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DEVELOPMENT OF *Polyporus adustus* Fries AND THE INFLUENCE OF THE MICROENVIRONMENT

by

© RICHARD JOSEPH LARCADE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "DEVELOPMENT OF *Polyporus adustus* Fries AND THE INFLUENCE OF THE MICROENVIRONMENT", submitted by Richard Joseph Larcade in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The mechanics of growth and development of *Polyporus adustus* Fries are described in relation to its response to the natural environment. Basidiocarp development is influenced by the interrelated factors of temperature, moisture and light, with relative humidity being of paramount importance. Alterations of the humidity in the microenvironment during primordial emergence and development, as well as its orientation on the wood surface, determine the ultimate physiognomy of the basidiocarp.

These studies of development were carried out primarily on fresh material and the effects of the environment were determined at experimental sites in the field using continuously recording equipment where possible. Both naturally and artificially infected logs were placed at the sites. The method used to infect sound logs has been found to be valuable in observing the development of *P. adustus* from the first stages of vegetative growth.

It has been found that the basidiocarp of *P. adustus* is a more complex structure than previously reported and three hyphal systems have been shown to exist. This polymorphic species exhibits a marked degree of adaptability to its environment and a

plasticity of form to best utilize the contours of its substrate. A large portion of the rapidly forming basidiocarps develops into the hymenophore when considering the total mass of the fruit body, resulting in the production of a large spore crop during a short period of time.

Observations of the vegetative mycelium on various artificial media have shown marked hyphal differentiation. Hyphal growth and development, and asexual spore formation, under specific cultural conditions, are evaluated with respect to their usefulness as criteria for classification and species identificiation.

Vegetative growth, basidiocarp development and the adjustment of the fungus thallus to the environment are discussed in terms of establishing interrelationships between species and formulating a natural classification of the Polyporaceae.

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INTRODUCTION

The Polyporaceae, encompassing a large, diverse group of fungi belonging to the Basidiomycetes, have elicited considerable interest from mycologists. These fungi are primarily wood decay species and are, therefore, economically important. Their destruction of living trees, lumber and structural timber has stimulated research in an attempt to characterize these fungi and gain an understanding of their growth processes.

Much of the literature characterizing the Polyporaceae emphasizes the macroscopic features or hyphal structure of basidiocarps and cultural characters of the vegetative mycelium as criteria for identification of the various species. The researchers of the 19th century were concerned mainly with taxonomic considerations and set up classification systems based on the gross morphology of the basidiocarp, creating artificial systems. This approach has given way to the investigation of microscopic characters of the basidiocarp and cultural studies of the vegetative mycelium as criteria used to characterize these fungi. In 1932, Corner first

published his work, introducing the concept that the basidiocarps of the Polyporaceae are composed of distinct "hyphal systems". Although Corner stated that this line of investigation was not meant to be a new taxonomic system, it was used in just that way by Cunningham (1947, 1954, 1965) and the result was yet another artificial and incomplete system.

The first extensive study of cultural characters of the vegetative mycelium was published by Nobles (1948, 1958b, 1965). Her work was based on the morphology of the vegetative mycelium; the starting point of her classification system was a determination of the presence or absence of extracellular oxidases, forming the first major division of these fungi. This information was used in conjunction with available information on basidiocarp morphology, the type of rot occurring in the wood substratum and interfertility phenomena.

Numerous other investigations are concerned with physiological aspects of these fungi such as light, temperature and moisture relations or with biochemical considerations. The vast majority of these studies have been carried out in strictly artificial environments without necessarily taking into consideration the fact that these organisms

develop abnormally under these conditions, or at least in a different manner than in their natural habitat.

While such literature has contributed valuable information characterizing the vegetative or reproductive phases of members of the Polyporaceae, few studies have been concerned with comprehensive investigations of the development of a basidiocarp from the vegetative mycelium in the natural substratum and in the natural environment. It was with the intention of contributing some information to this neglected area of research, that this study of the mechanics of growth and development of *Polyporus adustus* Fries, in relation to its environment, has been carried out. This particular fungus was chosen for several reasons. *P. adustus* is a species of wide distribution and, therefore, many specimens were readily available for examination. It is a polymorphic species which exhibits a marked plasticity of form, seeming to utilize the contours of its substrate in forming basidiocarps over a very brief period of time, a characteristic which is of interest when considering the ability of some organisms to readily adapt to best insure their survival. Finally, and of practical importance, *P. adustus* was found to be

of common occurrence in Alberta and, therefore, presented an opportunity to study the development of resupinate, effused-reflexed and imbricate basidiocarps as they form from the vegetative mycelium in the wood substratum, and their response to their environment.

The characterization of the vegetative mycelium of *P. adustus* under cultural conditions was another area that required further investigation. The descriptions published by earlier investigators appeared to be incomplete or conflicted with my observations made during preliminary studies. In order to resolve these discrepancies, an investigation was carried out on the growth and development of the vegetative mycelium on various media under strictly controlled conditions or selected physical conditions which had been studied in the field.

This study has been carried out in three phases. The first phase was concerned with the growth of the vegetative mycelium in the wood substratum, the subsequent formation of primordia and development of the different basidiocarp forms. The second phase, an investigation of the influence of the microhabitat on growth and morphogenesis, was carried out in the field. The final phase was an

investigation of growth of the vegetative mycelium in culture with the objective of satisfactorially characterizing this stage of *P. adustus*. This investigation also involved an attempt to induce basidiocarp formation in an artificial microenvironment based on information gained from field studies.

In order to present a complete and coherent account from the somewhat diverse information gathered, this work will be presented in two major sections. The first is an account of the growth of the vegetative mycelium and basidiocarp development in *P. adustus*. The second section is a compilation of data gathered on the influence of the microenvironment on growth and basidiocarp development. This section encompasses the results and observations made in the natural microhabitat, i.e., field studies, and those in the artificial microhabitat, i.e., studies of the vegetative mycelium on artificial media and basidiocarp induction under artificially simulated conditions. The diverse observations and results from these sections will be drawn together in the discussion to present a detailed picture of growth and development of *Polyporus adustus* and the influence of the environment upon these processes.

LITERATURE REVIEW

The Polyporaceae have been of interest to man for at least 2000 years. Lowe (1963) mentions investigations carried out by Dioscorides (Greek surgeon in the Roman army, 54-68 A.D.) on the medical uses of "the quinine fungus, *Fomes officinalis*" in his work on medical botany. Sixteen centuries later, in 1675, Steerbeck published his THEATRUM FUNGORUM, the first book exclusively concerned with fungi. Following the appearance of this work came the treatises of Linnaeus and the invaluable taxonomic studies of Persoon and Fries. This was a period devoted to bringing some order to this diverse group of organisms which were characterized and classified on the basis of gross morphology. In the past 200 years, a number of classification systems for the Polyporaceae, based on macroscopic characters, anatomical studies of the basidiocarp and cultural characters of the vegetative mycelium, have been devised. Taxonomic studies have resulted in the characterization of many members of the Polyporaceae, but these descriptions present a static picture based primarily on morphological features of the mature basidiocarp.

Hartig (1878) was one of the first investigators to describe in detail the action of polypore fungi upon wood and to point out their economic importance as wood-decay organisms. His work stimulated many workers to study the biology of these fungi. Buller (1906, 1909) investigated the development of *Polyporus squamosus* from the vegetative stage in wood through the development of basidiocarps. These studies involved attempts to inoculate sound living trees with spores from this wound parasite, but he did not appear to be concerned with observing the progress of decay in the trees or the processes leading to basidiocarp development. Buller found that the stipe and pileus development of *P. squamosus* were dependant upon light. Developing basidiocarps, when placed in the dark "grew into branched deerhorn-like structures, a foot in length, without any formation of pilei....[but pilei] formed when...exposed to light". He also found that the pileus becomes oriented horizontally and that the pores were positively geotropic. Buller evidently realized the influence of the environment on growth and morphogenesis, but did not present any data on these parameters.

Rhoads (1918) studied the macroscopic and microscopic aspects of development of *Polyporus pargamenus*. He studied the development of the different regions of the basidiocarp and described the spore characters in great detail. The cultural characteristics of the vegetative mycelium, grown from spore inoculum, were also examined. The germination mycelium, which lacked clamps, was termed "primary" and the clamped mycelium, "secondary". The terminology, which is now considered inaccurate from a developmental standpoint, appears to be that used by Falck (1909). Rhoads described the secondary mycelial system as consisting of "an intricately branched network of hyphae comprising both large hyphae with distinct thin walls, and hyphae of smaller diameter, formed by the repeated branching of the larger ones". The production and structure of asexual spores, oidia and chlamydospores, were stressed more than was hyphal development. Rhoads also mentioned the influence of the environment on morphogenesis. Primordia of *P. pargamenus* were found to develop into imbricate basidiocarps only when exposed to light. Primordia which were covered and received no light reverted to a fluffy vegetative mycelium. The effects of moisture on basidiocarp viability were also investi-

gated. These studies of the effect of environmental factors were based on evidence from observations of basidiocarp development. A similar study was carried out by Bayliss (1908) on *Polyporus (Polystictus) versicolor*. She made the observation that the pileus zonation is due to temporary cessation of growth in periods of decreased humidity occurring during development. When basidiocarps, growing in the field, were kept under conditions of high humidity by placing a basidiocarp-bearing branch partially in water and covering with a bell jar, hardly any zonation was evident. If the bell jar was removed for even an hour, the reduced humidity resulted in zone formation. It was also noted that the coloring of the pileus is affected by light. Basidiocarps grown in the laboratory in diffuse light were pale buff in color, while those exposed to light became darker brown. The combined factors of light and humidity gave the basidiocarp its characteristic multicolored zones.

In 1932 Corner began his studies of the Polyporaceae with a detailed description of *Polystictus xanthopus*. This basidiocarp was found to consist of four distinct systems of differentiated hyphae termed generative, skeletal, binding and

mediate, which differed "in thickness of wall, in manner of septation or branching, or in size...and each kind forms a system distinguished...by a common function" (Corner, 1932a). Generative hyphae were thin-walled, branched, longitudinal or interwoven and with clamp connections. Skeletal hyphae were described as "thick-walled, unbranched, aseptate, straight or slightly flexuous, longitudinal...with the lumen more or less obliterated in mature parts, but the apices thin-walled with dense contents". Binding hyphae had thick walls which obliterated the lumen in mature parts, were profusely branched, aseptate, narrow and interwoven. Mediate hyphae had slightly thickened walls, were sparingly or frequently branched, aseptate, flexuous and contained little cytoplasm. Corner further distinguished the "mycelial hyphae" which connected the basidiocarp with the substratum. Mediate and mycelial hyphae were not mentioned in Corner's later work (1953). The generative hyphae give rise to the other hyphal types and to the hymenium. The skeletal hyphae form the framework of the stipe and pileus. These hyphae are held together by the binding hyphae. Corner's study of *P. xanthopus*, in which he first presented his concept of hyphal systems, was followed by a study (1932b) of

the developmental morphology of *Fomes levigatus* and several related species having a brown, woody context. The construction of the developing basidiocarps involved the generative and skeletal hyphal systems. A comparison between *F. levigatus* and *P. xanthopus* was made on the basis of basidiocarp development, as well as construction. While it was observed that "the growing point of the primordium in *P. xanthopus* is positively phototropic and becomes diageotropic at a later stage after the formation of the pileus", *F. levigatus* was only "diageotropic". In both cases the hyphae emerging from the wood substratum were at first insensitive to these external stimuli and formed undifferentiated primordia. In 1953, Corner proposed that the polypores could be characterized by the construction of their basidiocarps. He suggested that there are three major kinds of basidiocarp construction existing in the polypores, termed monomitic, dimitic and trimitic, which have one, two and three systems of hyphae, respectively. A monomitic basidiocarp consists solely of generative hyphae. Two types of dimitic basidiocarps were distinguished; those consisting of generative and skeletal hyphae, and of generative and binding hyphae. Trimitic basidiocarps consist of all three hyphal

types. Cunningham (1947, 1954, 1965) utilized Corner's method of describing the construction of polypore basidiocarps as a basis for his classification system for the Polyporaceae.

Characterization of the vegetative mycelium of wood-decay fungi in culture was an area of mycological research which received little attention until Long and Harsch (1918) published their work dealing with identification of various fungi by their cultural characters on ten different artificial media. The media were selected to promote the development of specific characters in order to differentiate closely related species. In addition, attempts were made to induce basidiocarp formation in culture on agar slants of the different artificial media. They found light to be a necessary factor in basidiocarp initiation and stated that "aeration and humidity are two other factors which also enter into sporophore production on artificial media". The composition of the medium was considered to be of minimal importance in basidiocarp development. Although basidiocarps of some species were induced to form, it was noted that not one polypore developed a typical pileus.

Davidson, Campbell and Blaisdell (1938) used Bavendamm's (1928) method of growing wood-decay

fungi on media containing gallic or tannic acid. They characterized over 200 species on the basis of their vegetative growth and the presence or absence of a dark brown diffusion zone forming under mycelial mats resulting from oxidation of the acid in the medium. This reaction indicated whether or not a wood-decay fungus produced extracellular oxidases. Generally, fungi associated with brown rots (80%) gave a negative reaction, those with white rots (96%) a positive reaction.

Nobles was the first investigator to incorporate the observations made by earlier workers and those made personally to formulate a system for the identification of wood-destroying fungi on the basis of their cultural characters. In 1948 she published her key for the identification of these fungi based on host relationships, mycelial pigmentation, color changes in the agar, and morphological characters such as septation, asexual spore development and basidiocarp formation. In her later publications (1958b, 1965) Nobles revised her system and utilized the earlier studies on extracellular oxidase production as a first division which divided the species into two distinct groups. Subsequent divisions within each group were based on morphological characters.

All descriptions were originally based on observations of cultures from various inocula grown on 2% malt agar and on gallic (or tannic) acid agar. The gallic acid method was later replaced by dropping an alcoholic solution of guaiacum on the mycelium growing on the malt agar (1958a), a simple technique which gave the same results as the former method. In work done since 1958, both oxidase tests were used in descriptive studies, but the guaiacum technique was found satisfactory for routine identifications.

Many investigators, e.g., Long and Harsch mentioned earlier, have attempted to induce basidiocarp formation in culture in order to study their development along with that of the vegetative mycelium. Badcock (1941, 1943) devised a sawdust medium containing an "accelerator" which increased the growth of wood decay fungi. Placing this enriched medium in various culture vessels he induced basidiocarp formation in 81 species. He found that it was necessary to maintain cultures under conditions of fairly high, but not saturated, relative humidity, and expose them to light of moderate intensity. Tamblyn and DaCosta (1958) used a culture apparatus consisting of a wood block fitted over the mouth of

a jar filled with a modified sawdust medium based on that formulated by Badcock. The sawdust was inoculated with mycelium growing on agar. The mycelium grew throughout the sawdust medium and through the wood block and basidiocarps formed on the surface. These investigations, and others such as those of Hopp (1938) with *Fomes applanatus* and Lohwag (1955) with *Lenzites betulina* showed that morphogenesis was influenced by substrate composition, light, temperature and relative humidity.

The most extensive studies of the influence of the environment on morphogenesis has been carried out by Plunkett (1956, 1958, 1961). He has examined the influence of light, humidity, primordium orientation, temperature, Hydrogen-ion concentration and nutrition on morphogenesis in *Polyporus brumalis* and *Collybia velutipes*. Light was found to be important, although primordia of *P. brumalis* could form in the dark. Conditions of low humidity favored pileus development by enhancing the translocation rate into the growing tip of the stipe and the newly forming pileus. He examined the phototropic and geotropic responses in *P. brumalis* (1961) and found the growing stipe to be positively phototropic at first, but, as the pileus forms, the stipe becomes negatively geotropic. The positive phototropism is the dominant

reaction but once the pileus forms, light is prevented from reaching "the photoreceptive part of the stipe". Plunkett demonstrated the dominance of the phototropic reaction by directing a light source on the stipe apex after pileus development. The stipe grew toward the source, sometimes resulting in a fully inverted basidiocarp with the pores oriented in an upward vertical position. He suggested that the developing pores respond geotropically, but the response can be a positive or negative one ("...their growing margins may be under the influence of gravity and like the stipe hyphae may have two null positions for geotropic stimulation"), at least under cultural conditions. The dissepiments did not develop horizontally and Plunkett rejected the probability of development without geotropic influence, "a view reinforced by the almost invariable verticality of dissepiments in nature" (Plunkett, 1961).

The literature reviewed in the preceding pages formed the background for the present study of *Polyporus adustus*. Some of the methods used by other investigators for culturing the mycelium and for inducing basidiocarp formation have been followed or modified. However, the work concerning the effects

of environmental factors was of limited value since basidiocarp development under sufficiently controlled conditions could not be induced in *P. adustus*. It was necessary to study these effects in the field, an approach for which no published reports were available.

Polyporus adustus Fries.

1. Classification and Distribution.

Polyporus adustus is a widely distributed fungus and has been reported growing in North and South America, Europe, Africa, Asia, Australia and New Zealand. It was first described by Willdenow in 1787 as *Boletus adustus*. In 1821 Fries placed it in the genus *Polyporus*. Karsten (1879) transferred the species to the genus *Bjerkandera*. Murrill (1906) accepted this classification and gave it wide publicity in his publication on the Polyporaceae of North America. Pilat (1937) placed it in the genus *Gloeoporus*. This classification was given support by Cunningham (1965), based on his study of hyphal systems and a regrouping of the genera of Fries. Since the majority of investigators (Bose, 1930;

Corner, 1932; Overholts, 1953; Nobles, 1948, 1958b, 1964) recognize Fries' classification, his binomial is used in this thesis.

The species is generally considered a saprophyte although it has been reported as a wound parasite in apple and beech trees (Brooks, 1925; Wilson, 1909; Prior, 1913). Griffith and Barnett (1967) report it as being a mycoparasite in culture.

2. Basidiocarp Structure.

In spite of the wide distribution and polymorphic character of this fungus, it has not been the subject of intensive study by mycologists. Boyce (1938) briefly describes *P. adustus* as causing "a white mottled or cubical rot of dead hardwoods", its presence resulting in considerable loss of poplar and red gum logs and lumber in storage yards. He described the basidiocarp as annual, up to 3 inches wide, sometimes occurring in clusters and with a smoky-gray pore surface.

Ames (1913) discussed the structure of *P. adustus*, with particular attention to the hymenophore. She placed this fungus in the genus *Bjerkandera* on the basis of its thin, dense pileus and the fact that

the hymenophore arose from a distinct layer.

Lohwag (1940) studied the anatomy of the basidiocarp of *P. adustus*. He found the context to consist of hyphal bundles ("Hyphenbündeln") as well as individual hyphae. The pileus surface was described as a short trichoderm arising from the context. The occurrence of this fungus on both deciduous and coniferous wood was noted.

Corner (1953) described the basidiocarp of *P. adustus* as monomitic, consisting of generative hyphae only. These hyphae become thick-walled in older basidiocarps.

The description of this fungus given by Overholts (1953), in the major publication on the classification of the Polyporaceae, is based on macroscopic and certain microscopic features of the mature basidiocarp. *P. adustus* is described as having a sessile or effused-reflexed sporophore, tough to corky, usually imbricate, varying in color from white to tan, rarely zonate, finely tomentose or nearly glabrous, with a thin, even margin which is sterile below. The tube layer, which is gray and darkens with age, is separated from the context by a narrow, dark line. The tubes are narrow (5-7 per mm), the tramal tissue is brown, and the basidia are small.

The hyphae composing the basidiocarp are hyaline, profusely branched and have clamp connections. *P. adustus* is distinguished from closely related species on the basis of its darkly pigmented dissepiments (*P. fumosus* and *P. dichrous* have colorless, or nearly colorless, dissepiments).

3. Cultural Studies of the Vegetative Mycelium.

Most other studies of *P. adustus* were concerned with describing the vegetative mycelium in culture. Cartwright and Findlay (1930) presented a very basic description of the macroscopic characters of *P. adustus* grown on 2% malt agar. They observed it to be of even texture, cotton-wooly, rather felted and "becoming light fawn above". This description was elaborated upon by Cartwright (1932) when he investigated the microscopic characters of this fungus. The aerial hyphae remained undifferentiated, but the submerged hyphae varied in diameter and produced "small rod-shaped crystals". Moderately thick-walled chlamydospores developed and oidia occurred sparingly. *P. adustus* was distinguished from the closely related species, *P. fumosus*, on the basis of the macroscopic appearance of the mycelial mat,

asexual spore formation (profuse in *P. fumosus*) and the type of crystals produced.

Badcock (1939) utilized the studies of Rumbold (1908), Mez (1908), Bavendamm (1928) and Gilbert (1934) in order to identify wood-decay fungi, including *P. adustus* by the odor of their mycelium in culture. Badcock suggested that this would be a useful criterion in identifying these fungi when used in conjunction with observations of morphological characters.

