natural environment - wood tissue. Furthermore, the degree and pattern of branching and the kind of septation are the same. From these facts one can conclude that the mycelium of this species has a distinctive morphology which is expressed in both the natural and artificial environment. Such evidence does not support the commonly held idea expressed in the statement by Chesters (1968) that "The morphology of the mycelium of a fungus within its host substrate may be entirely useless as a diagnostic criterion, not because it lacks characteristic features, but because these are difficult to display and to observe". In this species the mycelium does

have morphological characters which are constant and can be adequately described. The mycelium of *F. igniarius* also has distinctive physiological characters such as maximum growth at 27°C and the wintergreen odor which are expressed under specific cultural conditions. Such characters can be used to identify the species and are more useful than structural features in identifying the varieties.

Natural taxa can be defined only when all stages of the life history of the organisms are fully described and understood. The mycelium is an important phase in the life cycle of these fungi but its use in classification has been limited to diagnostic keys designed for the identification of cultures isolated from decayed wood. Information on the structure and development of the vegetative mycelium in the natural substrate and in culture, which could be correlated with the description of the structure and development of the basidiocarp, is essential for satisfactory classification. It is suggested that parallel studies of hyphal structure and organization in the mycelium and in the basidiocarp of species of Polyporaceae would reveal many characters of taxonomic value.

In this investigation the mycelium of one species and its varieties has been intensively studied and the characters which are stable under variable conditions determined. In addition, a clear understanding of the mode of development of this phase of the life cycle has been gained and the relationship between characters expressed in the natural and cultural

environments established,

It will be necessary to carry out similar studies of the vegetative mycelium of other species of this genus and the family before the taxonomic significance of mycelial characters can be fully assessed. Such studies would also lead to an understanding of the development of this phase of the life cycle and the effect of factors of the micro-environment.

BIBLIOGRAPHY

BASHAM, J. T. 1958. Decay of trembling aspen. Can. J. Botany 36: 491-505.

BASHAM, J. T. 1960. Studies in forest pathology XXI. The effects of decay on the production of trembling aspen pulpwood in the Upper Pic region of Ontario. Can. Dept. Agr., Forest Biol. Div., Publ. No. 1060. 25p.

BERLINER, M. D. and R. H. Duff. 1965. Ultrastructure of *Armillaria mellea*. Can. J. Botany 51: 171-172.

BIRKINSHAW, J. H., W. P. K. FINDLAY and R. A. WEBB. 1940. Biochemistry of the wood-rotting fungi. 2. A study of the acids produced by *Coniophora cerebella* Pers. Biochem. J. 34: 906-916.

BONDARTSEVA, M. A. 1961. A critical review of the most recent classifications of the family of the Polyporaceae. Botanicheskii Zhurnal 46: 587-593. (Translation from the Russian by the Canada Department of Agriculture, 73250).

BOYCE, T. S. 1918. Embedding, staining of diseased wood. Phytopathology 8: 432-436.

BRACKER, C. E. and E. E. BUTLER. 1963. The ultrastructure and development of septa in hyphae of *Rhizoctonia solani*. Mycologia 55: 35-38.

BRACKER, C. E. and E. E. BUTLER. 1964. Function of septal pore apparatus in *Rhizoctonia solani* during protoplasmic streaming. J. Cell Biol. 21: 152-157.

BULLER, A. H. R. 1933. Researches on Fungi. Vol. V, Longmans, Green and Co., New York, pp416.

BURNETT, J. H. 1968. Fundamentals of Mycology. Edward Arnold (Publishers) Ltd. London.pp546.

BUTLER, E. E. and C. E. BRACKER. 1965. Proc. Am. Phytopathol. Soc. Symp. *Rhizoctonia solani*.

BUTLER, G. M. 1966. Vegetative Structures. In: The Fungi, 2, Chap. 3, 83-112 (Ainsworth, G.C. and Sussman, A.S., Eds., Academic Press, New York and London).

CAMPBELL, W. A. 1938. The cultural characteristics of the species of *Fomes*. Bull. Torrey Botan. Club 65: 31-69.

CAMPBELL, W. A., and R. W. DAVIDSON. 1941. Cankers and decay of yellow birch associated with *F. igniarius* var. *laevigatus*. J. Forestry 39: 559-560.

CARTWRIGHT, K. St. G. 1929. A satisfactory method of staining fungal mycelium in wood sections. Ann. Bot. (London) 43: 412-413.

CARTWRIGHT, K. St. G. 1930. A decay of Sitka Spruce timber caused by *Trametes serialis* Fr. Bull. For. Prod. Res., London., No.4.

CARTWRIGHT, K. St. G. and W. P. K. FINDLAY. 1958. Decay of timber and its prevention. 2nd Ed., H. M. Stationary Office, London.pp332.

