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THE UNIVERSITY OF ALBERTA

A BIOSYSTEMATIC STUDY OF *CORIOLUS HIRSUTUS* (FRIES) QUÉLET AND *CORIOLUS PUBESCENS* (FRIES) QUÉLET

by

© ROGER GERALD WILLIAM EDWARDS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

EDMONTON, ALBERTA

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled 'A biosystematic study of *Coriolus hirsutus* (Fries) Quélet and *Coriolus pubescens* (Fries) Quélet' submitted by Roger Gerald William Edwards in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Supervisor

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ABSTRACT

The five taxa *Coriolus hirsutus*, *C. pubescens*, *C. versicolor*, *C. velutinus* and *C. zonatus* compromise a species complex which has been reported to occur in Alberta. Interfertility tests between collections of these taxa have shown that only the species *C. hirsutus* and *C. pubescens* are extant in this area. The taxon *C. versicolor* which occurs in British Columbia and Eastern Canada has been shown to have a small degree of interfertility with the Alberta taxon *C. hirsutus*. The three taxa *C. hirsutus*, *C. pubescens* and *C. versicolor* are natural or biological species, since under natural conditions they are either spatially or genetically isolated and consequently do not interbreed. The two taxa *C. velutinus* and *C. zonatus* are considered to be forms of a species and will probably prove superfluous.

Since identification of individual basidiocarps used in this study was determined by compatible mating tests, positively identified material was available for assessing the variation within each species. It was found that the range of macroscopic variation in basidiocarps of *C. pubescens* was much greater than had been supposed and large ungulate basidiocarps have been described for the first time for this species. Stable macroscopic characters which may be used to distinguish between *C. hirsutus* and *C. pubescens* have been determined, and the macroscopic

variation within each species is given.

The microstructure of basidiocarps of *C. hirsutus* and *C. pubescens* has been studied and the growth and development of basidiocarps has been described and illustrated at the cellular level. A new approach to basidiocarp analysis has been utilized, based on the concept of basidiocarp tissue having a cellular construction where end cells differentiate into various types of discrete components, depending on their location within the basidiocarp. It has been found that these discrete structural components have characteristic forms for each species and their consequent value as taxonomic criteria is assessed.

The vegetative mycelium growing in artificial culture has been described at the cellular level. Both end cells and intercalary cells have been found to become differentiated into thick-walled structures. Taxonomic significance has been attached to the shape and size of these discrete structures.

Growth rates on artificial media of isolates of *C. hirsutus* and *C. pubescens* have been determined at different temperatures and the variation in optimum growth rates for the two species is discussed.

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CHAPTER I

INTRODUCTION

The Polyporaceae, a family of higher Basidiomycetes includes the majority of wood decay fungi and is therefore of considerable economic importance. Wood decay fungi are mostly saprobic, and are capable of infecting and decaying both standing dead trees and wood products. A few species are parasitic and are able to infect and decay living trees. It is on account of their economic importance that wood decay fungi have received the attention of research workers, not only to develop preservatives to prevent their growth and spread through wood products, but also to characterize and delimit species and develop a better understanding of the life history of species within this group.

The taxonomy of the family Polyporaceae has had a somewhat turbulent history, having been in a state of flux since its inception which can be traced back to Linnaeus. Although Linnaeus (1753) recognized the genera *Agaricus* and *Boletus*, in which he included a number of polypores, the International Code of Botanical Nomenclature states that the starting point for the nomenclature of polypores is the Systema Mycologicum, published by Fries (1821-32). In this work, Fries divided the fungi into four primary classes of which the "Hymenomycetes" were subdivided into six orders. The "Pileati", one of these six orders,

included the genus *Polyporus* Fries which contained, among others, the species *Polyporus hirsutus*, *P. pubescens*, *P. versicolor*, *P. velutinus* and *P. zonatus*. Fries (1851) later segregated the genus *Polystictus* Fries in which he included the species *Polystictus hirsutus*, *P. versicolor* and *P. zonatus*, while the remaining two species of this group were retained in the genus *Polyporus*. The difference between these two genera was that in the genus *Polyporus* the pores developed in a centrifugal manner, the oldest being closest to the base of the pileus, whereas in the genus *Polystictus*, the pores developed simultaneously from above downwards. Fries (1874) later abandoned the genus *Polystictus* since it did not appear among the ten genera of polypores included in the Hymenomycetes Europaei. The genus *Polyporus* was a large genus encompassing a diverse and unrelated group of polypores. Although Fries (1821) only used two genera of polypores, (*Polyporus* and *Daedalea*), Cooke (1959) has stated that about 300 genera of pore fungi have been described of which about 100 are valid.

Quélet (1886, 1888) subdivided the large Friesian genus *Polyporus* into a number of smaller ones. He proposed several new genera, one being the genus *Coriolus* Quélet which included the species *Coriolus hirsutus*, *C. pubescens*, *C. versicolor*, *C. velutinus* and *C. zonatus*.