Nobles (1948, 1958b, 1964) grew cultures of *P. adustus* from various types of inocula on 2% malt agar and gallic acid agar and used the guaiacum method. She found this fungus to develop a white mycelium. The thin-walled, undifferentiated hyphae rarely produced chlamydospores, but oidia formed from clamped hyphae or their simple septate branches. According to Nobles, isolates of *P. adustus* varied in their production of extracellular oxidases, as shown by the growth and color reaction on gallic acid agar and with the addition of guaiacum.

Zycha and Knopf (1966) presented a description of the vegetative mycelium of *P. adustus* which agrees with that of Nobles and they also describe crystals found in the substratum and the production

of chlamydospores, affirming Cartwright's observations.

Vandendries (1936) carried out compatibility studies on *P. (Leptoporus) adustus* and found it to be bipolar, as did Nobles (1964). Vandendries further observed that haploid cultures produced numerous oidia and chlamydospores while this was not true of diploid cultures.

4. Physiological Studies.

Kogl and Fries (1937) established that *P. adustus* required thiamine for growth. Further investigations of the physiological requirements of *P. adustus* did not appear in the literature until Henningsson published his extensive studies of fungi attacking birch and aspen wood (1967a, 1967b, 1967c). In his physiological studies he cultured fungi, including *P. adustus*, on wood blocks and four different artificial media. *P. adustus* was found to decompose aspen and birch wood rapidly. Temperature studies showed that the optimal temperature for vegetative growth was slightly higher than that for optimal decay rate. Carbon utilization studies demonstrated the ability of this fungus to metabolize

a wide range of pentoses, hexoses, disaccharides and the polysaccharide cellulose. It was assumed that this fungus lacks the enzyme invertase because of its inability to utilize saccharose, an excellent carbon source for most of the other fungi tested. This delignifying fungus also exhibited rapid growth on cellulose. Nitrogen utilization studies showed that *P. adustus* could utilize inorganic nitrogen sources. The suppression of nitrate assimilation caused by the presence of ammonium ions (Morton and MacMillan, 1954) occurs in *P. adustus*. This is caused by a feedback mechanism in which the ammonium ions inhibit nitrate reductase production (Kinsky, 1961). The decrease in pH in *P. adustus* cultures grown on NH_4NO_3 is the first indication "that this feedback mechanism functioned also in a basidiomycete".

Henningsson (1968) also carried out ecological studies, much of the work being based on his earlier laboratory studies. He found that the vegetative mycelium remains active throughout the winter because the daily temperature even during the winter (in Sweden) reaches a high enough level during part of the day to permit growth and, hence, decay activity. Fungi can decompose wood over a wide range

of substrate moisture content. These studies did not specifically mention *P. adustus*. However, it was noted that this fungus and *P. hirsutus* showed considerable antagonism in culture although they were generally found in the same region of log piles.

MYCELIAL GROWTH AND BASIDIOCARP DEVELOPMENT

Materials and Methods of Observation

Basidiocarps of *Polyporus adustus*, at various stages of development, were collected throughout central and southern Alberta. A small number of specimens were also collected from British Columbia, Saskatchewan, Montana and New York. Most collections included part of the wood in which the vegetative mycelium was growing. Records were kept of the geographic location, habitat and orientation of the basidiocarps of each collection. Note was taken of basidiocarps deviating from classical descriptions and of any other basidiomycetes colonizing the substratum. The routine procedure for collected specimens included macroscopic and microscopic examination of the basidiocarp, the culturing of dikaryotic vegetative mycelium from the wood and monospore isolates from viable basidiocarps. In addition to freshly collected material, herbarium specimens of *P. adustus* from the New York Botanical Garden, the Mycological Herbarium at Ottawa and the Cryptogamic Herbarium at the University of Alberta were examined.

The vegetative mycelium in the wood substratum was studied to determine its mode of growth and development prior to hyphal emergence and primordium formation. The material was prepared by cutting blocks measuring approximately 2 x 2-4 cm (width x depth from bark to log center) with a band saw, soaking the blocks in sterile distilled water at approximately 5°C for 24-48 hours and sectioning on a sliding microtome (American Optical Company) at 10-30 μ increments. The hyphae were best observed in radial sections but tangential and transverse sections were also made. Paraffin methods (Johannsen, 1940) were employed when the wood was extremely decayed or when a basidiocarp was attached. The fresh-cut wood sections were stained with safranin and anilin blue in picric acid following a modification of Cartwright's method (1929: Appendix I). The embedded material was sectioned on a Spencer rotary microtome at 10-20 μ increments and stained with safranin and fast green or safranin and anilin blue (Johannsen, 1940). All sections were dehydrated and mounted in permount diluted with toluene (1:1) and partially dried on a warming tray at 40°C .

Preparations of basidiocarp material involved paraffin methods, hand sectioning and dissection. Basidiocarps at developmental stages ranging from newly emerged primordia to mature, sporulating structures were fixed at the collection site in strong chrom-acetic acid (Johannsen, 1940). Occasionally specimens were fixed in FAA (50% ethanol) if they could not be used within 48 hours, but this fixative was unsatisfactory because of the damage done to the individual hyphae resulting in an unclear image after staining and mounting. Following fixation any excessively large specimens were trimmed down, washed in tap water and dehydrated using an ethanol-tertiary butyl alcohol series beginning with 10% ethanol (FAA-fixed specimens did not require rinsing and dehydration began with 3 changes of 50% TBA). Specimens were embedded in Tissue Mat (Fisher) and sectioned at 10-20 μ on a Spencer rotary microtome. The sections were mounted on slides with Haupt's adhesive flooded with 10% formalin to flatten the ribbons. Specimens were stained with safranin and fast green or safranin and anilin blue and mounted with the 1:1 permount-toluene mixture.

Fresh material was prepared by first soaking in distilled water until the hyphae were fully infla-

ted. The excess portion of the basidiocarp was trimmed away to leave only a small piece of the tissue under examination. In most cases this tissue was embedded in pith, placed in a Spencer hand microtome and sectioned at 20-40 μ increments with a sectioning razor (Clay Adams). Advantages of this method are that the hyphae are not altered physically and some spores may remain attached to the basidia (spores have never been retained when paraffin methods were used). These sections were stained with 1% aqueous β-phloxine. If permanent sections were desired, the sections were dehydrated and run through the staining and mounting procedures used for embedded material.

In order to study the hyphal structure of the basidiocarp, pieces of tissue were excised from the different regions and the hyphae were teased apart according to Teixeira's method (1956, 1962). This procedure has the advantage of permitting examination of longer sections of the basidiocarp elements. Dissecting needles filed down to a chisel edge were used to separate the individual hyphae under a Bausch and Lomb stereomicroscope with a supplementary 2x lens. The hyphae were stained with 1% aqueous β-phloxine or cotton blue in lactophenol.

Embedded and fresh basidiocarps were sectioned longitudinally along both their length and width and transversely, i.e., across the pore region and upward through the context. In most longitudinal sections a portion of the wood substratum was left attached to demonstrate the mode of growth of vegetative hyphae as they emerge from inside the wood to form basidiocarps. Sections of basidiocarps harvested at various stages of development from newly emerged primordia to sporulating specimens were studied in order to analyze the hyphal structure of the basidiocarps as development proceeds.

Herbarium specimens were studied microscopically using the hand sectioning or dissecting techniques. The material was mounted in 1% aqueous β-phloxine or in 3% KOH.

Photomicrographs were taken with a Zeiss photomicroscope. Macroscopic photographs were taken with a Leitz bellows camera (4" x 5" plate film). A Pentax 35 mm camera was used for photographs taken in the field.

Observations

Polyporus adustus is a saprophytic, lignin-attacking species in which the basidiocarp exhibits marked polymorphism. Variation ranges from large clusters of imbricate pilei to effused-reflexed to entirely resupinate structures and any combination of these (Figures 3, 4, 5, page 45). The most constant characteristic by which it is identified is its smoky-gray pore surface. The surface of pileate forms is sordid to tan in color and is generally azonate. This fungus causes a white mottled rot in deciduous wood primarily, but has been observed occasionally on coniferous wood. All specimens which have been collected during this study have been found to be restricted to damp, shaded habitats.

1. The Vegetative Mycelium.

In all observations of wood infected with *Polyporus adustus* the vegetative stage has been found to be a dikaryotic, clamped mycelium growing in the tracheids and vessels. The hyphae growing through the wood are narrow, thin-walled and usually hyaline. Occasionally the cytoplasm will appear

granular in some hyphae. Generally, one or two hyphae grow in a sinuous manner through the lumen of a tracheid or vessel (Figure 6, page 46). Branches from these hyphae grow transversely through the tracheary elements, passing either through the pit membranes or directly through the cell wall which is broken down by exogenous enzymes produced by the hyphae. Hyphal growth appears generally sparse throughout the wood, but as decay progresses dense aggregations form in individual vessels and less frequently in tracheids (Figure 7, page 46). These aggregations give the wood the white mottled appearance characteristic of the rot produced by this species. It should be noted that the white areas observed are actually hyphal masses and are not due to the cellulose residue left after lignin decomposition. In the early stages of decay individual hyphae grow only through the lumen of tracheids and vessels, but in the advanced stages the vegetative mycelium grows throughout the wood substratum. Growth is especially heavy toward the wood surface, that is, in the tracheary elements beneath the bark (if any is present) and/or on the cut or broken ends of logs and stumps (Figure 8, page 40).

In early stages of decay the enzyme activity of the hyphae is slight and cell walls are broken down only at points of hyphal penetration. As fungal growth increases, the tracheid and vessel walls are almost completely broken down leaving, in some cases, only primary wall material intact (Figure 9, page 47) and, in others, totally destroying the cell. This breakdown occurs in tracheary elements which become occluded with masses of hyphae. In such severely decayed wood hyphae grow through the parenchyma which appears uninfected in earlier stages of decay. The only elements which seem resistant to hyphal attack are the fibers. These cells have been found intact in areas of wood in which all other elements have been destroyed (Figure 10, page 47). The resistance of fibers to the catabolic action of the hyphal enzymes appears to be due to the thickness of their walls and to their occurrence in compact "bundles". The narrow, inaccessible lumen may also contribute to resistance, since secondary wall breakdown appears to be carried out by hyphae inside an individual cell. The mode of hyphal growth described here continues throughout the wood substratum without any observable differentiation in the individual hyphae. Modification of the hyphae directly under,

and involved in the formation of, a developing basidiocarp could not be observed.

2. Hyphal Emergence and Primordium Formation.

Prior to the emergence of hyphae from the side of a log, a compact mass of interwoven hyphae forms under the bark. A group of hyphae, in parallel formation, then emerges through a fissure in the bark and grows out over the wood surface forming a mat of densely interwoven hyphae (Figure 11, page 47). If the relative humidity in the microhabitat in which the fungus is growing remains near saturation for a period of 48 hours or longer during hyphal emergence, a mat of indeterminate size grows over the wood surface. The size of the mat is dependent upon the duration of the period of high humidity. When the humidity drops below a maximum of 80% linear spread ceases. If the microhabitat remains saturated for several days during mat formation a large growth will occur, but if the relative humidity is below 80% at this time linear spread ceases several hours after hyphal emergence, resulting in the formation of a "button" primordium (Figure 12, page 47). The physical position of emerging hyphae also influences the

ultimate physiognomy of the basidiocarp. Basidiocarps of *P. adustus* covering the cut end of a log or stump develop from numerous discrete primordia formed from hyphal masses emerging from the cut ends of tracheary elements. The larger clusters of imbricate pilei found on the side of a log or stump generally develop from one, or a few, large primordia which spread extensively over the bark surface. Extensive primordia have been observed on the ends of logs only if the wood has been broken off creating an overhanging, irregular surface.

Soon after cessation of radial growth, the emergent hyphae develop slightly thickened walls and form a highly compacted mat of interwoven hyphae. Within 24 hours a gray-brown pigment is produced over much of the surface of this mat, but the margin always remains unpigmented. The pigment is localized in the cell walls of hyphae which are slightly thicker walled than other hyphae of the primordium. This compacted, pigmented mat of felted hyphae, which has a dimpled surface, and in which radial growth has ceased is the basidiocarp primordium. At this stage there is as yet no discernable hyphal organization other than regular interweaving with a tendency to longitudinal orientation. The primordium becomes

thicker as the hyphae already present continue to branch but there is no increase in surface area. The primordium stage is of such short duration that it is seldom observed in the field.

3. Basidiocarp Development.

Pileate, effused-reflexed and resupinate basidiocarps differ in some developmental aspects and in certain anatomical features. The ultimate form of the mature basidiocarp is greatly influenced by physical orientation and topography of the wood surface as well as by the microclimate. The pileate form has been found to be the most common, although the type specimen at the New York Botanical Garden is effused-reflexed. All basidiocarp forms are similar in their early development prior to the time of pileus formation. The following detailed description applies to the development of a pileate basidiocarp.

After primordial formation is complete, development of the basidiocarp is initiated. The interwoven hyphae of the primordium become organized into loose strand-like associations which grow parallel to each other in a horizontal plane. Similar

hyphal associations are referred to as "Hyphenbündeln" by Lohwag (1940) and as hyphal strands by Butler (1957). Butler defines hyphal strands as "linear hyphal aggregates with the capacity to extend unidirectionally" and describes them as consisting of "loose aggregates of hyphae which increase in thickness by accretion of hyphae from the base" (1957). Although these definitions refer to the structure and development of vegetative hyphae, they can be applied to one system of hyphae in the developing basidiocarp of *P. adustus*. In addition to the system of hyphal strands, the undifferentiated hyphae from the primordium give rise to a system of interwoven hyphae which forms part of the basidiocarp. In the region of the hyphal strands these interwoven hyphae form a much less compact mass which enmesh the strands (Figure 13, page 48). No strands form at the base of the pileus where it is attached to the wood or at the margin which remains sterile. The base is composed of a compacted mass of interwoven hyphae which show a tendency toward vertical-parallel orientation (Figure 14, page 48). The interwoven hyphae at the margin also tend toward parallel orientation and are directed in the same plane as the pileus margin (Figure 15, page 48). Thus, the

context of the pileate basidiocarp is composed of a system of hyphal strands which appear to direct the course of pileus growth and a second system of less compact interwoven hyphae. The hyphae of both systems are morphologically similar, being thin-walled, clamped, 2.2-3.3 μ in diameter, with an average of 2.6 μ, and they resemble the vegetative hyphae. The hyphal strands grow out approximately perpendicular to the surface of the log from the primordium. Such growth may occur at numerous points on the primordium forming a large cluster of imbricate pilei or only at one or two points, forming a few separated pilei. Generally, the imbricate pilei will form on an extensive primordium situated on a standing stump or on the side of a smooth-surfaced log. The few, discrete pilei form on a button primordium or on an extensive primordium which is located on the underside of a log with only a marginal area growing in vertical orientation on a smooth wood surface.

The hyphae of the context give rise to the tomentum and the hymenophore. The fine tomentum is formed by profuse upward branching of the horizontally oriented hyphae at the upper region of the context and by vertical growth of terminal cells (Figure 16, page 48). These hyphae are morphologically similar

to the context hyphae but have a slightly larger average diameter (3.1 μ).

The hymenophore is composed of a more complex tissue than that of the other regions of the basidiocarp. The first stage of development is the formation of hyphal strands in close parallel arrangement at the lower limit of the context where the dissepiments will later form. The hyphae have a diameter of 3.3-4.4 μ with an average of 4.1 μ, and are wider than the other hyphae of the basidiocarp. These hyphae branch profusely with the branches growing in a downward direction to form the highly compacted, pigmented tissue which makes up the transition zone between the context and the dissepiments. Some hyphae of the transition zone gradually become oriented vertically downward showing a positive geotropic alignment and forming the tramal tissue which makes up the central part of the dissepiments. Other downward growing hyphae of precisely defined areas of the transition zone cease growth, a response which results in the formation of tubes (Figure 17, page 48). There is a significant difference in the mode of growth of the tramal hyphae (Figure 18, page 48) compared with those of the context. From measurements of the distance between septa, it appears that tramal

hyphae increase in length mainly by intercalary growth. The clamps on these hyphae have been found to be 100 μ to more than 200 μ apart while clamps on hyphae in the other regions of the basidiocarp are generally less than 50 μ apart and can be as close as 5-7 μ. Moreover, the tramal hyphae branch less frequently, are thicker walled than the context hyphae and are pigmented.

At the time of dissepiment formation there is a simultaneous development of the hymenium. This spore-forming layer develops by horizontal growth of short branches which grow out perpendicular to the dissepiments and are accompanied by similar growth of terminal cells. These short branches are cut off from the main hyphae by a slight constriction at the base of each and the formation of a simple septum. The result is a palisade of single-celled, clavate structures (Figure 19, page 49). It is believed that basal constriction rather than terminal swelling occurs because the terminus of the clavate cell is the same diameter as the hypha from which the branch, now considered a basidium, is derived. There is no subhymenium. The basidia are quite small and appear similar to other terminal cells. They have an average measurement of 8 x 4 μ which falls within the

range of the tramal hyphae (3.3-4.4 μ). Unless basidiospores are present no sterigmata are evident. The four spores produced on a basidium are hyaline, subglobose to oval and measure 5-6 x 3-4 μ. Under adverse conditions the production of basidiospores ceases abruptly and those already produced are discharged. Spores are rarely found in specimens examined more than 24 hours after collection. There is no evidence of a thickening hymenium which would result in more than one spore crop per tube. Under constant temperature and moisture conditions in the laboratory, hyphal tips have been observed to grow from the trama between the basidia and extend into the pores, but these remain undifferentiated and develop during a cessation of basidiospore production. Basidiocarps persist only one growing season but the basidia making up the hymenium will mature asynchronously and consequently basidiospores are produced during favorable periods of the growing season. Basidia can be induced to produce spores in the laboratory by alternate wetting and drying of the basidiocarp.

4. Variations in Basidiocarp Development.

a. Resupinate and Effused-Reflexed Basidiocarps.

The development of resupinate and effused-reflexed basidiocarps is the same in the early stages as that of the pileate form. The mycelium emerges from the wood and grows over the surface. Once linear spread ceases the mat becomes felty, the central surface hyphae develop thickened, pigmented walls and dimpling of the pigmented region occurs.

The resupinate basidiocarp (Figure 5, page 45) will develop on the underside of a log or fallen tree or on the side of the substratum where it can utilize furrows in the bark or fissures in the wood to form a positively geotropic hymenophore. The context, which is in direct contact with the wood surface, is composed of hyphae organized for the most part in parallel orientation. But, these hyphae do not aggregate to form the distinct strands found in pileate basidiocarps. Rather, the hyphal organization very closely resmebles that of the base of the pileus (see Figures 20 and 14, pages 48-49). The surface where the pigmentation develops is composed of compacted, interwoven, profusely-branched hyphae, similar to the transition zone forming beneath the pileus context. It is from this tissue that the dissepiments are formed. Wherever the upper surface of the basidiocarp is oriented obliquely or parallel to the ground,

groups of hyphae grow down in a positively geotropic manner to form the hymenophore (Figure 21, page 49). This same development occurs in the effused-reflexed basidiocarp, but at the upper, vertically oriented margin the hyphae grow horizontally away from the substratum surface to form the reflexed part of the basidiocarp. This reflexed growth resembles development of a pileus.

b. Daedaloid Pore Surface.

A common feature of the imbricate, resupinate and effused-reflexed forms is that part of the basidiocarp spreads along the wood surface. In all these forms there is a positive geotropic orientation of the tubes. The basidiocarp of *P. adustus* is a very plastic structure and will form tubes if there is the slightest oblique orientation of the context. The hymenophore will take on a daedaloid appearance in some areas, typically on imbricate forms where a resupinate area of a basidiocarp meets with a pileus, and on effused-reflexed forms where the hymenophore becomes oriented horizontally from a vertical position. The pores will be daedaloid until the pileus context is oriented parallel to the ground and then the tube mouths become even, forming the regular sur-

face that has been reported to be characteristic of *P. adustus*. This same daedaloid hymenophore surface occurs over large areas of resupinate and effused-reflexed basidiocarps having an oblique orientation.

c. Zonation.

Some investigators have stated that zonation of the pileus is characteristic of *Polyporus adustus*. I have found that this is not a constant feature but occurs only when the developing basidiocarp is subjected to adverse climatic conditions. Extremes in temperature and dry conditions might not be severe enough to kill the entire basidiocarp, but the terminal cells of the growing margin will cease growth and become pigmented (Figures 22 and 23, page 50). This pigmentation differs from that of the hymenophore hyphae in that the terminal cells do not have thickened walls and the pigmentation is in the cytoplasm. When favorable conditions return, lateral branches develop behind the terminal cells and normal growth is resumed. The dead pigmented cells persist in the context and tomentum and give the basidiocarp its zoned appearance.

Figure 1. Site II showing logs surrounding hygrothermograph. This photograph was taken when logs were first put out in April, 1969.

Figure 2. Site II as it appeared during the latter part of July, 1969. The light recorder is in the photograph; the Rustrak recorder and battery are at lower right.