CHESTERS, C. G. C. 1968. Morphology as a Taxonomic Criterion. In: The Fungi, 3, Chap. 20, 517-540 (Ainsworth, G.C. and Sussman, A.S., Eds., Academic

Press, New York and London).

CHIARRAPPA, L. 1959. Wood decay of the grapevine and its relationship with black measles disease. Phytopathology 49: 510-519.

CORNER, E. J. H. 1932. A *Fomes* with two systems of hyphae. Trans. Brit. Mycol. Soc. 17: 51-81.

COWLING, E. B. 1957. A partial list of fungi associated with decay of wood products in the United States. Plant Disease Reptr. 41: 894-896.

COWLING, E. B. 1961. Comparative biochemistry of the decay of Sweetgum sapwood by the white-rot and brown-rot fungi. U.S. Dept. Agr. Tech. Bull., 1258.

CUNNINGHAM, G. H. 1954. Hyphal systems as aids in identification of species and genera in the Polyporaceae. Trans. Brit. Mycol. Soc. 37: 44-50.

DAVIDSON, R. W., T. E. HINDS and F. G. HAWKSWORTH. 1959. Decay of aspen in Colorado. Rocky Mt. Forest and Range Exp. Sta. Pap. No. 45:pp14.

de GROOT, R. C. 1960. Environmental factors affecting spore discharge of *Fomes igniarius* (L ex Fr.) Kickx. Progress Report. Dept. of Plant Pathology, Univ. of Wisconsin, Madison, Wisconsin.

FISHER, E. 1934. Observations on *Fomes pomaceus* (Pers.) Lloyd infecting plum trees. Trans. Brit. Mycol. Soc. 19: 102-113.

FRITZ, CLARA W. 1923. Cultural criteria for the distinction of wood-destroying fungi. Proc. and Trans. Roy.

Soc. Canada 17: 191-288.

FRITZ, CLARA W. 1954. Decay of poplar pulpwood in storage. Can. J. Botany 32: 799-817.

GIESY, R. M. and P. R. DAY. 1965. The septal pores of *Coprinus lagopus* in relation to nuclear migration. Amer. J. Botany 52: 287-293.

GIRBARDT, M. 1958. Über die Substruktur von *Polystictus versicolor* L. Archiv. für Mikrobiologie 28: 255-269.

GIRBARDT, M. 1962. Licht and elektronenoptische unter-Suchungen an *Polystictus versicolor* (L.). Planta 58: 1-21.

GOOD, H. M. and JANE I. NELSON. 1962. Fungi associated with *Fomes igniarius* var. *populinus* in living poplar trees and their probable significance in decay. Can. J. Botany 40: 615-624.

GOOD, H. M. and W. SPAINIS. 1958. Some factors affecting the germination of spores of *Fomes igniarius* var. *populinus* (Neuman) Campbell, and the significance of these factors in infection. Can. J. Botany 36: 421-437.

HARRISON, C. H. 1942. Longevity of the spores of some wood-destroying Hymenomycetes. Phytopathology 32: 1096-1097.

HEMMI, T. and S. KURATA. 1931. Pathological studies on *Polyporus betulinus* (Bull.) Fr. Forsch. Pflkr. Tokyo, I, Cit. fr. Biol. Abstr., 7 (4), 7664, 1933.

HENNINGSSON, B. 1965. Physiology and decay activity of the birch conk fungus *Polyporus betulinus* (Bull.) Fr. Studia Forestalia Suecica 34: 1-75.

HENNINGSSON, B. 1967. Physiology of fungi attacking birch and aspen pulpwood. Studia Forestalia Suecica 52: 1-55.

HIRT, R. R. 1949. Decay of certain northern hardwoods by *Fomes igniarius*, *Poria obliqua* and *Polyporus glomeratus*. Phytopathology 39: 475-480.

HOPP, H. 1936. Appearance of *Fomes igniarius* in culture. Phytopathology 26: 915-917.

HOSSFELD, R. L., J. C. OBERG and D. W. FRENCH. 1957. The appearance and decay resistance of discolored aspen. Forest Products J. 7: 378-382.

HUXLEY, H. E. and G. ZUBAY. 1961. Preferential staining of nucleic acid containing structures for electron microscopy. J. Biophys. Biochem. Cytol. 11: 273-296.

HYDE, J. M. and C. H. WALKINSHAW. 1966. Ultrastructure of basidiospores and mycelium of *Lenzites saepiaria*. J. Bacteriol. 92: 1218-1227.

LaFUZE, H. H. 1937. Nutritional characteristics of certain wood-destroying fungi, *Polyporus betulinus* Fr., *Fomes pinicola* (Fr) Cooke, and *Polystictus versicolor* Fr. Plant Physiol. 12: 625-646.

LOWE, J. L. 1957. Polyporaceae of North America. The genus *Fomes*. State Univ. Coll. of Forestry at

Syracuse Univ. p97.