1

2

Figure 3. Imbricate basidiocarps, the common form of development. (X0.7)

Figure 4. Effused-reflexed basidiocarp. The reflexed portion is indicated by the arrow. Note the daedaloid pore surface around the arrow. (X1.9)

Figure 5. Resupinate basidiocarp which developed on the underside of a poplar log. (X1.0)

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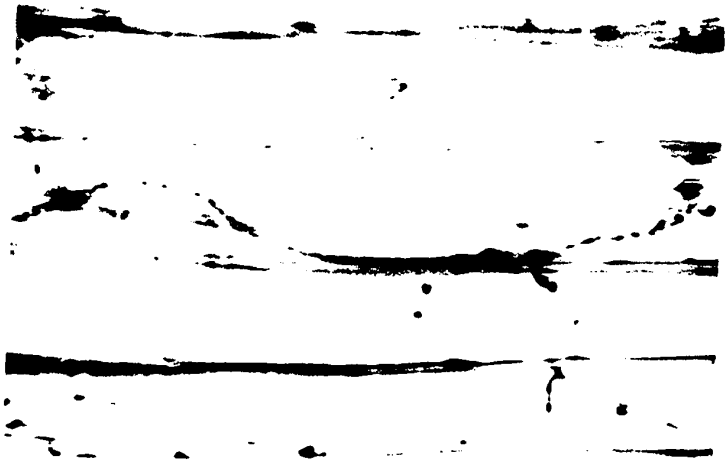
Figure 6. Individual hypha growing in a sinuous manner through a poplar wood tracheid. (X629.8)

Figure 7. Hyphal aggregation in a vessel of poplar wood. (X160.5)

Figure 8. Cross section of wood just beneath basidiocarp. Note that tracheary elements are filled with masses of hyphae, formed by profuse branching. (X512)



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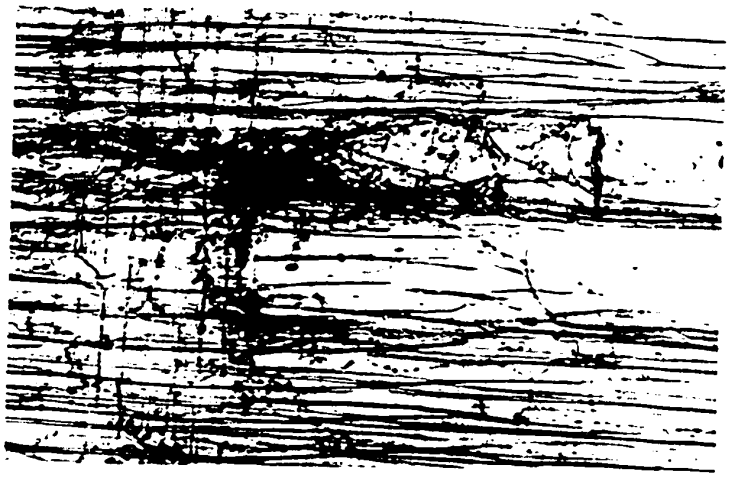

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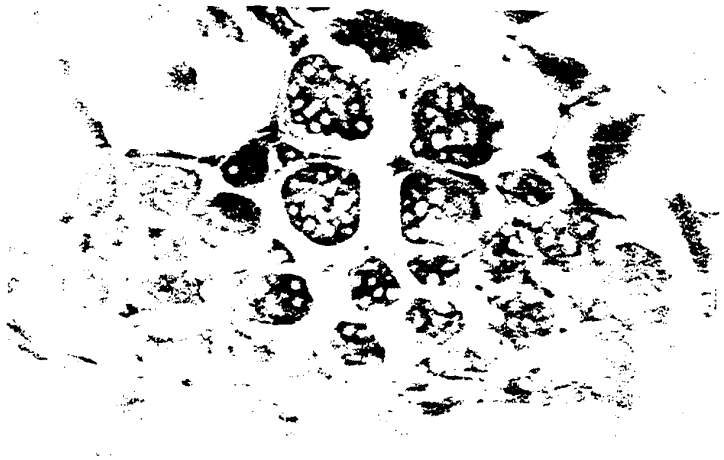

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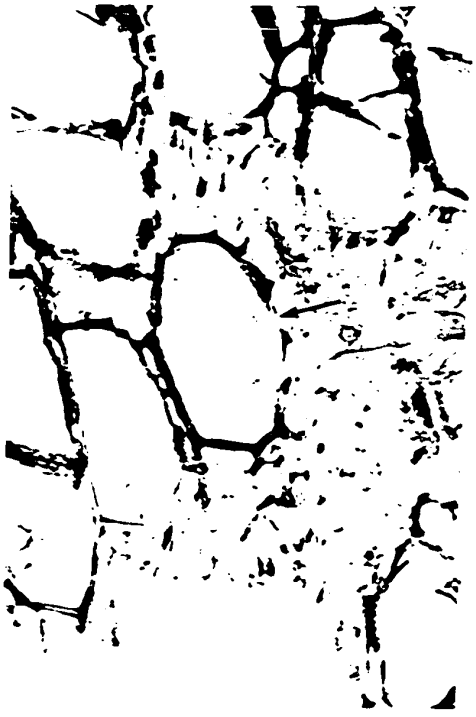
Figure 9. Wood section showing the breakdown of the secondary, lignified tracheid walls. The primary wall is left intact (arrow). (X1000)

Figure 10. Wood in advanced stage of decay. Only bundles of thick-walled fibers remain intact. (X128)

Figure 11. Hyphae emerging through a fissure in the bark of a log from Site III. (X63)

Figure 12. A button primordium (arrow) on the end of a log at Site II. (X1.6)



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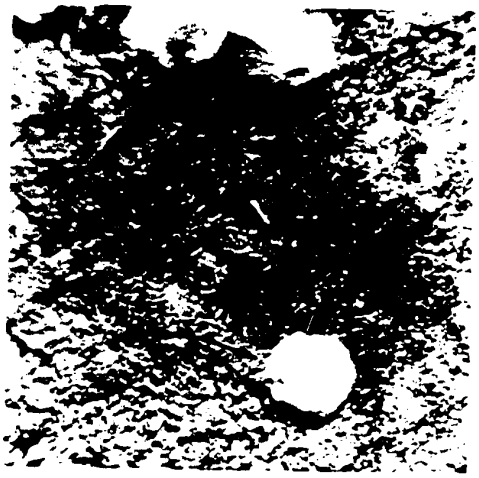

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Figure 13. Context of a pileate basidiocarp. Note the hyphal strands which are enmeshed by the system of interwoven hyphae. (X511)

Figure 14. The base of a pileus, composed of closely packed hyphae showing a general parallel alignment. (X1000)

Figure 15. Hyphae in parallel alignment, making up the sterile margin of the pileus. Compare with Figure 13 showing the construction of the context behind the margin. (X1000)

Figure 16. The pileus surface, composed of short hyphae arising directly from the context. (X1000)

Figure 17. The transition zone, from which hyphae are beginning to grow downward to form dissepiments. Cessation of this growth in precisely defined areas (arrow) results in tube formation. (X250)

Figure 18. Dissepiment, showing tramal hyphae in close parallel alignment and the hymenium. (X400)

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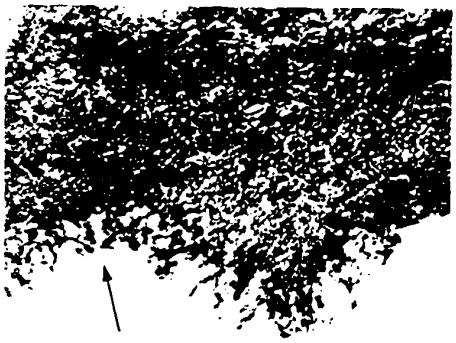

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Figure 19. The hymenium of *P. adustus*, a palisade of single-celled, clavate structures. (X1000)

Figure 20. Resupinate basidiocarp showing context (C), composed of parallel hyphae without strand formation. The transition zone (T) is morphologically similar to that of pileate forms. (X400)

Figure 21. Resupinate basidiocarp developing in a bark furrow. Note the formation of dissepiments only where the hyphae are able to align themselves in a positively geotropic manner. (X25)

19

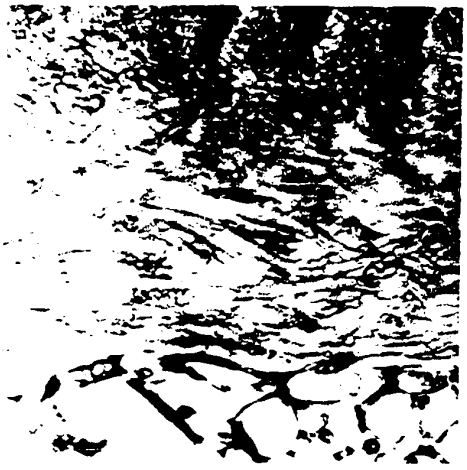

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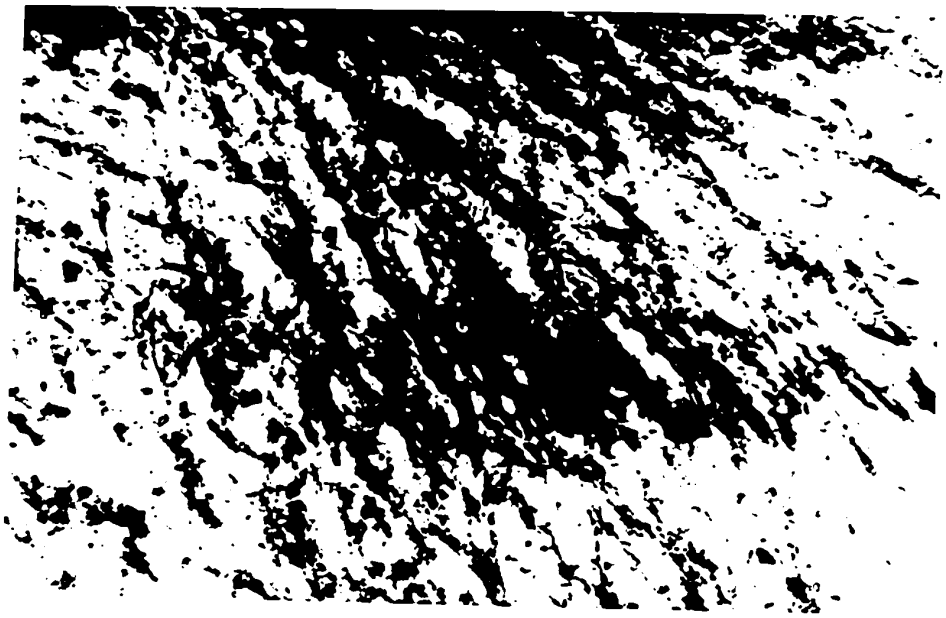
Figure 22. Zonate region of context of revived basidiocarp. (X170)

Figure 23. Pigmented hyphal tips in context which give zoned appearance to pileate basidiocarps. Renewed growth occurs by lateral branches developing behind pigmented apical cells. (X1072)



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23

THE INFLUENCE OF THE MICROHABITAT ON GROWTH AND DEVELOPMENT

Materials and Methods

The term "microhabitat" as used here refers to the localized area in which *Polyporus adustus* is growing. It is affected by changes in the microclimate, i.e., temperature, moisture and light, and also by the physiological environment of the wood substrate (See Smith, 1966. pp. 13, 87-91). According to this definition the microhabitat includes the limited environment in which *P. adustus* grows in nature and that created artificially in the laboratory. Thus, the methods used in studying the microhabitat include those carried out in the field and in the laboratory. The cultural studies were intended primarily to confirm or help to explain the data compiled in the field.

A. The Natural Environment

In order to observe growth and basidiocarp

development of *P. adustus* and to measure the environmental parameters affecting it under partially controlled conditions, three study sites were set up in the field. The sites chosen differed in the density of the tree canopy, exposure and drainage. Each was considered to be a microhabitat in which *P. adustus* could be studied (Figures 1 and 2, page 44).

Site I was located on the Bocock Farm, north of St. Albert, Alberta at the ecotone between a poplar grove and the grass and shrub border of a small pond. The area was approximately 100 ft north of the pond. There was a light aspen and balsam poplar canopy just immediately over the site which received diffused but bright sunlight during the day. There was moderate ground vegetation 3-5 decimeters high. The ground was flat but drainage appeared good and the area was dry compared to the other sites.

Site II was in the center of a large aspen and balsam poplar grove approximately 500 ft southeast of the same pond. The tree canopy was moderate and the sunlight reaching the study area was diffused unless a strong wind disturbed the trees, in which case bright direct light could penetrate. Ground vegetation in the immediate vicinity grew to be about 1.0-3.0 decimeters. The relative humidity appeared

to be generally high. There were numerous fallen, decaying trees. One large one bordering the experimental area was covered with *P. adustus*. The topography was slightly sloped and drainage was good.

Site III was located in the University Forest Reserve which is on the tree covered north-facing slope of the North Saskatchewan River Valley adjacent to the campus. This site was on a small plateau toward the bottom of the steep inclination. The tree canopy was very dense and consisted primarily of spruce and poplar. Ground vegetation grew to 2.0-3.0 decimeters on the plateau. The site was always damp due to the collection of water draining off from above. This microhabitat was the most sheltered, appeared to receive less light and direct rainfall (due to the heavy spruce canopy) and was protected from wind.

1. General Methods

Forty poplar logs were placed at each site. Twenty-five were artificially infected with *P. adustus* and 15 were naturally infected. The artificially infected logs were prepared by first cutting recently-felled, sound poplar trees into 1 ft lengths. A

hole was drilled into the center of each log and they were autoclaved at 15 lbs steam pressure for 1 hour for 3 consecutive days.

Prior to sterilization of the logs, inoculum was prepared using 2 wood mycelium isolates of *P. adustus* which had been maintained on malt agar slants. The stock cultures were transferred to the center of Blakeslee's malt agar plates (Blakeslee, 1915). After the colonies spread half way across the plates 5 mm plugs were cut from the margin using a cork borer. These were aseptically transferred to sterile 3 dram glass vials containing poplar sawdust enriched with Badcock's accelerator medium (Badcock, 1941). The vials were capped with small glass beakers and the cultures incubated at 26°C until the mycelium was growing throughout the sawdust medium. At this time the logs were autoclaved and one vial was inserted into the hole previously drilled into each log and sealed over with plasticine.

The naturally infected logs were cut from 3 dead standing aspen poplar trees found on the edge of a shallow lake. Basidiocarps of *P. adustus* were growing on each and the characteristic mottled rot caused by this fungus could be seen in the wood. The trees were brought back to the laboratory where

the wood was examined microscopically and cultured on Nobles' malt agar to determine that the wood was definitely infected with *P. adustus* and whether other fungal species were present. Bacteria and yeasts were found in wood taken from near the log surface and *Penicillium* sp. and *Hormiscium* sp. were isolated but the only basidiomycete found in these logs was *P. adustus*.

The aritficially and naturally infected logs were set out in 2 piles at each site during the first week of May, 1969. Data were compiled on the temperature, moisture (relative humidity, rainfall, moisture content of wood) and light conditions of each microhabitat during the first season. This information was correlated with observations of the emergence of hyphae from the wood to form a primordium and the subsequent development of basidiocarps.

The progress of the vegetative mycelium in artificially and naturally infected logs was studied during the growing season from June 1969 to August, 1970. Logs were removed periodically from each site and brought back to the laboratory. They were then cut transversely with an electric band saw and each block was labelled. With aritificially infected logs the wood block at the point of inoculation was identi-

fied as "0". The blocks cut to one side of "0" were identified consecutively as +1, +2. etc. and to the other side as -1, -2, etc. The naturally infected logs were labelled in the same manner with "0" being the center of each log. Then the wood blocks, each measuring approximately 3 cm thick and 15-30 cm in diameter, were cut into 4 sectors. The sectors were placed in containers containing sterile distilled water and soaked for 24-48 hours at 5°C. After soaking, they were sectioned on the sliding microtome at 10-30 μ increments, stained using the modified Cartwright method previously described (p. 26) and mounted in toluene-diluted Permount. The sections were studied microscopically in order to observe the growth of the vegetative mycelium through the sound (artificially infected) wood and the changes occurring in the wood in the advanced stages of decay (naturally infected) prior to hyphal emergence and primordial formation.

Developing (primordial stages) and maturing basidiocarps were photographed in situ. Some were removed and prepared for microscopic study and others were observed as they developed.

2. Measurement of Environmental Parameters

a. Temperature and Relative Humidty.

A recording hygrothermograph was set out at each site to give continuous data on temperature and relative humidity conditions in the microhabitat. Two of these instruments were Short and Mason model 2362P.P. and the third was a Lambrecht model 252. Each was enclosed in a protective casing of wood with screening on the sides and bottom. Before using them in the field the hygrothermographs were calibrated by the Electronics Division of the University Technical Services Department. During the entire period in which data were collected the accuracy of the instruments was checked against readings obtained with a Bendix model 566 psychrometer. This was done at least weekly when the charts were changed and generally was done bi-weekly. At the same time the charts were changed the hair humidity sensors were washed with distilled water, in order to remove any debris and to regenerate the hairs. The maximum and minimum readings for temperature and humidity were recorded on a daily basis. Weekly averages were computed and the information was compared for each microhabitat and also with the meteorological data compiled by the Meteorological Branch of the Department of Transport at the Edmonton Industrial Airport.

b. Rainfall.

The total rainfall occurring in each microhabitat was measured with a rain gauge. The gauge consisted of a glass funnel surrounded with an acetate splash shield which emptied into a 50 ml graduated cylinder which was placed in a larger cylinder to catch any overflow. The acetate shield extended 10 cm below the funnel mouth and served as a cap for the outer cylinder to prevent evaporation. The gauges were checked after every rainfall. The actual rainfall was determined by dividing the volume of water in the cylinder by the ratio of the funnel to cylinder area:

$$\text{Actual rainfall} = \frac{\text{total water in cylinder}}{19}$$

(ratio of cylinder to funnel area)

c. Substrate Moisture Content.

The moisture content of the logs was measured to determine whether there was any correlation between the moisture available to the vegetative hyphae and fluctuations in the microhabitat. At weekly intervals corings were taken from 3 logs at each site with an increment borer and were immediate-

ly placed in preweighed screw-top vials. In the laboratory the wet weight of the wood + vials was determined using a Mettler balance. The vials were then uncapped, dried at 90°C for 48 hours and placed in a desiccator containing $CaCl_2$ for another 48 hours. The caps were replaced and the dry weight of the wood + vials was determined. Because of differences in the size (and consequently the weight) of each core, all data were recorded as percent moisture content.

d. Light.

Continuous measurements of light intensity were taken at Sites I and II using recorders built by the Electronics Division of the Technical Services Department. Each recorder consisted of a series of silicon photoelectric cells mounted on an aluminum base to form a circle and sealed in a lucite dome. Light striking the cells sent a continuous electrical current to a Rustrak model 88 recorder attached to the light sensing apparatus. The intensity was recorded on a 0.00-1.00 milliampere scale and imprinted on a continuously moving chart. Power was supplied to the recorders by 12-volt truck batteries which were recharged every 3-4 days. The chart readings

were converted to foot-candles by means of a standard curve made for each recorder by calibrating the milliampere readings with foot-candle readings taken with a Canadian Research Institute light meter. Light meter readings were taken weekly at both sites and compared to the milliampere vs. foot-candles standard curve to check the accuracy of the recorders.

Because of the high cost of these recording apparatuses and the lack of information available concerning their performance in the field over a prolonged period, only two were built. Therefore, the light intensity at Site III was recorded periodically with the same C. R. I. light meter used to calibrate the recorders. The reasons for placing the instruments at Sites I and II in preference to III were the unavailability of level ground on which to place the bulky equipment and the difficulty in transporting the heavy batteries to this area to which there was no vehicular road. Also, the light intensities appeared much lower and more constant than at the other sites and could be more easily evaluated by spot checks.

Periodically the recorded portions of the charts were removed from the Rustrak recorders and the data was tabulated for each site. The minimum,

maximum and average milliampere readings/day were recorded and converted to foot-candles using the standard curve. The data from Site III consisted of foot-candle readings and the time at which they were taken. Data from the three sites were compared.

B. The Cultural Environment

Laboratory experiments involved growing *Polyporus adustus* on synthetic and natural substrates in microhabitats which were entirely artificial or which simulated the natural environment. The procedures used in the study of the cultural environment will be discussed under four headings: general methods; temperature and vegetative growth; substrate composition and the vegetative mycelium; basidiocarp induction in the artificial environment.

1. General Methods

All methods used in cultural studies were standardized to reduce experimental error. Inoculum was obtained from wood and from spores. (See Appendix II for methods of spore isolation). Duplicate stock cultures were maintained on half-strength Nobles'

malt agar slants (Nobles, 1958) at 5°C . Inoculum was prepared by transferring a small piece of mycelium from a stock tube to the center of plates containing 15 ml of solidified medium. When the mycelium had grown to within 10-15 mm of the plate edge, 5 mm plugs were cut with a sterile cork borer at the colony margin and transferred to culture vessels. When inoculating solid medium the mycelial surface of the plug was always placed in contact with the agar. The use of a mycelial suspension inoculum was discarded because of discrepancies in growth rate and hyphal morphology found in replicate cultures. Possibly, the differences could be traced to the strong tendency of *P. adustus* to sector, since the sectored region might exhibit differences in growth and morphology. Extensive observations of monokaryotic cultures, following the procedures outlined by Nobles (1965), have shown considerable variation in isolates from the same basidiocarp.

The media used in this study (with the exception of the sawdust medium) could be adapted for liquid or solid cultures. Liquid media were solidified with 1% Oxoid agar (w/v) or 2% Difco Bacto agar (w/v). The water soluble media components were dissolved in one-half the final volume of water and

cold sterilized using a Millipore filter apparatus. The agar and other non-filterable or non-soluble constituents were added to the remaining volume of water and autoclaved at 15 lbs pressure for 20 minutes, allowed to cool and added to the cold-sterilized constituents. This procedure was employed in order to eliminate the possibility of denaturing proteins and precipitating any of the metallic ions which had been added. Liquid media were dispensed in 50 ml aliquots into matched, sterile 250 ml Erlenmeyer flasks, plugged with sponge stoppers and finally covered with disposable plastic beakers to retard evaporation. All flasks used in liquid culture experiments utilizing synthetic media were washed in detergent (Organisol, Fisher Scientific Co.) and soaked in a dichromate-sulfuric acid solution. They were rinsed with several changes of tap water, twice with distilled water and dried in a hot air oven. All solidified media were poured in disposable, pre-sterilized 8.5 cm plastic Petri dishes. All media were incubated for 48 hours prior to inoculation to check for contamination and duplicate sterility controls were maintained for the duration of each experiment.

For all cultures grown on liquid media dry

weight was employed as the index of growth. The contents of each flask were poured into a Buchner funnel containing a tared 9 cm disc of Whatman No. 3 filter paper, washed with 250 ml of distilled water, dried to constant weight in a hot air oven at 90°C and stored in a desiccator for 48 hours before reweighing. Quantitative data were based on the arithmetic mean of 3-5 replicates.

As cultural studies progressed, it was found that liquid cultures were not entirely satisfactory for growth studies of *Polyporus adustus*. The results of highly standardized growth experiments did not yield replicate results in 3 different runs which were carried out. However, liquid cultures were useful in establishing trends and were used in preliminary experiments. These included formulating growth curves to evaluate the media used for these studies, screening of carbon and nitrogen sources to be used in utilization experiments and determining the optimal hydrogen ion concentration.

Most experiments were carried out using solidified media. These solid cultures offered a less precise index of growth than liquid cultures, i.e., linear growth and subjective observations vs. biomass determination. However, this disadvantage

was outweighed by two advantages: solid media cultures yielded replicate results in all runs and such cultures allowed morphological studies of the mycelium (all liquid-culture experiments required that cultures also be grown on solid media for morphological observations). Linear spread of the mycelium (mm) from the inoculum plug/day was the criterion used to determine growth. The quantitative data are presented in terms of the arithmetic mean of the 10-15 replicates set up for each experimental variable studied. Morphological data are presented as a qualitative estimate of vegetative growth and of hyphal differentiation based on visual examination of each culture. Photomicrographs of the vegetative mycelium were taken with a Zeiss photomicroscope to illustrate hyphal differentiation.

2. Temperature and Vegetative Growth

The growth of *P. adustus* at selected temperatures over a 27° range was studied. Cultures were grown on Blakeslee's malt (Blakeslee, 1915) and glucose-asparagine basal medium (Lilly and Barnett, 1951). Ten replicate plates of each medium were inoculated with a polyspore inoculum and incubated

at 10°, 15°, 20°, 26°, 30° and 37°C (20 plates/temperature). The linear spread of the colonies was measured daily. The results were recorded as the arithmetic mean of the 10 replicates/medium for each temperature. Cultures were evaluated on the basis of daily radial growth and the time required to cover the 8.5 cm plates.

3. Substrate Composition and the Vegetative Mycelium

Experiments were carried out to evaluate the various media used in cultural studies. For morphological observations it was desirable to use a medium on which *P. adustus* would grow at a moderate rate and exhibit normal development, i.e., produce a vegetative mycelium similar to that occurring in natural substrate. The three media tested were Blakeslee's malt, which served as a positive control, Lilly's and Barnett's glucose-asparagine basal medium, and Henningsson's medium B (Henningsson, 1967a). Cultures were grown on both liquid and solid medium and incubated at 26°C . Triplicate sets of flasks were harvested at 3 day intervals until the cultures showed evidence of autolysis. Cultures on solid media were evaluated on the basis

of linear spread and their hyphal morphology. Growth was found to be greatest on Blakeslee's malt medium closely followed by medium B and glucose-asparagine basal medium. The moderate growth rate achieved in the synthetic media made them more desirable for studies involving morphological observations. It was decided to use the glucose-asparagine basal medium for substrate utilization experiments because it was less complex to prepare and supported equivalent growth. Blakeslee's malt medium was used in temperature studies and also served as a standard for all experiments. Henningsson's medium B was used on a limited scale in nitrogen utilization experiments.

Growth of *Polyporus adustus* in glucose-asparagine basal medium at pH 2.0, 3.0, 4.0, 4.5, 5.0, 6.0, 6.5, 7.0 and 8.0 was examined. Triplicate sets of cultures were grown in liquid medium in which the pH was adjusted with 6N NaOH or 6N HCl. Cultures were harvested after 14 days. *P. adustus* grew best at pH 4.5-6.5. Since growth was almost comparable within this range, it was decided to adjust the pH of media only if it fell outside these limits. However, in cases where several experiments were closely related to each other the

media was adjusted to 5.5.

a. Carbon Utilization

Carbon utilization studies were carried out on solidified basal medium with 0.5% asparagine (w/v). A medium containing 1% glucose served as a positive control. A medium with no carbon source served as a negative control. The carbon sources tested were the pentoses xylose and ribose, the hexoses galactose and mannose, the disaccharides cellobiose, maltose and sucrose and the polysaccharide cellulose. These were incorporated into the basal medium containing 0.5% asparagine (w/v) at a level equivalent to 1% glucose. Each series of 15 replicates/carbon source was incubated at 26°C . Carbon utilization was evaluated on the basis of daily radial growth (mm) and on visual evaluation of colony vigor. Pieces of the mycelium were removed, put on slides and stained with 1% aqueous β-phloxine for microscopic examination. Records were kept of the presence of hyphal types and of structures which developed on the different carbon sources.

b. Nitrogen Utilization

Nitrogen utilization experiments were car-

ried out using solidified basal medium with 1% glucose (w/v). A medium containing 0.5% asparagine served as a positive control while a medium containing glucose and no nitrogen served as a negative control. The inorganic nitrogen sources tested were KNO_3 and $(NH_4)_2SO_4$. The organic sources tested were the amino acids glycine, L(+)-lysine, DL-aspartic acid, N-dimethyl amino succinic acid, L-glutamic acid and L-cysteine, and urea. All nitrogen compounds were added to the basal medium with glucose at a level equivalent to the nitrogen in 2 g of asparagine (424 mg/liter). Each series of 15 replicates was incubated at 26°C . Nitrogen utilization was evaluated in the same manner as carbon utilization.

4. Basidiocarp Induction in the Artificial Environment

Since basidiocarp development did not proceed beyond a very early primordium on artificial medium, experiments were carried out on natural wood substrate under laboratory conditions.

Blocks of decayed poplar, sound poplar and sound birch wood, 5mm x 5mm x 5mm, were placed in

10.0 x 8.0 cm culture dishes or in quart canning jars lined on the bottom with absorbent cotton. These were autoclaved at 15 lbs pressure for one hour on 3 consecutive days. Half the blocks were then aseptically transferred to culture dishes containing Blakeslee's malt agar. Inoculum was placed in a hole previously drilled in the block which was then plugged with a piece of sterile cork or the inoculum was placed directly on the agar medium on which the block was resting. The cultures were incubated in the dark at 26°C until the blocks were covered with a mycelial mat. The cultures were then divided into 3 groups. Group I remained in the incubator (dark, 26°C). Group II was placed on a shelf by a window and received bright diffuse light. Group III wood blocks were removed from the culture vessels and placed in a large covered tank on a bed of moist, sterile peat. The humidity was varied by spraying a fine mist of water with an aspirator flask attached to an air jet. Substrate moisture was altered by adding water to the peat or letting it dry out. The blocks received light measured at 80 foot-candles during the day and were subjected to the ambient temperature of the laboratory.

The method of Tamblyn and DaCosta (1958) was also used to induce basidiocarp development. Wide mouth glass bottles, 11 mm x 5 mm diameter, were filled with poplar sawdust containing 5% Badcock's accelerator medium (w/w) (Badcock, 1941) and 170% moisture content. The mixture was tightly packed and the bottles were covered with small jars and autoclaved at 15 lbs pressure for 1 hour. The medium was inoculated with mycelial plugs placed on the sawdust surface. Additional sterile medium was packed on top of the inoculum, the jars were replaced and the cultures were incubated at 26°C in total darkness. After the vegetative mycelium was growing throughout the sawdust medium, a water-saturated, sterilized poplar or birch block was fitted over the bottle mouth. Two wells, 4 cm in diameter x 1 cm deep, were drilled on opposing faces of the block prior to soaking and sterilization. One fitted snugly over the bottle mouth and was secured with molten paraffin. The blocks were covered with sterile polyethylene sheeting to prevent desiccation. After the vegetative mycelium became established over the block surface, the plastic sheeting was removed and the entire apparatus was transferred to a large sterile tank containing 2 cm of water in

the bottom and supplied with constantly changing air. The well on the top of the wood block was kept filled with sterile distilled water and the water level at the bottom of the tank was kept constant.

Sections of artificially and naturally infected logs were placed in tanks and subjected to variations in atmospheric humidity in the laboratory. Sound 6 inch sections of poplar logs, 5-6 inches in diameter, were inoculated and placed in the chambers used for wood block cultures described above. Naturally infected logs, 6-12 inches long, were placed in trays in a fume hood and subjected to the same conditions as the artificially infected logs.

The experiments utilizing natural substrates were carried out to determine under which artificial environment basidiocarp development in *Polyporus adustus* would occur. These data were compared to those compiled in field studies.

Results and Observations

A. The Natural Environment

1. General Observations

During the first season after the logs were inoculated with *P. adustus*, the vegetative mycelium grew sparsely through the tracheids and vessels of the wood. Examination of the wood sectors showed that hyphae had grown to a maximum of 5.0 cm in either direction from the point of inoculation or a total of 10.0 cm through the tracheary elements of a 30.5 cm (1 foot) log. Although hyphal growth was equivalent in most of the logs studied at the 3 experimental sites, it was generally greater in those at Site II. In all logs examined, hyphal growth was proceeding as described earlier, i.e., "one or two hyphae grow (ing) in a sinuous manner through the lumen of a tracheid or vessel. Branches from these grew transversely through the tracheary elements, either through the pit membrances or directly through the cell wall which is broken down by exogenous enzymes produced by the hyphae".

Basidiocarps developed on the naturally infected logs. These were abundant at Site III and moderate at Site II, but few developed at Site I. Only vegetative growth occurred in the artificially infected logs during the first season they were put out.

Periodic observations were made at Site III during the next year (the other sites had been disturbed and all the logs removed) to determine to what extent growth would occur in these artificially infected logs. When the site was checked on August 1, after approximately 2 weeks of considerable rainfall, numerous basidiocarps had developed and more primordia were forming. Both button and indeterminate primordia had formed and gave rise to typical effused-reflexed and imbricate basidiocarps (Figures 24, 25, 26, page 75). The wood was examined and was found to show signs of incipient decay only. No aggregations of mycelium were present to give the mottled appearance of advanced decay. Microscopic examination showed the hyphae growing through the lumen of the tracheids and occasionally in vessels. This growth was very similar to that observed the previous year and, in addition, there was considerably more branching with a number of hyphae growing trans-

Figure 24. Basidiocarps on artificially infected logs at Site III. Development occurred 15 months after inoculation. (X0.3)

Figure 25. Effused-reflexed basidiocarp on artificially infected log. Note pigmented central region and white margin. The yellow patch at the lower right is the plasticine used to seal the hole into which the inoculum vial was inserted. (X0.8)

Figure 26. Small, imbricate basidiocarp on the end of an artificially infected log. (X0.8)

versely through numerous adjacent tracheid walls across the wood section. An unidentified imperfect fungus was observed growing through the parenchyma, but the hyphae of *P. adustus* was not found in these cells.

2. The Influence of Environmental Parameters on Growth and Development

a. Temperature and Relative Humidity

The average weekly temperature ranges recorded at Sites I, II and III and that recorded at the Edmonton Industrial Airport are given in Figure 27. Primordia appeared first at Site III and were fully mature within 7 days. The average maximum temperature at this site remained around 20°C for a month prior to hyphal emergence. Primordia appeared at Sites I and II 10 days after primordia first formed on logs at Site III. The central surface became pigmented within 48 hours and pores formed within 72 hours. The temperatures at these sites showed a greater fluctuation than occurred at Site III and covered a wider degree range. The average weekly maximum and minimum relative humidty readings are shown in Figure 28. At all sites,

Figure 27. Comparison of the weekly mean temperature range of the microhabitats and the macrohabitat.

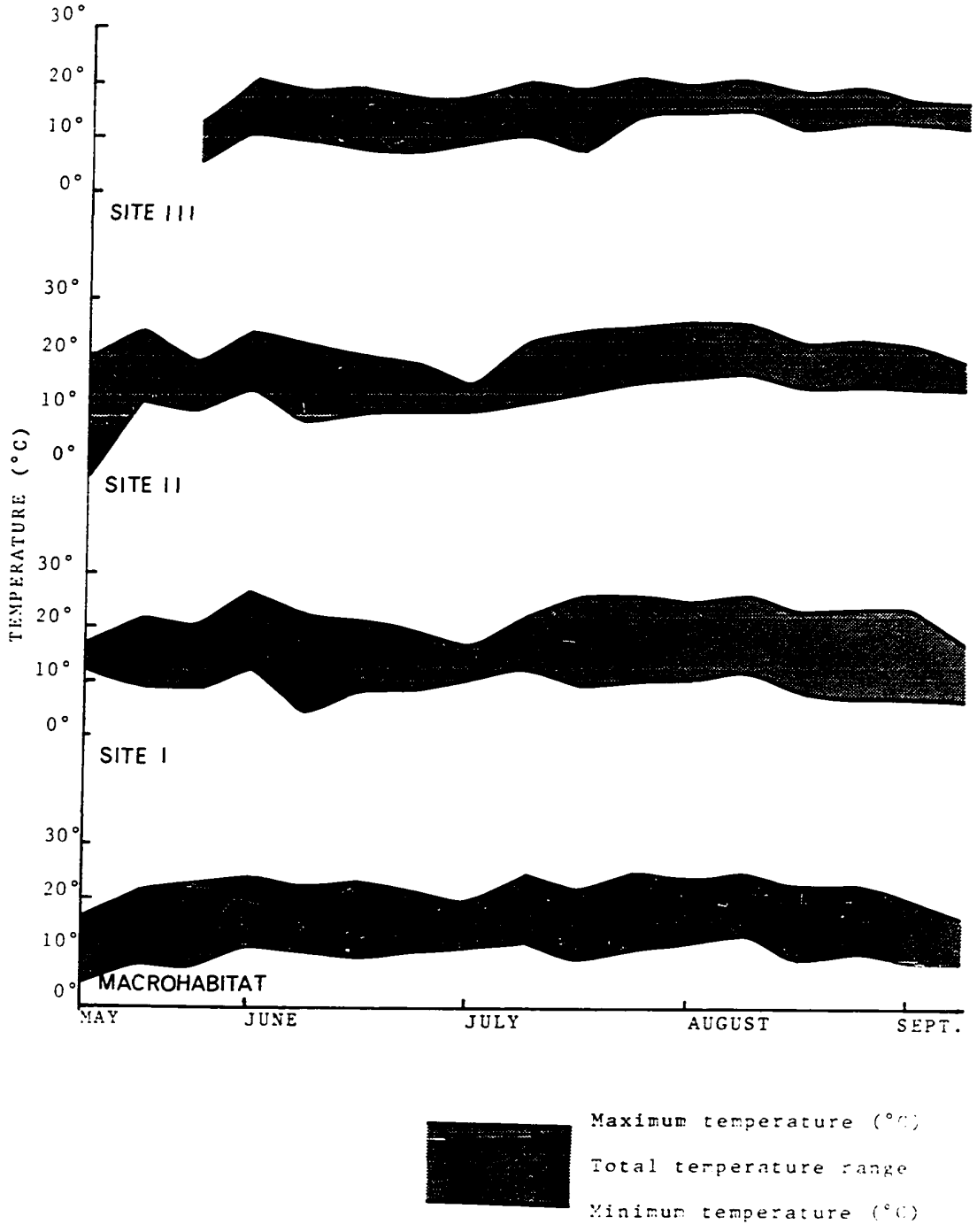
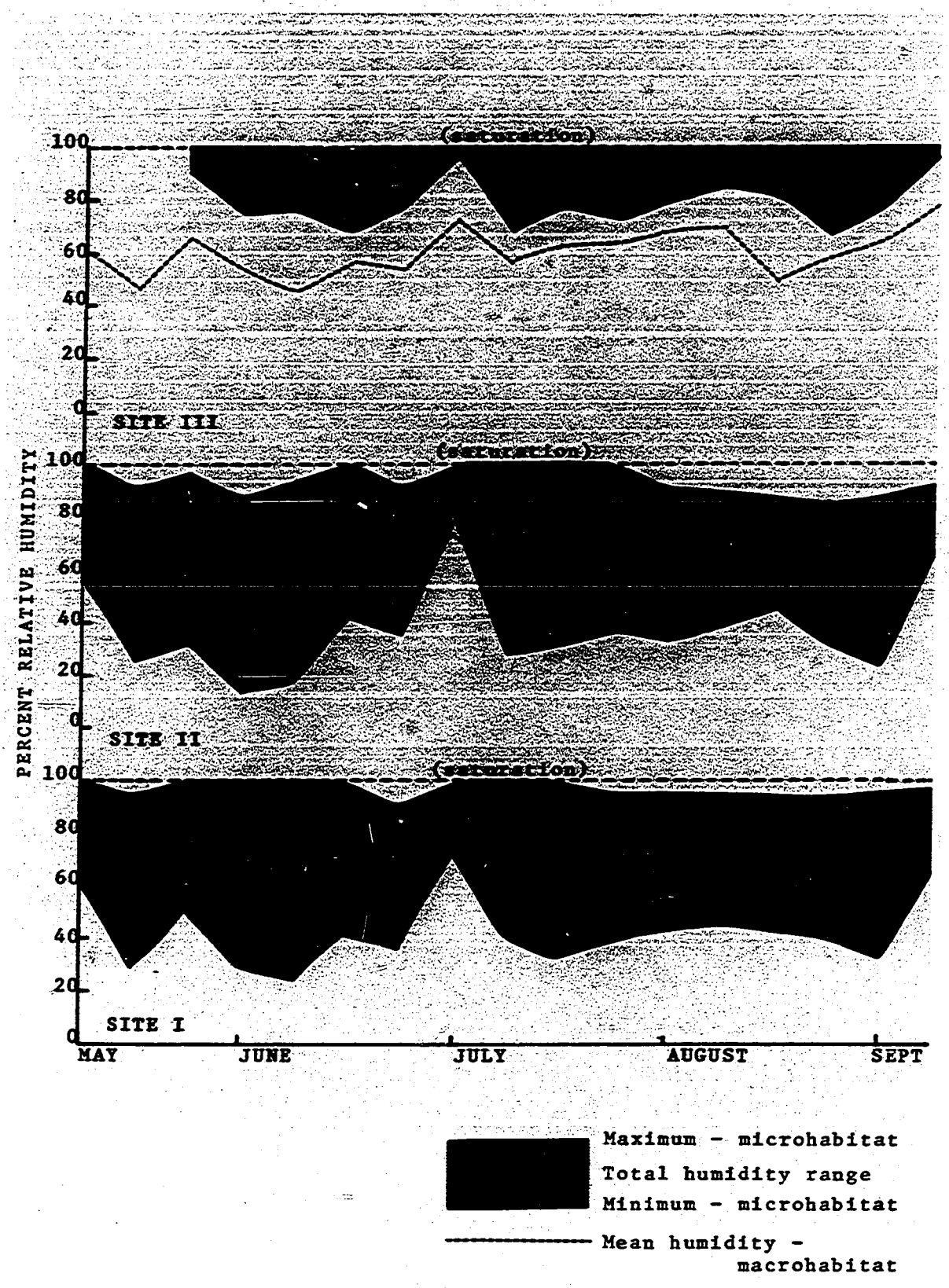

30°
20°
10°
0°
SITE III
30°
20°
10°
0°
SITE II
30°
20°
10°
0°
SITE I
30°
20°
10°
0°
MACROHABITAT
TEMPERATURE (°C)
MAY
JUNE
JULY
AUGUST
SEPT.
Maximum temperature (°C)
Total temperature range
Minimum temperature (°C)

Figure 28. Comparison of the weekly mean relative humidity range of the microhabitats and the mean humidity of the macrohabitat.