LU, B.C. 1965. Fine structure in fruiting bodies of *Coprinus*, with special emphasis on chromosome structure. Ph.D. Thesis, Univ. of Alberta.pp104.

LUFT, J. H. 1956. Permanganate - a new fixative for electron microscopy. J. Biophys. Biochem. Cytol. 2: 799-801.

LYR, H., H. ZEIGLER and C. HEFFE. 1959. Enzymausscheidung und Holzabbau durch *Phellinus igniarius*. Quel. und *Collybia velutipes* Curt. Phytopathology 35: 173-200.

MALHOTRA, S. K. and R. T. EAKIN. 1967. A study of mitochondrial membranes in relation to elementary particles. J. Cell. Sci. 2: 205-212.

MANOCHA, M. S. 1965. Fine structure of *Agaricus* carpophore. Can. J. Botany 43: 1329-1333.

MARCHANT, R., A. PEAT and G. H. BANBURY. 1967. The ultrastructural basis of hyphal growth. The New Phytologist 66: 623-629.

McALEAR, J. H. and G. A. EDWARDS. 1959. Continuity of plasma membrane and nuclear membrane. Exptl. Cell. Res. 16: 689-692.

MEINECKE, E. P. 1929. Quaking aspen. A study in applied forest Pathology. U.S. Dept. Agr., Tech. Bull. 155,pp33.

MIKALAIKEVICIUS, V. 1958. Kai Karie drebulines pinties (*Phellinus tremulae* Bond e. Borriss.) biologijos

klausimai. (Some questions of the biology of *P. tremulae*). Darb. Liet. Miš. ùk moks. tyr. Inst. Kaunas. 3: 187-203. (Russian summary). Abstr. in Rev. Appl. Mycol. 39: 56 (1960).

MOLLENHAUER, H. H. 1959. Permanganate fixation of plant cells. J. Biophys. Biochem. Cytol. 6: 431-437.

MOORE, R. T. 1963a. Fine structure of mycota. I. Electron microscopy of the discomycete *Ascodesmis*. Nova Hedwigia Zeit. Kryptogamenkunde 5: 263-278.

MOORE, R. T. 1963b. Fine structure of mycota. 10. Thallus formation in *Puccinia podophylli* aecia. Mycologia 55: 633-642.

MOORE, R. T. and J. H. McALEAR. 1961a. Fine structure of mycota. 5. Lomasomes - previously uncharacterized hyphal structures. Mycologia 53: 194-200.

MOORE, R. T. and J. H. McAlear, 1961b. Fine structure of mycota. 8. On the aecidial stages of *Uromyces caladii*. Phytopathologische Zeit 42: 297-304.

MOORE, R. T. and J. H. McALEAR. 1962a. Fine structure of mycota. 3. Reconstruction from skipped serial sections of the nuclear envelope and its continuity with the plasma membrane. Exptl. Cell Res. 24: 588-592.

MOORE, R. T. and J. H. McALEAR. 1962b. Characterization of the golgi dictyosome of the fungus *Neobulgaria pura*. Fifth Inter. Cong. on Electron Microscopy. (SS. Breese, Jr. Ed.) UU-7. Academic Press, New York.

MOORE, R. T. and J. H. McALEAR. 1962c. Fine structure of mycota. 7. Observations on septa of Ascomycetes and Basidiomycetes. Amer. J. Botany 49: 86-94.

MOORE, R. T. and J. H. McALEAR. 1963a. Fine structure of mycota. 4. The occurrence of golgi dictyosome in the fungus *Neobulgaria pura* (Fr.) Petrak. J. Cell Biol. 16: 131-141.

MOORE, R. T. and J. H. McALEAR. 1963b. Fine structure of mycota. 9. Fungal mitochondria. J. Ultrast. Res. 8: 144-153.

MOUNCE, IRENE. 1929. Studies in forest pathology. II. The biology of *Fomes pinicola* (SW.) Cooke. Can. Dept. Agr. Bul. 111 n.s.

NAIR, N. G., N. H. WHITE, D. M. GRIFFIN and S. BLAIR. 1969. Fine structure and electron cytochemical studies of *Sclerotium rolfsii* Sacc. Austral. J. Biol. Sci. 22: 639-652.

NOBLES, M. K. 1948. Studies in forest pathology. VI. Identification of cultures of wood-rotting fungi. Can. J. Res. C. 26: 281-431.

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

NOBLES, M. K. 1965. Identification of cultures of wood inhabiting hymenomycetes. Can. J. Botany 43: 1097-1139.

NOECKER, N. L. 1938. Vitamin B_1 in the nutrition of four

species of wood-rotting fungi. Am. J. Botany 25: 345-348.