PERCENT RELATIVE HUMIDITY
100
80
60
40
20
0
SITE III
(saturation)
SITE II
(saturation)
SITE I
MAY
JUNE
JULY
AUGUST
SEPT
Maximum - microhabitat
Total humidity range
Minimum - microhabitat
Mean humidity - macrohabitat

whenever the relative humidity of the microhabitat remained high for a prolonged period of time, primordia formed. The logs at Site III were subjected to conditions of higher relative humidity than those at the other sites and the largest and greatest number of basidiocarps developed at this site. Conditions of high relative humidity during the first week of July preceded hyphal emergence and primordium formation at all three sites. Observations of basidiocarp development on artificially infected logs the following season gave further credence to the suggestion that a prolonged period of humidity induced, or at least enhanced, the processes leading to morphogenesis. Basidiocarps developed at Site III after a rainy period during the second half of July. During this period in the first season, Site I showed the greatest fluctuation in humidity, 73-100%, compared to 82-100% and 98-100% for Sites II and III respectively. Pigment production by the hyphae of the undifferentiated mat did not occur while the humidity remained above 80%. At Site III, 72 hours elapsed before the humidity dropped sufficiently for cessation of hyphal growth over the wood surface and for pigmentation to develop. While the humidity remained close

to saturation the hyphal mat continued to spread over the wood surface resulting in relatively large primordia compared to those at Sites I and II. Clusters of small imbricate basidiocarps developed from these "indeterminate" primordia within 72-96 hours after pigmentation occurred. The logs at Site II were subjected to relatively drier conditions than those at Site III and hyphae began to emerge from the wood after a 10 day interim following the end of the period of high humidity. Cessation of linear growth and the production of pigment occurred 48-72 hours after emergence. Mature, small, pileate basidiocarps developed within a week after the hyphae first appeared. A similar situation was found at Site I but only two small button primordia formed on a log at the bottom of the log pile. Pigmentation was produced within 24 hours and small, individual, pileate basidiocarps developed. It should be noted that only at Site III did the relative humidity of the microhabitat always remain higher than the average mean readings taken in the macrohabitat. The humidity at the more exposed Sites I and II was more closely correlated with the average mean humidity recorded in the macrohabitat. This discussion of morphogenesis in relation to relative humidity

data refers only to the first appearance of primordia and their subsequent development into basidiocarps. New basidiocarp development continued during the study period mainly at Sites II and III. At Site I only three other small stunted clusters of imbricate basidiocarps formed and these were on two protected logs at the bottom of the pile.

b. Rainfall

The actual rainfall occurring in each of the microhabitats is shown in Figure 29. The data are presented for each day of measurable precipitation. Formation of new primordia and continued growth of basidiocarps already present occurred either at the time of (in the case of Site III), or several days following (Sites I and II) a major rainfall.

c. Substrate Moisture Content

The average moisture content of the wood substratum at each site is given in Figure 30. The data show a general tendency toward decreasing moisture in the logs as the season progressed. Only at Site I is there a good correlation between a substantially rainy period (August 2-9) and increased

Figure 29. The total rainfall at each microhabitat.

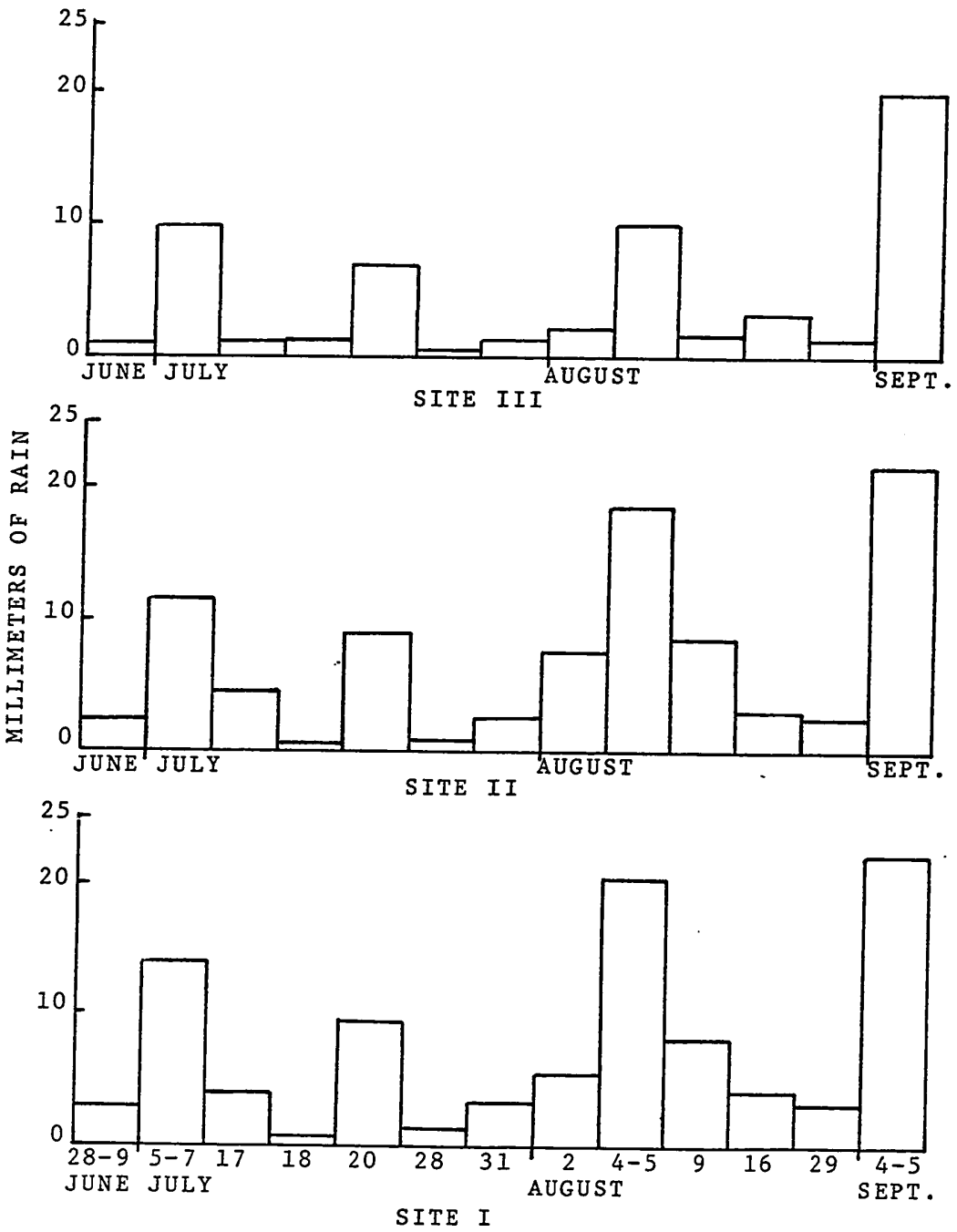
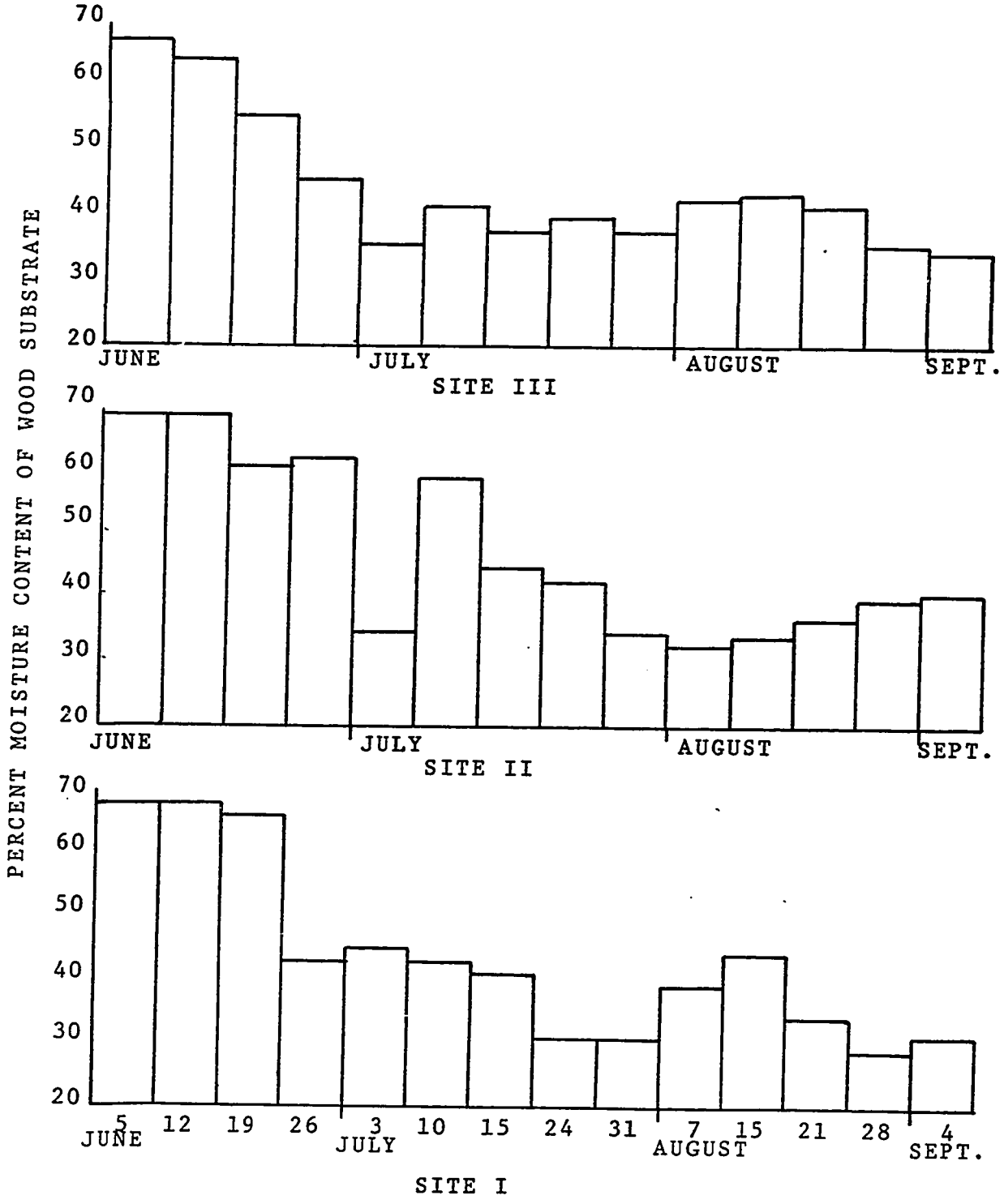

25
20
10
0
JUNE JULY
AUGUST
SEPT.
SITE III
MILLIMETERS OF RAIN
25
20
10
0
JUNE JULY
AUGUST
SEPT.
SITE II
25
20
10
0
28-9 5-7 17 18 20 28 31 2 4-5 9 16 29 4-5
JUNE JULY
AUGUST
SEPT.
SITE I

Figure 30. The mean percent moisture content of the wood substratum at each microhabitat.

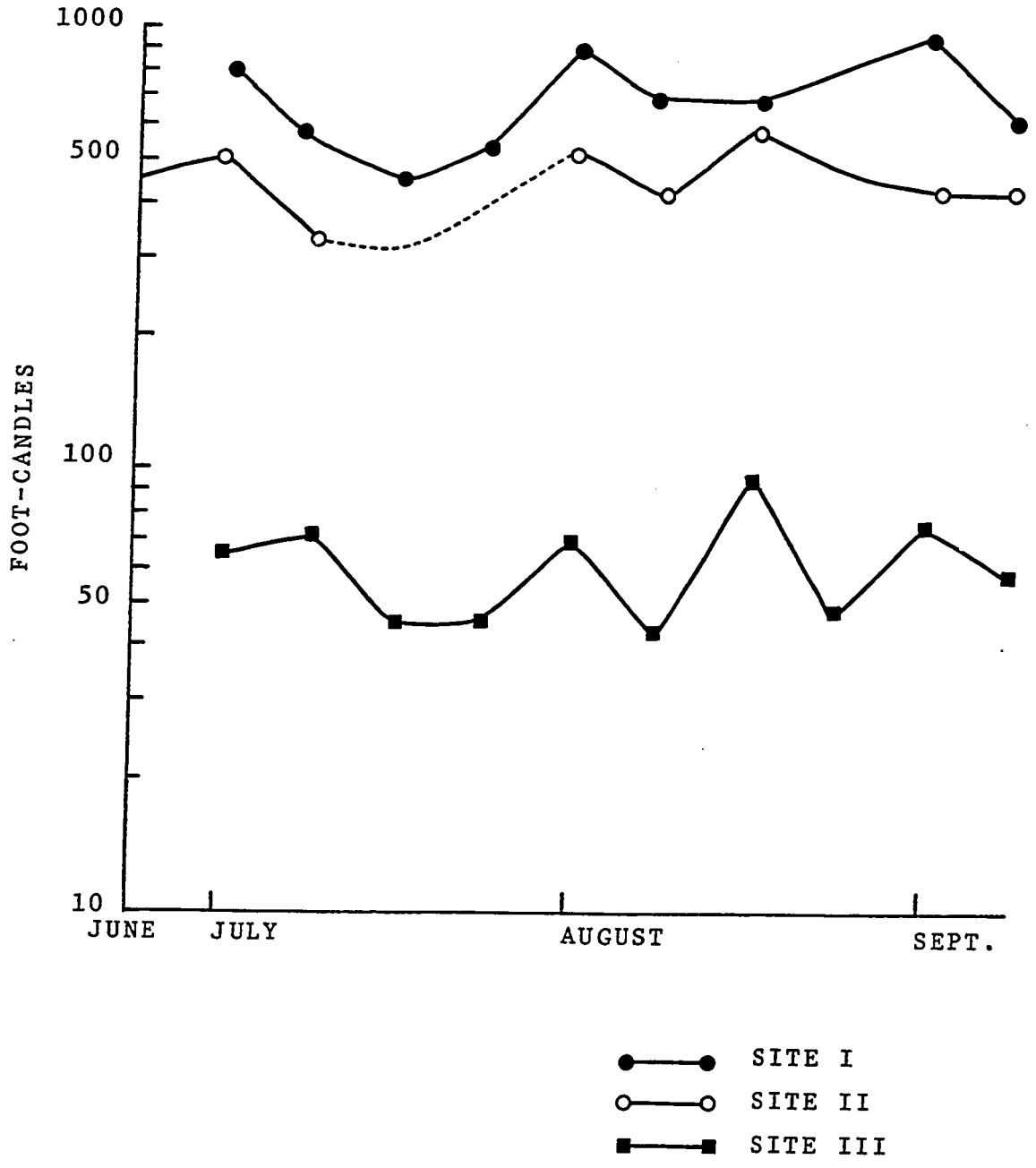

PERCENT MOISTURE CONTENT OF WOOD SUBSTRATE
70
60
50
40
30
20
JUNE
JULY
AUGUST
SEPT.
SITE III
SITE II
5 12 19 26 3 10 15 24 31 7 15 21 28 4
SITE I

moisture content of the logs. The data does seem to imply that the logs will pick up moisture either from the soil on which they are resting or from their humidity-saturated habitat rather than directly absorbing rain water. A comparison of Figures 28, 29, and 30 shows that there is a closer correlation between the relative humidity of the microhabitat and substrate moisture content than there is between rainfall and moisture content. Under these field conditions it was not possible to correlate substrate moisture content and the quantity of vegetative growth.

d. Light

The continuously recorded data from Sites I and II were broken down into measurements of the average weekly mean light intensity. Data for Site III are based on the mean light meter readings taken morning and afternoon at 2-day intervals. Mean weekly light intensity data are given in Figure 31. The light reaching the exposed logs at Site I was of greater intensity than that reaching Sites II and III and partially accounts for the hotter and drier microclimate. The light reaching the logs at the cool, damp microhabitat of Site III was of

Figure 31. The weekly mean light intensity at each microhabitat.



* o---------o : Data from spot readings during equipment breakdown.

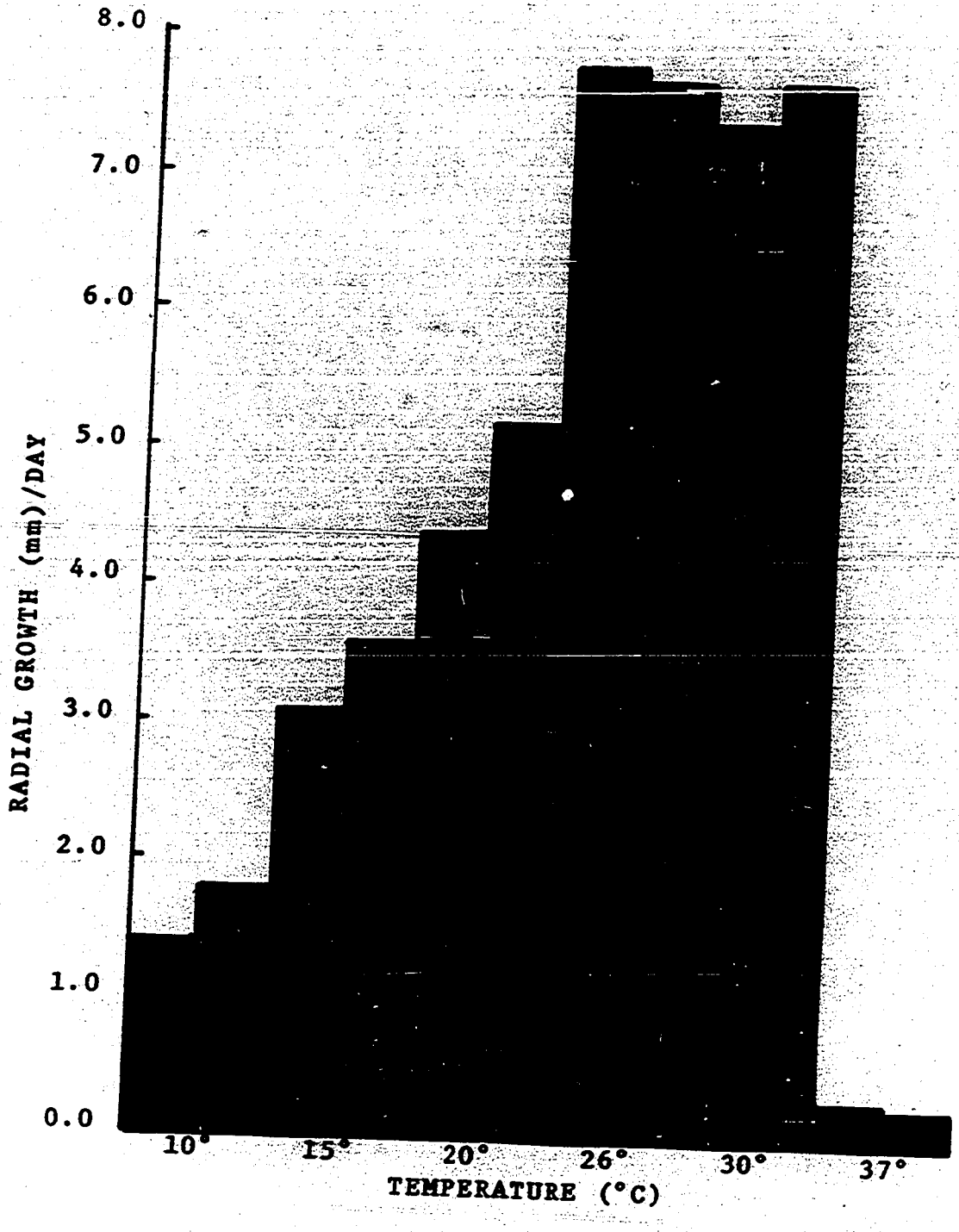
considerably lower intensity than that at the other sites. The light conditions at Site II fell between the extremes of Sites I and III but the average mean intensities were closer to those at Site I. It should be noted that all light data are quantitative and do not take qualitative aspects into account.

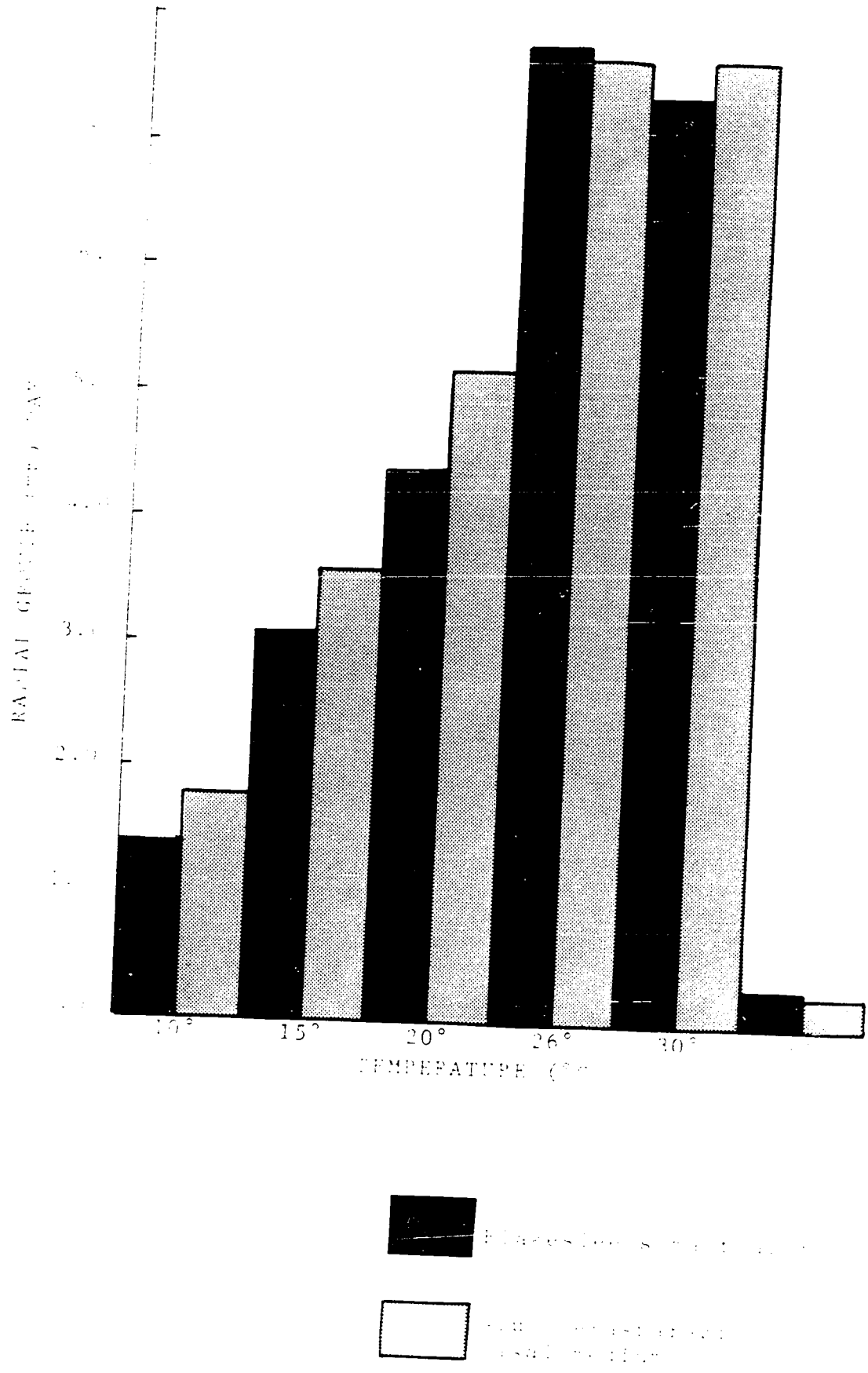
B. The Cultural Environment

1. Temperature and Vegetative Growth

Measurements of the mean daily radial growth of *Polyporus adustus* on malt and on glucose asparagine basal media at selected temperatures are presented in Figure 32. Growth was generally better on the basal medium with the exception of cultures incubated at 26°C . Growth was approximately the same on both media at this temperature and was also similar at 30°C . Incubation at 26-30°C resulted in the maximum rate of vegetative growth under the cultural conditions used in this experiment with the 8.5 cm plates being covered in 6 days. Although there was some measurable growth at 37°C, it did not occur until 10 days after inoculation and was very erratic. The high incubation temperature caused

Figure 32. The effect of temperature on the growth of the vegetative mycelium in culture.


8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
RADIAL GROWTH (mm)/DAY
10°
15°
20°
26°
30°
37°
TEMPERATURE (°C)
Blakeslee's malt agar
Glucose-asparagine basal medium


15°
20°
26°
30°

partial desiccation of the media and this was accompanied by the production of a brown pigment in the cytoplasm of the inoculum hyphae which diffused into the medium. Immediately following pigment production traces of hyphal growth were recorded. This pigmentation appeared similar to that produced by the hyphal tips of a developing basidiocarp under adverse conditions, resulting in pileus zonation (see page 43).

The optimum temperature range for growth of the vegetative mycelium in culture did not occur often in the natural environment. A temperature of 26°C did occur at Sites I and II at the time primordia first formed. The temperature at Site III rarely rose above 20°C, the maximum being 23°C recorded once during the entire study period. Numerous primordia formed at this site when the maximum recorded temperature was 17°C .

2. Substrate Composition and the Vegetative Mycelium

Cultures of *Polyporus adustus* grown on various artificial media showed some variation in morphology as well as differences in growth rate. The morphology of the vegetative mycelium was first

studied using cultures grown on Blakeslee's malt agar. These cultures served as a standard against which all others were compared.

As mentioned previously, observations of monospore isolates and their pairings showed considerable variation in cultural characters. All cultures consisted mainly of branched, flexuous, simple-septate hyphae averaging 3.2 μ in diameter, with granular, vacuolate cytoplasm. Two other types of hyphae were observed; unbranched, simple-septate hyphae with a uniform diameter (2.1 μ), and broad, aseptate hyphae, 5-7 μ in diameter. The proportion of these hyphal types differed among the isolates. There was also variation in the production of oidia and chlamydospores, few to many being produced in the different isolates. When compatible monospore isolates were paired, the flexuous hyphae and those of uniform diameter developed clamp connections. Oidia, formed by hyphal fragmentation, occurred in varying numbers in different pairings. Chlamydospore formation was markedly suppressed, and they did not develop in all diploid cultures. Differences were also observed in the surface morphology of the mat (growth rate, density, color).

The following studies were concerned with

the dikaryotic mycelium, grown from polyspore inoculum, which exhibited a stable morphology and developed all hyphal types and asexual structures observed in monospore isolates and pairings when grown on Blakeslee's malt agar.

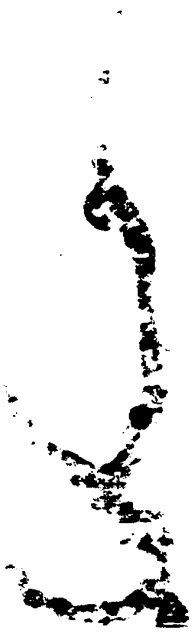
New vegetative colonies were composed of flexuous, profusely branched hyphae with clamp connections and an average diameter of 2.3 µ (Figure 33, page 91). These hyphae gave rise to two other types of hyphae. The most abundant type was an unbranched hypha of uniform diamter with clamp connections, and a diameter of 1.3-2.2(3.0) µ (Figure 34, page 91). The second type were wide (4.2-8.4 (10.5) µ) simple-septate hyphae which arose at clamp connections of the flexuous hyphae (Figure 35, page 91). These hyphae gradually increased in diameter as they grew from the clamp from which they originated. A clamp connection occasionally formed on these hyphae. Other hyphae were found in some older cultures which were of slightly narrower diameter, averaging 5.0 µ, with widely spaced clamps (150-200 µ apart) from which branches may grow. As these hyphae aged they became vacuolate and developed thickened, pigmented walls (Figure 36, page 91). They closely resemble the pigmented tramal hyphae of

Figure 33. Branched, clamped, flexuous hypha. This type comprises the greater part of the vegetative mycelium and gives rise to other hyphal types in culture. (X1280)

Figure 34. Unbranched, clamped hypha of uniform diameter. (X629.8)

Figure 35. Wide hypha arising from clamp of branched, flexuous hypha. (X511)

Figure 36. Broad, aseptate hypha with slightly-thickened, pigmented walls. (X629.8)



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the basidiocarp, differing only in their somewhat larger diameter. Broad, aseptate hyphae also were found but these appeared to be a degenerate form of the clamped or simple-septate hyphae. The wide, clamped hyphae contained oblong crystals in their granular cytoplasm. These crystals were also found in the medium on which aging cultures were growing.

Two types of asexual spores are produced, oidia and chlamydospores. Chlamydospores were observed infrequently (although they were abundant in some monokaryotic cultures) and developed only on malt extract and maltose media after 7-10 days incubation (Table I). They were either terminal or intercalary and formed on the flexuous, branched, clamped hyphae (Figure 37, page 93). Oidia were formed by hyphal fragmentation in all cultures throughout the growth period. All hyphal types were observed undergoing this process in which the cytoplasm became vacuolate and broke up into discrete aggregations along the length of a hypha. This was generally followed by formation of simple septa and fragmentation. Infrequently, the breaking up of the cytoplasm was followed by constriction along the hypha between the cytoplasmic aggregations, after which septa formation and fragmentation occurred.

Figure 37. Terminal chlamydospore on branched, flexuous hypha. (X1280)

Figure 38. Hypha fragmenting during oidia formation. Arrow points to one of numerous septa along the length of the hypha. Note that no constriction has occurred at the septa. Resulting oidia will be rectangular. (X511)

Figure 39. Short chain of oidia (o). Arrow points to constriction which occurs before septation. Oval oidia (beneath arrow shaft) are easily distinguished from rectangular oidia seen in Figure 38. (X1008)

Figure 42. Abnormal development of hyphae on solidified glucose-lysine basal synthetic medium. Note bulbous swellings and simple septa. (X511)

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The resulting spores were round to oval in shape. In order to minimize the confusion in describing which type of oidia formed in the different cultures, they will be distinguished as "oidia" and "oidia (o)". It was decided to use the term oidia since it is commonly used in cultural descriptions. However, according to Alexopoulos (1962), spores forming by hyphal fragmentation are arthrospores. This is the common process of asexual spore formation in *P. adustus*, but these "arthrospores" will be termed oidia for the sake of consistancy. The rounded spores, while not conforming to the definition of oidia given by Alexopoulos, at least resemble them morphologically, and these spores will be termed oidia (o) to indicate their oidial appearance. Formation of oidia (o) has been observed only in the hyphae of uniform diameter, but these more frequently form oidia in the same manner as other hyphae. The square to rectangular oidia (some of which have clamps attached) are easily distinguished from the round to oval oidia (o) in Figures 38, 39, page 93.

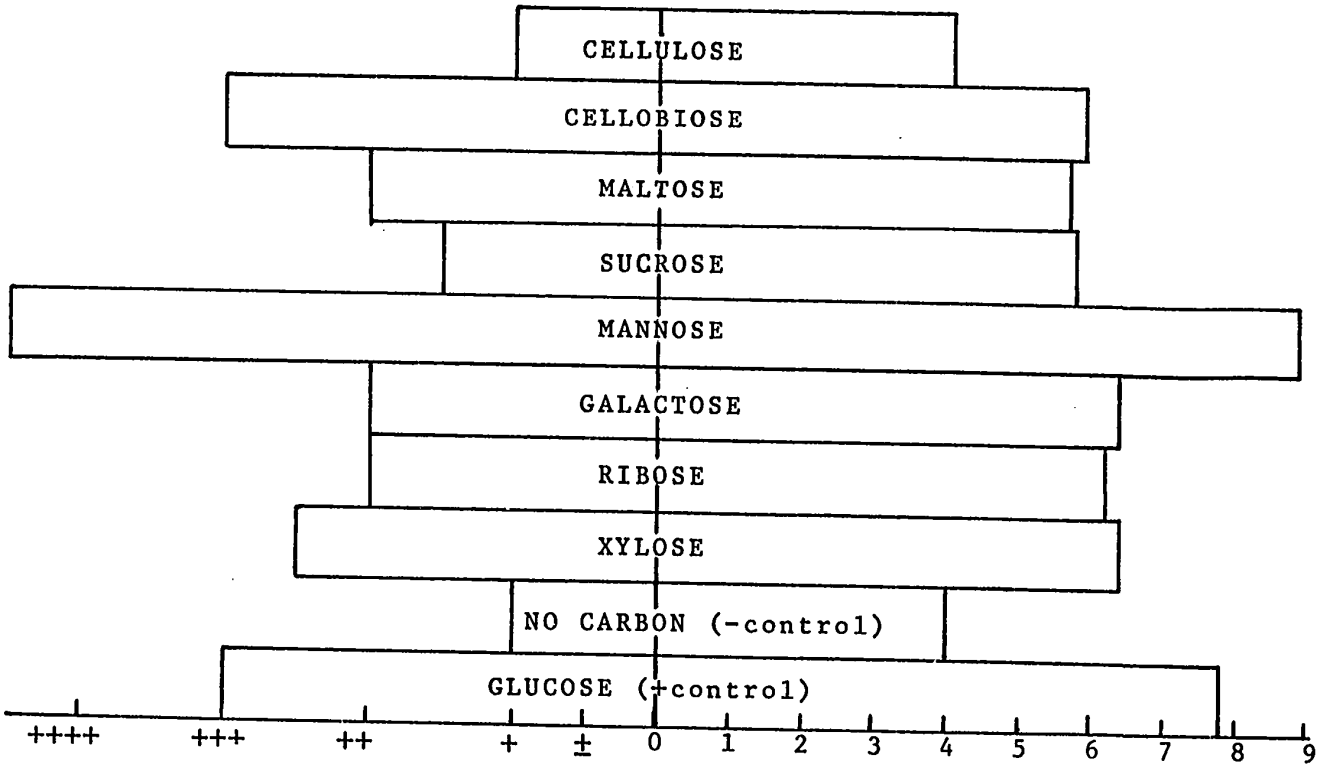
Infrequently, hyphae were observed which produced large numbers of very short branches which grew to no more than 10-20 μ. These resembled the

binding hyphae described by Corner (1932a) which occur in some basidiocarps. Hyphal strands (see Butler, 1957) also occurred frequently in cultures growing on the media indicated in Tables I and II. The hyphae produced a brown pigment to a greater or lesser degree in most media which resulted in the colonies appearing sordid to brown in color.

a. Carbon Utilization

The data presented in Figure 40 show that the vegetative mycelium of *P. adustus* grows exceedingly well on a medium containing the hexose mannose as the sole carbon source. The other pentoses, hexoses and disaccharides used in these studies supported approximately equivalent growth with the exception of sucrose, on which the total mycelial mass was visibly sparse compared to the other cultures, especially toward the colony periphery. Cellobiose was also an excellent carbon source, and, while the growth rate was slightly less on this medium than on glucose (which served as a positive control), it supported greater hyphal differentiation. These data are summarized in Table I. It should be noted that chlamydospores were produced only by vegetative mycelium utilizing maltose. Hyphae resembling binding

Figure 40. The effects of carbon source on growth of the vegetative mycelium.



QUALITATIVE EVALUATION OF GROWTH

0 No growth; complete inhibition

± Inhibition after initial growth

\+ Sparse mycelium

MEAN RADIAL GROWTH (mm)/DAY

++ Light-moderate mycelium

+++ Dense mycelium

++++ Extremely dense mycelium

Table I. The effect of carbon source on colony morphology, hyphal development and asexual spore formation in the vegetative mycelium.

++++: extremely abundant

+++: abundant

++: moderate

+: few (2-5 observed/slide)

Carbon Source	General Colony Description	Branched, Flexuous, Clamped	Hypha: Unbra Clam of Un Diame
Glucose (+ control)	Dense, floccose mycelium covers plates in 6 days. Becomes matted and develops brown pigmentation.	+++	+
No Carbon (- control)	Sparse, floccose mycelium covers plates in 8 days. Colony remains extremely sparse toward periphery. Light brown pigmentation develops.	++	++
Xylose	Moderate-dense, floccose mycelium covers plates in 8 days. Light brown pigmentation develops after only 4 days.	+++	+
Ribose	Light, floccose mycelium covers plates in 8 days. Colonies become sordid but no brown pigmentation develops.	+++	++
Galactose	Light, floccose mycelium covers plates in 8 days. Colonies become felted and develop light brown pigmentation.	++	++
Mannose	Extremely dense, floccose mycelium covers plates in 4-5 days. Colonies become felted and develop brown pigmentation. Buff exudate diffuses into medium.	+++	++
Sucrose	Light, floccose mycelium covers plates in 8 days. Remains sparse at periphery. Colonies become sordid and pigmented at interface between light and sparse growth.	++	++
Maltose	Light, floccose mycelium covers plates in 8 days; sparse at periphery. Colonies become sordid and develop discrete areas of brown pigment.	++	++
Cellobiose	Dense, floccose mycelium covers plates in 8 days. Colonies become felted and sordid. Brown pigmentation develops over entire colony.	+++	++
Cellulose	Sparse-light, floccose mycelium covers plates in 8 days. Colonies become felted-tufted and remain white in color.	++	++

Hyphal Types

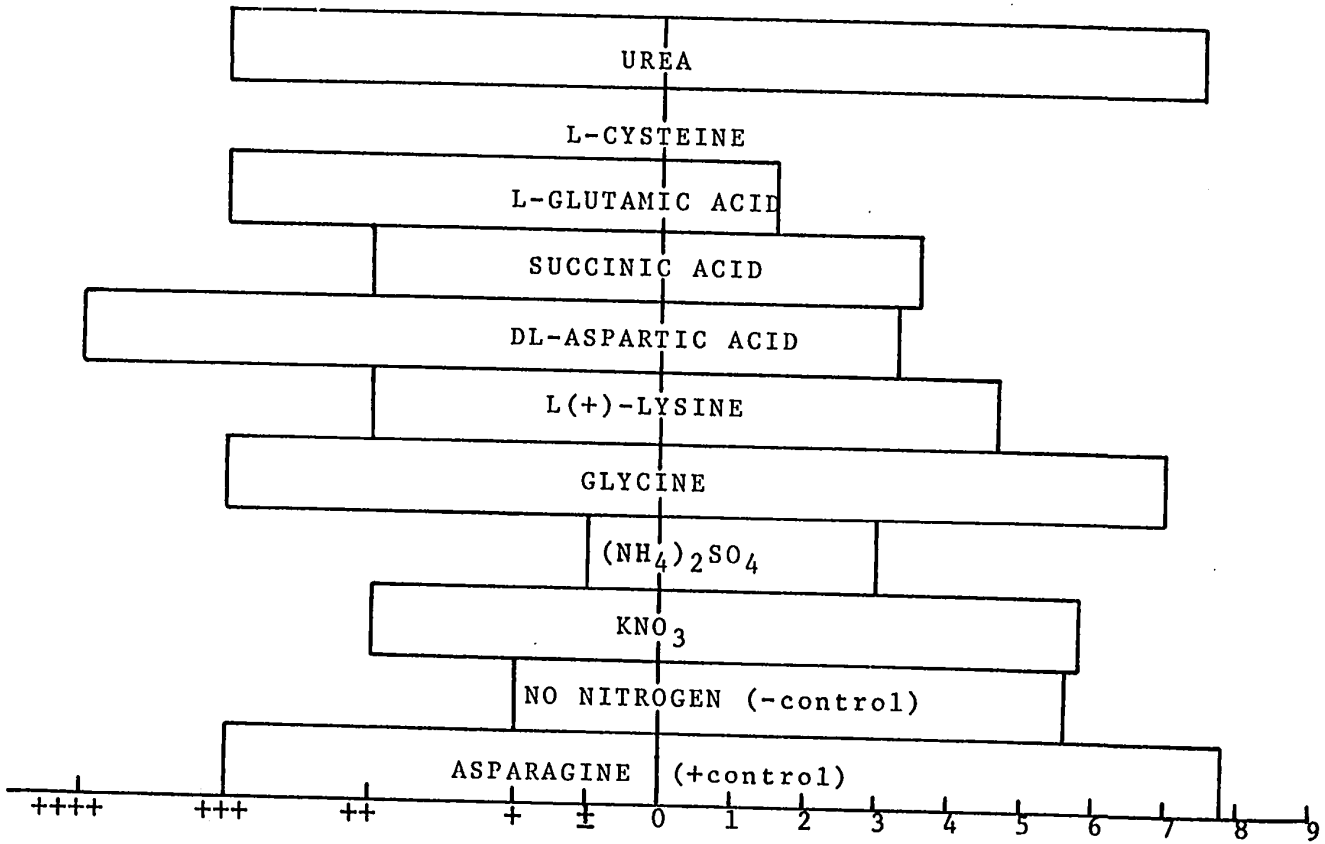
Unbranched, Clamped, of Uniform Diameter	Wide, Simple-Septate	Asexual Spores		Other Characters
+	+	Oidia:	++	Wide, aseptate hyphae with thickened walls
++	+	Oidia:	++	Collapsed, wide, aseptate hyphae with thickened walls.
		Oidia(o):	+	
+	-	Oidia:	++	Hyphae with numerous short branches. Wide aseptate hyphae with thickened walls. Crystals.
++	+	Oidia:	++++	Wide, aseptate hyphae with thickened walls. Strands.
++	+	Oidia:	+++	Wide, clamped hyphae with thickened walls. Crystals
++	+	Oidia:	++	Wide, clamped hyphae with thickened walls.
++	+	Oidia:	+++	Wide, aseptate hyphae with thickened walls.
++	+	Oidia:	++	Wide, aseptate hyphae with thickened walls. Hyphae with numerous short branches. Strands. Crystals.
		Chlamydo-spores:	+	
++	+	Oidia:	+++	Wide, clamped hyphae with thickened walls (clamps 150-200 μ apart). Strands.
++	-	Oidia:	+++	Wide, clamped hyphae with thickened walls. Strands. Crystals.

hyphae developed on media containing maltose and on that containing the pentose xylose. Oidia (o) were observed forming from hyphae supplied with no carbon source.

b. Nitrogen Utilization

A summary of quantitative and qualitative growth of the vegetative mycelium utilizing various nitrogen sources is given in Figure 41. The effects of nitrogen on growth are much more marked than those of carbon. The organic nitrogen sources, urea and asparagine, are utilized readily by *P. adustus*. Inorganic nitrate (KNO_3) supported light growth. A severe drop in pH (5.5-2.5) occurred when ammonium served as the nitrogen source resulting in complete inhibition of growth. Numerous amino acids were tested in liquid Henningsson's B and basal synthetic media. On the basis of mycelial growth achieved by *P. adustus* on media with various amino acids certain ones were chosen for these experiments. *P. adustus* grew very well on glycine, which proved to support growth equivalent to that on asparagine. Cultures grown on aspartic acid medium showed a 10 day period of inhibition after which growth was excellent. This is considered to be inhibition

Figure 41. The effects of nitrogen source on growth of the vegetative mycelium.