NORDIN, V. J. 1954. Studies in forest pathology. XIII. Decay in sugar maple in the Ottawa-Huron and Algoma Extension Forest Region of Ontario. Can. J. Botany 32: 221-258.

NUTMAN, F. J. 1929. Studies of wood-destroying fungi. I. *Polyporus hispidus* Fries. Ann. Appl. Biol. 16: 30-64.

OSHIMA, N. 1953. Studies on *Fomes igniarius* (L.) Gill. M.Sc. Thesis, Colorado Agr. and Mech. Coll.pp169.

OVERHOLTS, L. O. 1953. The Polyporaceae of the United States, Alaska and Canada (Prep. for publication by J. L. Lowe) Univ. Mich. Press, Ann Arbor,pp466.

PROCTOR, P. 1941. Penetration of the walls of wood cells by the hyphae of wood-destroying fungi. Yale. Sch. Forest. Bull., 47. p31.

RILEY, C. G. 1952. Studies in forest pathology. IX. *Fomes igniarius* decay of poplar. Can. J. Botany 30: 710-734.

ROBBINS, W. J. and ANNETTE HERVEY. 1955. Growth substance requirements and the growth of *Stereum murrayi*. Mycologia 47: 155-162.

ROBBINS, W. J. and ANNETTE HERVEY. 1958. Wood, Tomato, and malt extracts and growth of some Basidiomycetes. Mycologia 50: 745-752.

ROBBINS, W. J. and ROBERTA MA. 1941. Biotin and the growth

of *Fusarium avenacearum*. Bull. Torrey Botan. Club 68: 446-462.

ROFF, J. W. 1964. Hyphal characters of certain fungi in wood. Mycologia 56: 799-804.

SABATINI, D. D., K. G. BENSCH and R. J. BARRNETT. 1962. New fixatives for cytological and cytochemical studies. Proceedings Fifth Inter. Cong. for Electron Microscopy, Philadelphia, 2: 13.

SCHMID, R. and W. LIESE. 1968. Structural and histochemical observations on fungal fiber hyphae. Electron Microscopy. Vol. II, Academic Press, New York and London, p415.

SEDLMAYR, M., E. S. BENEKE, and J. A. STEVENS. 1961a. Physiological studies on *Calvatia* species. I. Vitamin requirements. Mycologia 53: 98-108.

SEIFERT, K. 1966. Chemischer Abbau der Buchenholz-Zellwand durch den Weissfäulepilz *Polystictus versicolor* (Linn.) Fr. - Holz als Roh-u. Werkstoff, 24.

SHATKIN, A. and E. L. TATUM. 1959. Electron microscopy of *Neurospora crassa* mycelia. J. Biophys. Biochem. Cytol. 6: 423-426.

SHOPE, P. F. 1931. The Polyporaceae of Colorado. Ann. Missouri Botan. Garden 18: 287-456.

SILVERBORG, S. B. 1959. Rate of decay in northern hardwoods following artificial inoculation with some common heartrot fungi. Forest Sci. 5: 223-238.

STATES, J. S. 1969. Some aspects of basidiocarp

morphogenesis in *Gleophyllum (Lenzites) saepiarium* (Fries) Karsten, a xerophytic polypore. Ph.D. Thesis. Univ. of Alberta, p174.

VERRALL, A. F. 1937. Variation in *Fomes igniarius* (L.) Gill. Minn. Agr. Expt. Sta. Tech. Bull. 117, p41.

WALL, R. E. 1962. Comparative studies of the varieties of *Fomes igniarius* (L. ex fr.) Kickx. Ph.D. Thesis, Univ. of Wisconsin, p97.

WARD, E. W. B. and N. COLOTELO. 1960. The importance of inoculum standardization in nutritional experiments with fungi. Can. J. Microbiol. 6: 545-556.

WELLS, K. 1964a. The basidia of *Exidia nucleata*. I. Ultrastructure. Mycologia 56: 327-341.

WELLS, K. 1964b. The basidia of *Exidia nucleata*. II. Development. Amer. J. Botany 51: 360-370.

WELLS, K. 1965. Ultrastructural features of developing and mature basidia and basidiospores of *Schizophyllum commune*. Mycologia 57: 236-261.

WILLETS, H. J. and F. D. CALONGE. 1969. The ultrastructure of stroma of the brown-rot fungi. Arch. Microbiol. 64: 279-288.

WILSENACH, R. and M. KESSEL. 1965. On the function and structure of septal pore of *Polyporus rugulosus*. J. Gen. Microbiol. 40: 397-400.

WOLPERT, H. 1924. Studies in the physiology of fungi. The growth of certain wood-destroying fungi in relation to the H-ion concentration of the media. Ann. Mo.

Bot. Gdn. 11: 48-96.

ZALOKAR, M. 1961. Electron microscopy of centrifuged hyphae of *Neurospora*. J. Biophys. Biochem. Cytol. 9: 609-617.