QUALITATIVE EVALUATION OF GROWTH

0 No growth; complete inhibition

± Inhibition after initial growth

\+ Sparse mycelium

MEAN RADIAL GROWTH (mm)/DAY

++ Light-moderate mycelium

+++ Dense mycelium

++++ Extremely dense mycelium

Table II. The effect of nitrogen source on colony morphology, hyphal development and asexual spore formation in the vegetative mycelium.

++++: extremely abundant

+++: abundant

++: moderate

+: few (2-5 observed/slide)

Nitrogen Source	General Colony Description	Branched, Flexuous, Clamped	Unbranched Clamped, of Uniform Diameter
Asparagine (+ control)	Dense, floccose mycelium covers plates in 6 days. At first white and floccose, becoming sordid and then developing dark brown pigmentation.	+++	+
No Nitrogen (- control)	Very sparse mycelium covers plates in 7 days. Remains matted-tufted and white.	++	+
KNO_3	Light, matted mycelium covers plates in 7 days. No pigmentation develops.	++	+
$(NH_4)_2SO_4$	At first, moderate-dense floccose mycelium grows rapidly. After 4 days growth slows and terminal hyphae show dendritic growth. pH drops from 5.5-2.5 and complete inhibition occurs after 9 days.	++	-
Glycine	Dense, floccose mycelium covers plates in 6 days. Colonies become matted-tufted. Light brown pigmentation develops at periphery.	++	+
L(+)-Lysine	Light, matted mycelium covers plates in 9 days. Colonies remain white, with only an occasional discrete pigmented area.	++	++
DL-Aspartic Acid	Growth inhibited at first; inhibition overcome after 10 days incubation and plate is then covered in 14 days. Extremely dense mycelium develops brown pigmentation.	++	+
N-dimethyl aminosuccinic Acid	Light, floccose mycelium covers plates in 12 days. Becomes matted tufted. No pigmentation develops.	++	++
L-Glutamic Acid	Dense, floccose mycelium grows at very slow rate. Colonies become sordid but without dark pigmentation. Brown exudate diffuses throughout agar medium.	++	+
L-Cysteine	No growth. Complete inhibition.	-	-
Urea	Dense, floccose mycelium covers plates in 6 days. Becomes tufted and develops light brown pigmentation.	++	+

Hyphal Types

Unbranched, Clamped, of Uniform Diameter	Wide, Simple-Septate	Asexual Spores	Other Characters
+	+	Oidia: ++	-
+	-	Oidia: ++	-
+	+	Oidia: +++	-
-	-	Oidia: + Oidia(o): ++	Hyphae at colony periphery are swollen and bulbous. Strands.
+	+	Oidia: ++++	Wide, clamped hyphae with thickened walls. Strands.
++	-	Oidia: + Oidia(o): +	Bulbous swellings on hyphae in pigmented areas of colony. Strands.
+	+	Oidia: ++	-
++	-	Oidia: ++	-
+	-	Oidia: ++ Oidia(o): ++	Strands.
-	-	-	-
+	++	Oidia: ++ Oidia(o): ++	Wide, aseptate hyphae with thickened walls.

rather than a lag phase because a comparison with the negative control shows that the vegetative mycelium will grow to some extent when no nitrogen source is added to the medium. No inhibition occurs in cultures grown on succinic acid, which is chemically related to aspartic acid. Daily growth is more rapid but the total mass of mycelium is less. A very dramatic example of inhibition is seen in cultures utilizing cysteine as a nitrogen source. There is absolutely no growth, even on the inoculum plug.

The morphology of the vegetative mycelium of *P. adustus* is summarized in Table II. Hyphal differentiation was less pronounced than on media with various carbon sources, but there was abnormal hyphal development on certain nitrogen sources. Notable in this respect were $(NH_4)_2SO_4$ and lysine. The hyphae growing on these media developed bulbous swellings in some regions of the colonies (Figure 42, page 93). These same colonies also formed rounded oidia (o) in addition to typical oidia.

3. Basidiocarp Induction in the Artificial Environment

All cultures of *Polyporus adustus* grown on

birch and aspen wood blocks showed excellent vegetative growth. But no basidiocarps developed on cultures incubated at 26°C in the dark (Group I) or those placed in diffused sunlight (Group II). Rudimentary, dimpled, unpigmented primordia formed in some cultures after 6 months but they never developed beyond this stage. The wood block cultures of Group III, subjected to low light intensity and periodic drying, developed mature, resupinate basidiocarps within 14 days after being placed in the large covered tanks. These basidiocarps were not large and remained entirely resupinate, but were structurally normal and produced viable spores.

The cultures of *P. adustus* grown following the Tamblyn-DaCosta method formed only the rudimentary primordia which were observed in other wood block cultures. When several of these apparatuses were removed from their saturated environment and placed in the tanks used for the Group III cultures discussed in the preceding paragraph, small basidiocarps developed. Pigmentation developed only after the cultures were removed from a saturated environment and placed in one with variable humidity.

Resupinate basidiocarps developed on both artificially and naturally infected logs which were

subjected to variations in atmospheric humidity. Basidiocarps formed on the sound, artificially infected logs within 14 days after the humidity in the tanks was allowed to drop below saturation. The wood showed little evidence of hyphal penetration, but the surface where it was inoculated was covered with a dense mycelial mat. All primordia which formed were of the indeterminate type. No discrete button primordia formed in culture. The basidiocarps which developed on the naturally infected logs were of considerably larger size and there was no development of an undifferentiated vegetative mycelium over the wood surface other than the normal hyphal growth which precedes primordium formation.

DISCUSSION

"In seeking a suitable basis on which to examine the relationship of environment and form, the use of many promising criteria has been ruled out by lack of adequate information, especially of observations on the fungi in their natural habitats." This statement made by Austwick (1968) points out the lack of information compiled on the development of basidiomycetes under natural conditions. The studies encouraged by Austwick have potential applications in many areas of scientific research and have a very real application in formulating a natural classification of the Polyporaceae. Several years earlier Lowe (1963), discussing the classification of the polypores, stated that "exact morphological knowledge of species is the key to present success". Exact morphological knowledge can only be obtained by detailed study of all stages of growth from the vegetative phase to the production of spores by a mature basidiocarp. Extensive studies of individual species result in compilation of data that will not necessarily be useful in identification of specimens. Yet, such work on large numbers of species

is the only basis on which generalizations can be made for the genera of this family. This study has been concerned with elucidating the stages of growth and development of *Polyporus adustus*, and determining the influence of the environment on these processes. I believe that the results from these investigations have provided a more comprehensive description of the developmental morphology of this fungus than has been presented previously.

The use of artificially infected logs has proven to be a valuable technique for controlled studies of growth and development in the natural environment. This method shows great promise for studies of the natural development of wood-decay fungi from the earliest stages of infection. With some general knowledge of their environmental requirements, even exotic species can be studied by simulating conditions in controlled environment chambers. The real value of this method lies in the fact that a fungus can be grown in its natural microhabitat, eliminating the problems of substrate composition, humidity, aeration, etc., inherent in the cultural environment.

A. Development of *Polyporus adustus* in the Natural Environment

The studies of growth and development of the vegetative mycelium in the natural environment have revealed that *P. adustus* grows rapidly through the tracheary elements of sound wood. During observations of artificially infected logs over two growing seasons, this growth was found to be sparse, with little apparent breakdown of cell walls. No aggregations of hyphae developed in the vessels or tracheids and decay was only in the incipient stage when basidiocarps first developed on the wood surface. Formation of basidiocarps before the wood is severely decayed and subsequent development over a period of years, discounts the hypothesis that starvation is necessary for fruiting in *P. adustus* and perhaps the majority of species. The rapid rate of vegetative growth has been noted by von Schrenk (1907), but he stated that the sapwood of a large log of red gum was completely destroyed within a year if the logs were permitted to lie in the woods or along stream banks. Decay did not occur to this extent in the aspen logs used in this study. It is possible that the vegetative mycelium derives its nutrition

from the carbon and nitrogen compounds and other nutrients which are found in a free state in the wood and not bound up in the cell walls. As these nutrient sources are used up, *P. adustus* may produce exogenous enzymes to break down the cell wall material. The utilization of carbon and nitrogen sources will be discussed later.

The vegetative mycelium, observed in wood sections from both artificially and naturally infected logs and from the collections made during this study, was invariably dikaryotic. Zycha and Knopf (1966) reported the isolation of numerous monokaryotic colonies, but there are no reports of *P. adustus* developing haploid basidiocarps in nature. Leonard and Dick (1968) induced haploid basidiocarp formation in cultures of *Schizophyllum commune*, using cell-free extracts of "Fruiting-inducing substance (FIS)" from the mycelium of *S. commune* and *Hormodendrum cladosporioides*. FIS has been found in a number of fungi. Leonard and Raper (1969) found that induced haploid fruiting in *S. commune* is controlled by a single gene, fis^+, which appears to segregate independently of the incompatibility factors. The probability of hormonal regulation of fruiting in *P. adustus* cannot be overlooked, and

this is an area of research that would merit further study. However, until contrary information is found, it will be assumed that, in nature, dikaryotization is a necessary stage preceding basidiocarp formation in the life cycle of *P. adustus*.

The vegetative mycelium remains undifferentiated while growing in the wood substratum. This lack of hyphal modification makes the vegetative stage unsatisfactory for taxonomic descriptions since the mycelium of many basidiomycetes resembles that of *P. adustus*. It has been argued by some investigators that the hyphae which grow in the wood do have characteristic features and that one only has to prepare better sections and study them more closely. My observations of *P. adustus* gave no evidence to support this supposition. The mycelium retained the same hyphal characteristics from the time it first started growing through the wood to stages of advanced decay. The only observable changes were an increase in the numbers of hyphae in a cell and the aggregations of hyphae which gave the wood its mottled appearance. It is concluded that vegetative stages cannot be described fully when growing in wood substrate, as Chesters (1968) has stated, "not because it lacks characteristic

features, but because these are difficult to display and to observe". Only under optimal growth conditions in culture can these hyphae fully express their genotypic potential. The constant, reliable characters of the vegetative mycelium under strictly defined conditions, are valid criteria for taxonomic descriptions.

Two distinct forms of primordial development occur, the discrete button and indeterminate mat. The great range of size variation is influenced by the relative humidity at the time of hyphal emergence. The influence of this factor will be discussed later. The type of primordium which develops, its orientation, and the topography of the surface on which it forms determine the ultimate physiognomy of the mature basidiocarp. A button primordium forming on the smooth vertical surface of the cut end of a log will develop into an imbricate basidiocarp with approximately 1-3 pilei. The same primordium developing in a furrow of the bark on the side of a log will be resupinate or effused-reflexed because the hyphae forming the dissepiments will be able to grow in a positively geotropic manner without first forming a pileus. A large

indeterminate primordium which forms on the side of a smooth-surface log will exhibit all forms of development. The part of the primordium on the underside of the log will remain resupinate, that on the side will develop imbricate pilei, the region in between these vertically and horizontally oriented surfaces will have a daedaloid pore surface and develop into an effused-reflexed structure. These are the most predictable developmental patterns for this fungus, but other variations are found depending on the factors mentioned above and also on climatic changes.

Until the time of basidiocarp development, there is no discernible hyphal organization in this fungus. Definite organization does occur in the basidiocarp, but there is no modification resulting in skeletal and binding hyphae. Corner (1953) has classified *P. adustus* as monomitic because of this lack of hyphal modification. If only hyphal anatomy is considered, the basidiocarps of species such as *Polyporus sulphureus* and *Polystictus (Polyporus) versicolor* appear strikingly differentiated and complex compared with *P. adustus*. However, observations of numerous basidiocarps of *P. adustus* at

different stages of development have shown them to be more complex than described by Corner. Pileate basidiocarps consist of two hyphal systems: one in which hyphal strands grow in parallel alignment, and a second of individual interwoven hyphae. Both systems develop in the pileate portion of effused-reflexed basidiocarps. Resupinate forms show no evidence of strand development, and are morphologically similar to the base region of the pileus, i.e., the hyphae are in parallel alignment but not grouped into strands. The anatomical studies of the different basidiocarp types indicate that strand formation is a structural modification which serves the same purpose as skeletal hyphae. The second system of interwoven hyphae holds the hyphal strands together to form the dense, compacted context. Although two systems are found in mature, pileate basidiocarps, three systems are actually involved in development. The generative hyphae form the primordium and give rise to the system of strands, which then grow out from the wood surface in horizontal orientation. These strands lead the growth of the basidiocarp and maintain its shape. These same generative hyphae give rise to the system of interwoven, or felted, hyphae which enmesh the strands.

Corner (1953) has said that "all the hyphae of the fruit body are identical in manner of growth and branching, so that they must be called monomitic". In the light of this statement, it can only be concluded that *P. adustus* does not fall into this monomitic group. Rather, three hyphal systems are present which function in the same way as those of trimitic basidiocarps. In the case of the basidiocarp of this fungus, at least, the differences between monomitic, dimitic and trimitic can be considered to be only structural, concerned with cell wall thickness and persistence of septa in the hyphae.

Hymenophore development is similar in all basidiocarp types. The constant features of this region, in particular the pigmentation of the walls of the hyphae of the transition zone and the trama, help distinguish *P. adustus* from the closely related species, *Polyporus fumosus*. This fungus is generally larger than *P. adustus*, but there is a considerable overlap in the size range. Unfortunately, no fresh specimens of *P. fumosus* were available so that only herbarium material was studied. In this dried state, the basidiocarp construction of both species was so similar as to be indistinguishable. However,

the tramal hyphae of *P. fumosus* do not develop pigmented walls. This pigmentation occurs only in the transition zone between context and hymenophore. The grayish color of the pore surface is due to the pigmentation of the transition zone which is visible at the top of each tube. Bruising results in pigmentation of tramal hyphae, but the pigment never develops in all the tramal hyphae as it does in *P. adustus*.

Until the development of the basidiocarp of *P. fumosus* is studied, we must rely on distinctive, constant characters of the mature basidiocarp such as tramal hypha pigmentation, to distinguish these two species. Descriptions of mature basidiocarps cannot be discarded since this is the stage at which these fungi are generally collected and at which they consequently must be identified. Furthermore, this is the stage to which the name of the taxon is attached. Whatever the shortcomings of Overholts' key of the Polyporaceae, it cannot be denied that it is very pragmatic in its approach and is workable for distinctive species. However, studies of developmental morphology must be carried out on individual species in order to devise a natural classification of these fungi and to formulate

a system which permits distinctions to be made between closely related species. It is only by intensive studies of individuals that generalizations can be made for the different species and genera. This method of study necessitates the collection of fresh specimens at various stages of development. Fresh specimens should be emphasized because the use of herbarium material can lead to erronious conclusions. Corner (1950) and Lowe (1963) have pointed out the problems of working with dried material. All hyphae are not retained; thin-walled hyphae have a tendency to disappear during drying. The thin-walled hyphae making up the context of *P. adustus* and similarly constructed species may not persist and studies of herbarium material will give a false picture of development and ultimate basidiocarp construction.

Obvious difficulties are involved in carrying out developmental studies as advocated here. First, there is the problem of finding sufficient material to study within a reasonable distance from the laboratory. Another problem is finding a particular fungus in a substratum that does not contain other fungi which may be antagonistic and thus affect the normal development of the species

being studied. These difficulties can be overcome if logs are artificially inoculated using the technique described in this thesis, and are set out in a microhabitat resembling that in which the fungus is usually found growing. Using this technique, the life cycle of wood-decay fungi can be studied from the earliest stages of vegetative growth through to the development of mature basidiocarps.

B. Influence of the Microhabitat on Growth and Development

1. The Natural Environment

It has been stated that the vegetative hyphae of *P. adustus* remain unmodified when growing in the natural environment. Whether this is due to conditions with respect to available nutrients, aeration, moisture, light, structure of the substrate (the surface on which the hyphae grow differ markedly from the surface of agar medium), or competition with other organisms is not known. Investigators have shown that any one or a combination of these factors may influence growth and differentiation of hyphae.

Temperature studies carried out under cultural conditions revealed that optimal growth of the vegetative mycelium of *P. adustus* occurs between 26 and 30°C . But this temperature range did not occur in the natural environment at Site III, where the largest and the greatest number of basidiocarps developed. According to Henningsson (1967a), the "temperature for optimal radial growth [of *P. adustus*] seemed to be somewhat higher than that for optimal decay activity". The range for optimal radial growth (25-30°C) found by Henningsson, is in agreement with the findings of this study. He found optimal decay activity to occur at 20°C . The maximum temperature at Site III, where basidiocarp development was greatest, was around 20°C . But examination of the vegetative mycelium in artificially infected logs during the first growing season indicated that vegetative growth through the wood was most rapid in logs at Site II, where the temperature was generally higher. If one can equate the penetration by hyphae through wood cells with decay activity, then it would seem that, at least in the natural environment, no clear cut statement can be made relating the factor of temperature to radial growth, decay activity and morphogenesis. Hawker (1966) states

that "temperature has a profound effect...on reproduction...largely as a result of its effect on the many complex chemical and physical processes involved in vegetative growth". She indicates that an increase in temperature should favor fruiting, which is not the case with *P. adustus*.

The correlation between moisture content of the logs, and vegetative growth and decay activity was not very striking. Vegetative growth generally proceeded at a faster rate in the logs at Site II, which were usually found to have a higher moisture content than logs at the other sites. However, even at the individual sites the moisture content depended upon whether a log was protected at the bottom of a pile or exposed at the top. These comparative field studies indicate that vegetative growth increased with an increase in substrate moisture content, although growth occurred in all logs to a greater or lesser extent. Henningsson (1967c) has shown that *P. adustus* exhibits greatest decay activity when the moisture content of aspen is 80%, although he found that this process continues over a wide moisture range (35-160%). I have found that vigorous growth takes place at a moisture con-

tent as high as 170%. Henningsson remarks that the influence of substrate moisture content on decay activity is not sufficiently known, the difficulty of such studies being that fungi, by their own metabolic processes, alter the original moisture content.

Because of scanty and contradictory information we do not understand the effects of substrate moisture content or temperature on growth and development, but we do know that relative humidity profoundly influences hyphal emergence and primordium formation. If hyphae emerge from the wood into an environment in which the humidity falls below 80%, growth ceases soon after emergence, the protective gray pigment develops and the resulting button primordium will begin to differentiate within 24 hours after it first appeared. Under conditions of high humidity (above 80%) an indeterminate primordium forms over a large area of the wood surface. The pigment which always develops in the walls of the surface hyphae composing the central region of the primordium, and in the walls of the transition zone and tramal hyphae, is first produced when the emergent hyphae are subjected to a drop in atmospheric

humidity. Smith (1966) suggests that pigmentation of cell walls protects the exposed hyphae against desiccation. The pigmentation of the hymenophore may be associated with spore production. It has been found that the hymenium of *P. adustus* is very sensitive to drying conditions. The pigmented walls of the tramal hyphae might function in maintaining moisture levels in the tramal tissue and basidia so that sporulation can occur over a wider range of humidity condtions.

The humidity also affects the developing pileus. If dry condtions occur for a short period of time the apical cells of the hyphae cease to grow and become pigmented, then lateral branches develop behind these terminal cells. Zonation of the pileus, which is occasionally seen, is the result of cessation of growth of the terminal cells, pigmentation, and renewed growth by the formation of lateral branches. When desiccation occurs for a prolonged period the basidiocarp dies.

Under cultural conditons, desiccation also resulted in cytoplasmic pigmentation, followed by development of lateral branches which seemed able to grow (although at a very slow rate) under conditions which had previously inhibited growth.

It is possible that mild desiccation causes acceleration of, or a shift in, the normal metabolic pathway to compensate for the changing external environment. The pigment which accumulates may be a byproduct of these processes. Desiccation of apical hyphae has also been shown to stimulate the translocation of larger quantities of nutrients to the apex (Plunkett, 1956), a process which can result in increased metabolism and accumulation of byproducts. Although the processes related to pigmentation of desiccated hyphae have not been determined, it seems safe to assume that this is a protective mechanism which prevents water loss from basidiocarp hyphae and stimulates formation of lateral branches, resulting in continued growth. When *P. adustus* can be induced to fruit under sufficiently controlled conditions, more may be learned about this pigmentation phenomenon.

Differentiation into mature basidiocarps occurs more rapidly under dry conditions, although the resulting structures are considerably smaller than basidiocarps forming under conditions of optimal humidity. The more rapid development is likely due to an increased nutrient supply to the apical hyphae under conditions of rapid transpiration,

which result in an increase in metabolic rate (Plunkett, 1956). This ability to mature rapidly in a potentially hostile environment is important to the survival of *P. adustus*. Spores from these basidiocarps are rapidly discharged under dry conditions. These spores are dispersed to other suitable substrata in which the vegetative mycelium can grow even if the atmospheric humidity is below the level at which basidiocarp development can occur.

Low light intensities were found to favor primordium growth and basidiocarp development. Basidiocarps developed more readily when subjected to average intensities of approximately 40-100 foot-candles. The effect of light, as determined by this strictly comparative study, is believed to be related to humidity variations rather than to the control of fruiting by a photochemical response on the part of *P. adustus*. As the light intensity increased, the humidity at each site decreased, resulting in limitations to the extent of primordial formation and to cessation of pileus development. As was mentioned earlier, no attempt was made to formulate an action spectrum for morphogenesis. This aspect of light studies was not investigated

for two reasons: the first was the fact that basidiocarps could not be induced to form readily in culture under controlled conditions; the second reason was equipment limitations. Since the light recorders used in this study utilized silicon cells which are sensitive only to visible light, the results obtained do not represent the full spectrum. Further, it has been reported by Freyman (1968) that aspen and conifer tree canopies absorb different spectra and consequently the light reaching the ground will differ in spectral distribution. The effect of the spruce-aspen canopy at Site III and the aspen canopy at Sites I and II on the light at ground level cannot be determined. Although qualitative light studies may yield some information about phototropic responses in *P. adustus*, light is not a critical factor in basidiocarp development. Plunkett (1956) suggested that there is an interaction of light and humidity which influences development. Basidiocarps actually developed under deciduous and coniferous canopies and they were induced in the laboratory under conditions of low light intensity (80 foot-candles). More important is the fact that, regardless of the intensity (within the limits noted in these experiments), basidiocarp

development did not occur beyond a rudimentary, pigmented primordium, when the relative humidity was maintained above 80%.

From these studies it can be concluded that there is an interaction of environmental factors, and when a specific set of conditions is achieved by this interaction, basidiocarp development takes place. Relative humidity is the most critical factor involved, but the humidity is altered by changes in temperature, by drying effects of light and by rainfall.

There is considerable plasticity of form in *P. adustus* basidiocarps and dissepiments will form wherever the irregular surface of a log or stump allows positively geotropic growth to occur. On the underside of logs, overhanging ends of irregularly broken logs, or on the underside of furrowned bark, dissepiments will form without the development of a pileus. On imbricate and effused-reflexed basidiocarps, tubes develop on resupinate areas which show the slightest downward-oblique orientation with level ground. This response results in some regions of the pore surface developing a daedaloid appearance. A smooth, even pore surface develops

only when a pileus or resupinate basidiocarp is horizontally oriented. The form of development allows the hymenophore to form without extensive development of other basidiocarp regions. This adaptation permits production of large numbers of spores during a short period of time. The products of metabolism and the energy produced by these processes are not expended in development of extensive, highly modified basidiocarps, but rather are channeled into the sporulation process before adverse environmental conditions occur which cause the death of the basidiocarp.

The development of the system of strands is essential for pileus formation. These parallel aggregations of hyphae develop in an approximately perpendicular orientation to the primordium or resupinate region of a basidiocarp. The area in which strand formation, and consequently pileus development, occurs is most likely an area in which dissepiments are not able to develop. It follows that the strong tropic responses of the strands and the dissepiments play a role in limiting pileus formation to regions of the basidiocarp on which tubes cannot form. It is reasonable to assume that a minimal degree of hyphal modification and basidiocarp plasticity are an advantage to a fungus which

develops rapidly during a relatively brief period of favorable environmental conditions. Furthermore, the precise conditions required for development restrict *P. adustus* to a limited number of habitats which are shaded and damp.

2. The Cultural Environment

Species identification using the vegetative mycelium must be carried out in culture to allow complete expression of hyphal features. But until a workable system is available for identifying wood-decay fungi using cultural characters, study of the mycelium in culture must always be accompanied by study of basidiocarps on the wood surface if they are available. The approach used by Nobles is an indispensable framework for identification of wood-decay fungi, but this study has shown that the use of one medium can limit the expression of hyphal characters. More extensive studies along the lines of those carried out for *P. adustus* will be necessary before Nobles' system is really workable. In her classification, Nobles' has not used features associated with hyphal differentiation, an aspect of the vegetative mycelium that offers great promise

in taxonomic studies. A study of the expression of hyphal characters on various media was done on a limited scale by Long and Harsch (1918), but this method has been neglected by more recent investigators in the interests of formulating a general classification system in which a standard medium is used. If the vegetative mycelium is to be used for the purposes of classification or taxonomy, the development of hyphal types or "systems" (i.e., such as strand formation), and asexual spore formation, must be carefully investigated for each species in the genus. With the information available, a key based on hyphal characters of the vegetative mycelium is not workable.

Studies of the mycelium of *P. adustus* indicate that hyphal differentiation occurs to a greater degree than has been reported previously. The branched, clamped, flexuous hyphae which develop from the inoculum give rise to the unbranched, clamped hyphae of uniform diameter and the broad, simple-septate hyphae. These three hyphal types, and formation of oidia are constant features of the mycelium grown on Blakeslee's malt agar or glucose-asparagine basal synthetic agar. Substitution of equivalent amounts of different carbon and nitrogen

sources affects the rate of growth and the total mass of mycelium produced.

Carbon utilization experiments have shown mannose to be an excellent source for vegetative growth, followed by glucose, cellobiose and xylose. Moderate vegetative growth occurred on the medium containing maltose, but this disaccharide allowed greater hyphal differentiation than any of the other carbon sources. Bille-Hansen (1953) found that three species of *Coprinus* grew well and fruited on maltose, compared to fruiting by only one species on glucose and sucrose. Maltose permitted zygospore production in *Phycomyces blakesleeanus* (Hawker, 1957). Possibly, the utilization of maltose by *P. adustus* results in the formation of substances that cause hyphal differentiation. It is doubtful that this sugar occurs in nature (Lilly and Barnett, 1951). The substances which induce hyphal differentiation in nature may be different from those active in culture. It is not known whether there is the requirement for a specific substance, available both in maltose and a naturally-occurring compound, which in involved in hormonal

regulation of differentiation. Although we have yet to determine processes involved, there is no doubt that the carbon and nitrogen sources available to *P. adustus* influence growth and hyphal development.

Cellulose did not support good growth and differentiation in *P. adustus*, probably due to the inability of this fungus to produce β-glycosidases in sufficient quantity to break down the cellulose into cellobiose units. The utilization of cellobiose by *P. adustus* may occur in nature when cellulose is degraded by bacteria or other fungi colonizing the wood substratum.

The ability of this fungus to utilize xylose, whose condensation product, xylans, may constitute up to 25% of the woody tissue (Meyer and Anderson, 1952), and cellobiose, which occurs after cellulose is hydrolyzed in the wood, gives *P. adustus* an advantage over other fungi which are not able to utilize these carbon sources. The ability to use free sugars might permit *P. adustus* to become established in wood cells before decay activity actually occurs. Galactose and mannose, both utilized by this fungus, occur free in the cytoplasm of many plants, but in small amounts. The

use of free sugars would partially account for the fact that *P. adustus* grows rapidly through wood and produces basidiocarps within 15 months of infection, while causing only limited decay. Basidiocarp formation, with only slight penetration of the wood substratum was also observed in the laboratory. It may be only after the free sugars are exhausted that this fungus actively breaks down the xylose and lignin incorporated in the cell wall.

Nitrogen utilization studies revealed that *P. adustus* grew very well when supplied with asparagine, urea or glycine. Growth occurred when different amino acids were incorporated in the media, with the exception of cysteine. Complete inhibition occurred in the presence of cysteine. Whether this is a form of competative inhibition, as occurs in *Neurospora crassa* (Nicholas, 1965) or feed-back inhibition which suppresses methionine synthesis, is unknown. Merrill and Cowling (1966) found aspartic acid, glutamic acid, glycine and lysine to occur in aspen wood as free amino acids. Although aspartic acid inhibited growth for 10 days, this inhibition was overcome and this amino acid supported excellent growth, although at a slower rate than with lysine

or glycine. The inhibiting effect of Ammonium sulfate on growth was due to a severe drop in pH. Henningsson (1967a) suggests that this is caused by "the unilateral uptake of cations and the exudation of organic acids". Nitrate supported sparse vegetative growth. Henningsson found that *P. adustus* and *Libertella betulina* were the only fungi commonly occurring in aspen and birch that utilized nitrate as the sole source of nitrogen. The ability to utilize nitrates is of questionable value since, normally, plants rapidly reduce the nitrogen to other forms. These studies have shown that *P. adustus* can utilize a wide range of nitrogen sources occurring in its natural substratum.

These different sources of carbon and nitrogen which can be utilized by *P. adustus* affect the relative numbers of each hyphal type occurring in a mycelium, as well as the degree of oidia formation. Furthermore, only certain sources result in development of structures such as oidia (o) and chlamydospores. These structures can be induced to form by growing the vegetative mycelium on specific media. Once the hyphal characters expressed by a species on various media are determined, the

development of specific structures can be regulated. This type of study shows promise in classification and taxonomy of wood-decay fungi. Similar cultural studies of the vegetative mycelium of related species should be carried out in order to determine relationships between these species based on the vegetative stage. It will then be possible to state which features of the vegetative mycelium provide reliable criteria for species identification.

Under cultural conditions, the vegetative hyphae of *P. adustus* show a greater degree of modification than the hyphae of the basidiocarp. Perhaps hyphal modification is suppressed in this fungus growing in the natural environment in favor of rapid growth and development.

I believe that further studies of the morphology of the Polyporaceae will show that the species can be divided into two major groups on the basis of the pattern of development. In one group, which would include, for example, *Gloeophyllum saeparium* and species of *Fomes*, the basidiocarps develop slowly over several growing seasons, producing modified hyphae which protect the actively growing hyphae from adverse environmental conditions.

In the other group, which would include *Polyporus adustus*, *P. fumosus* and *P. pargamenus*, growth is rapid and the hyphae do not undergo extensive modification. Hyphal modification is suppressed in favor of production of large numbers of basidiocarps under favorable conditions. In both groups of polypores there are characteristic hyphal systems based, not only on modifications resulting in skeletal and binding hyphae, but on the development of *functional* systems which develop under defined sets of environmental conditions and serve to adapt the species to the environment.

C. The Developmental Morphology of *Polyporus adustus*

The information gained from this study can now be used to formulate a picture of the developmental morphology of *P. adustus*. This picture includes features of the vegetative and reproductive stages, both of which are necessary to an understanding of the development of this fungus. In discussing the use of morphology in taxonomic treatments of fungi, Chesters (1968) stated that criteria can be selected from all phases of the life cycle as long as they are constant and can be accurately

described in qualitative and quantitative terms. This very basic, logical frame of reference has been adhered to in this work. Consequently, in formulating a description of *P. adustus* I have considered only constant features of the mycelium.

The vegetative hyphae, growing in poplar wood, are narrow, clamped and hyaline. These hyphae grow through the lumen of tracheids and vessels. Branches grow transversely through the pit membranes or directly through the cell walls. Hyphal aggregations form in the vessels, and occasionally in the tracheids, and vessels are the first cells broken down by the activity of this fungus; then the parenchyma is attacked. The fibers remain intact even in severely decayed wood. A compact hyphal mass develops under the bark, and hyphae fill the tracheary elements near the wood surface at sites of basidiocarp formation.

The vegetative mycelium, growing on Blakeslee's malt or glucose-asparagine basal synthetic agar at 26°C, covers an 8.5 cm Petri dish in 6 days and consists of three main hyphal types. The first to appear are branched, clamped, flexuous hyphae with an average diameter of 2.3 μ. These

are the most prevalent hyphae and give rise to two other less abundant types. One of these is a clamped hypha of uniform diameter (1.3-2.2(3.0) μ). The second is a wide (4.2-8.4 (10.5) μ), simple-septate hypha which arises from a clamp connection of a flexuous hypha. As the culture ages these wide hyphae lose their septa and their cytoplasm and collapse. After 7 days incubation, oidia form by fragmentation of all hyphal types. Chlamydospores form in malt agar cultures and also when maltose is substituted in the basal synthetic medium. On urea-asparagine medium, oval oidia, termed oidia (o), develop by repeated constriction along the length of a hypha of uniform diameter followed by septation. These can be observed in short chains or as individual spores in cultures after 7-10 days incubation.

Primordia vary greatly in size from a small button to an extensive mat growing over the wood surface. The hyphae are unorganized except for a general parallel orientation. In all primordia the central surface is dimpled and is smoky-gray in color. The pigment is in the slightly-thickened walls of the hyphae.

Basidiocarps are imbricate, effused-reflexed or resupinate. The larger imbricate pilei are sessile but small, substipitate pilei form between the larger ones. The pileus surface is finely villose-tomentose to glabrous in weathered specimens, sordid to tan in color, and occasionally zonate. It is composed of terminal hyphae, 3.1 μ in diameter, growing upward from the context. The context consists of strands of clamped hyphae 2.2-3.3 μ in diameter, running in parallel orientation. A second system of interwoven hyphae enmesh the strands. The pileus base and the sterile margin consist of hyphae in close parallel alignment, but no strand formation occurs. The hyphae of the margin remain unpigmented. All resupinate basidiocarps and resupinate areas of other types show this parallel alignment without strand formation. Strand formation occurs only in pileate structures. A transition zone of profusely-branched, felted hyphae (3.3-4.4 μ in diameter) with pigmented walls, forms on the lower surface of the context. These hyphae grow downward in a positively geotropic manner to form the dissepiments. The tramal hyphae have pigmented, slightly-thickened walls, widely spaced clamps, a diamter of 3.3-4.4 μ, and are in close parallel alignment. The hymenium

develops by horizontal branching of the hyphae lining the tubes and appears as a palisade of single-celled, clavate structures. Sterigmata are evident only when basidiospores are present. The four spores on each basidium are hyaline, sub-globose to oval and measure 5-6 x 3-4 μ.

The most distinctive macroscopic characteristic of this species is the pigmented transition zone and tramal tissue. This has been found to be a constant characteristic which distinguishes this species from *P. fumosus*, which is normally pigmented only in the transition zone. Tramal pigmentation, usually the result of bruising in *P. fumosus*, does not occur uniformly through the tissues.

The description of the mycelium is based on constant cultural characters on defined media under specific conditions used in this study. The basidiocarp characters are those which can be observed in primordia and during development in the natural habitat, but not in over-mature or herbarium specimens. This description is not meant to be used for taxonomic purposes. It is first necessary to carry out similar developmental studies on a wide

range of individual species. Such studies should lead to the formulation of an acceptable breakdown of this diverse family into naturally related groups, based on the mode of hyphal development of the mycelium and its adjustment to the environment. The whole range of genotypic expression of these fungi must be examined along with their adjustment to their physical and chemical environments in order to fully understand the development of the members of the Polyporaceae and their relationships with each other.

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A P P E N D I C E S

APPENDIX I

Procedure for staining mycelium in wood sections (adapted from Cartwright, K. St. G., 1929).

1.	1% aqueous safranin	5 minutes
2.	Rinse in distilled water	5 minutes
3.	Picro-anilin blue:	
	Heat to simmering and stain	30 minutes
4.	Rinse in distilled water	5 minutes
5.	Dehydrate:	
	50% ethanol	1 minute
	70% ethanol	1 minute
	85% ethanol	1 minute
	95% ethanol	1 minute
	100% ethanol	1 minute
6.	Clearing solution*	60 seconds
7.	Xylene + few drops 100% ethanol	5 minutes
8.	Xylene	10 minutes

* Clearing solution:

Xylene	25 ml
Absolute ethanol	25 ml
Clove oil	50 ml

APPENDIX II

Spore Isolation Technique

The apparatus for collecting spores consisted of a small watch glass inside a Petri dish. After this apparatus was autoclaved, 1 ml of sterile distilled water was pipetted into the watch glass.

A sporulating section of basidiocarp was rinsed in a 30 µg/ml streptomycin solution for 15 seconds and was glued to the lid of the Petri dish with rubber cement. The lid was replaced on the dish after the cement dried. Spores were discharged into the watch glass, which was directly under the basidiocarp. The resulting spore suspension was diluted with 9 ml of sterile distilled water and one ml was then pipetted onto the surface of one-half strength Nobles' malt agar. Germinating spores were isolated with a chisel-edge dissecting needle, and incubated at 26°C on full strength malt agar. Pure cultures were transferred to one-half strength malt agar slants and stored at 5°C.

APPENDIX III

The Weekly Mean Range of Light Intensity (foot-candles) at Sites I and II.

	SITE I		SITE II	
Date	Minimum	Maximum	Minimum	Maximum
June 24-30	10	4800	10	4500
July 1-7	10	4800	10	3400
July 8-14	10	3600	10	2550
July 15-21	10	4400	10	-
July 22-28	10	4300	10	-
July 29-Aug. 1	10	4500	10	4200
August 5-11	10	5200	10	3800
August 12-18	10	5600	10	5280
August 19-25	10	7000	10	6650
Aug. 26-Sept. 1	10	7000	10	6600
September 2-8	10	5000	10	4200

APPENDIX IV

Formulae for Media Used in Cultural Studies

1. Malt Extract Agar (Blakeslee, 1915)

Dextrose	20 gm
Peptone	1 gm
Malt extract	20 gm
Agar	20 gm
Distilled water	1000 ml

Modification: 10 gm Oxoid agar substituted for 20 gm Bacto-Agar.

2. Malt Extract Agar (Nobles, 1948)

Difco Bacto malt extract	12.5 gm
Difco Bacto-Agar	20.0 gm
Distilled water	1000.0 ml

Modification: (1) 10 gm Oxoid agar substituted for 20 gm Bacto-Agar. (2) 6.25 gm malt extract added for half-strength medium.

3. Basal Synthetic medium (Lily and Barnett, 1951)

KH_2PO_4	1.0 gm

$MgSO_4 \cdot 7H_2O$	0.5 gm
Fe^{+++}	0.2 gm
Zn^{++}	0.2 mg
Mn^{++}	0.1 mg
Biotin	5.0 µg
Thiamine	100.0 µg
Distilled water to make	1000.0 ml
Glucose	10.0 gm
Asparagine	2.0 gm
Agar (for solid media)	20.0 gm

Modification: (1) 10 gm Oxoid agar substituted for 20 gm Bacto-Agar. (2) Biotin was omitted. (3) Equivalent amounts of carbon and nitrogen source were substituted for glucose and asparagine.

4. Medium B (Henningsson, 1967a)

Glucose	20.0 gm
KH_2PO_4	1.0 gm
$MgSO_4 \cdot 7H_2O$	0.5 gm
Ammonium tartrate	2.5 gm
$CaCl_2 \cdot 2H_2O$	0.3 gm
NaCl	0.1 gm

$ZnSO_4$	8.79	mg
$CuSO_4$	0.393	mg
Fe-citrate	5.0	mg
H_3BO_3	0.057	mg
$(NH_4)_6MO_7O_{24}$	0.368	mg
$MnSO_4 \cdot 7H_2O$	0.061	mg
Thiamine-HCl	100.0	µg
Redistilled water to make	1000.0	ml

5. Badcock's Accelerator Medium (Badcock, 1941)

Maize meal	2.5	gm
Bone meal	1.5	gm
Potato starch	0.75	gm
Sucrose	2.0	gm
Malt extract	0.5	gm

Add to 100 gm sawdust.