Murrill (1905, 1907) accepted many of the genera of Quélet, including the genus *Coriolus*. His comprehensive works must be considered the foremost treatment of North

American polypores at that time. He recognized 74 genera of polypores of which many were original. He used both anatomical and morphological characters to define and separate the genera. In his later work, Murrill (1907-8) included *Coriolus nigromarginatus*, *C. pubescens* and *C. versicolor* among the 40 species of the genus *Coriolus*. The name *Coriolus nigromarginatus* (Schw.) Murrill is considered synonymous with *Coriolus hirsutus* (Fries) Quélet, the latter taking preference by virtue of its priority.

A more recent treatment of North American polypores is that by Overholts (1953). His approach was conservative and was based on the Friesian system. Overholts accepted 8 genera, of which the genus *Polyporus* included the species *Polyporus hirsutus*, *P. pubescens*, *P. versicolor* and *P. velutinus*. The taxon *Polyporus zonatus* was considered by Overholts to be a form of *P. versicolor*.

Bondartsev (1953) has published the largest volume on polypores to date. He included 270 species in 61 genera. He recognized the genus *Coriolus* and listed *C. hirsutus*, *C. pubescens*, *C. versicolor* and *C. zonatus* among the 11 species of this genus. He considered *C. velutinus* to be synonymous with *C. pubescens*. The classification system outlined by Bondartsev appears to be the most natural yet proposed and since it is now readily available, it is the system adopted in this thesis.

A classification system based largely on microscopic characters of the basidiocarp has been published by

Cunningham (1948, 1965). In his earlier work he accepted the genus *Coriolus* which included *C. hirsutus*, *C. versicolor*, *C. velutinus* and *C. zonatus* among the 6 species of this genus. The genus *Coriolus* was later abandoned by Cunningham and the four taxa just mentioned were placed in the genus *Trametes* Fries which now contained a total of 24 species. Cunningham did not recognize the taxon *Coriolus pubescens*.

Various other authors have rearranged the classification of the Polyporaceae to suit their own ideas with the result that the situation is extremely confused.

The confused state of the taxonomy of the Polyporaceae is primarily caused by the polymorphic nature of the basidiocarps of these fungi. Basidiocarps of a particular species show considerable variation in size, colour, degree of pubescence, pore surface and in the many other characters which are used to identify a species. In the past, collectors have gathered specimens of polypores, and divided them into species mainly on their macroscopic appearance. Typical specimens of each species are readily identified, but intermediate forms may be pushed towards one species or another, according to the whim of the collector. This has inevitably led to the formulation of artificial 'form' species where all collections having an appearance which falls within a certain range are referred to as one species.

The five taxa, *Coriolus hirsutus*, *C. pubescens*, *C. versicolor*, *C. velutinus* and *C. zonatus* are considered

to be a closely related, natural group of polypores, having many characteristics in common, while differing primarily in degrees of zonation, degree of pigmentation, degree of pubescence, etc., rather than in the presence or absence of a particular morphological or anatomical character. Taxonomic characters which have a variable expression are not reliable criteria on which to base a species description. Consequently the species description for the five taxa given above overlap in many characters and the limits of each taxon have been poorly defined. One taxon appears to grade into another and consequently there is no clear indication of how many good species there are in this group and of the limits of each species.

The traditional taxonomic procedure for delimiting a particular species is to collect a number of individuals which are thought to belong to the species and then to circumscribe the species so that the allowable variation is provided for. This approach may be quite effective in dealing with discrete, easily recognized species, but where problems of polymorphism occur, there are severe limitations to the use of this method. Firstly, individual specimens which have been assembled by a taxonomist and were considered by him to be conspecific on the grounds of comparative anatomy and morphology, may in fact be members of several species. Secondly, individual specimens which the taxonomist has rejected as not being within the expected range of variation for a particular species may be

perfectly good examples of that species, but are unrecognized as such by the taxonomist. Consequently, the final species description falls short in its objective of exactly circumscribing the species. A third limitation, and perhaps the most important, is that imposed by the definition of the term 'species', since what may be a good species to one taxonomist may be unacceptable to another.

In an effort to get away from the traditional approach, with its inherent limitations, it was decided to attempt a new approach which would eliminate these particular problems. As a first step, it was necessary to define exactly what was meant by the term 'species' as it applies to the species complex under study.

A morphological species concept has been favoured in the past and is partly responsible for the present confusion since it is almost impossible to apply to a polymorphic species subject to environment-induced phenotypic variation. A biological species concept, which considers that individuals having a high degree of interfertility belong to the same species, presents practical problems in its application to most organisms. Certain groups of polypores are an exception, and the biological species concept may be applied to them with ease, since they can be readily grown and test mated in culture and a compatible pairing can quickly be recognized by the formation of clamp connections. The tremendous advantage of using interfertility tests to determine conspecificity is that the result obtained is

almost always unequivocal and not subject to different interpretations by other workers. Interfertility tests therefore provide proof that two individuals are members of the same species. In this study, all individuals showing a high degree of interfertility are considered to be conspecific.

The new approach taken in this work was to begin with a few typical specimens of each species and, using these as a basis, to test pair them with other, morphologically different individuals, so that a large collection of individuals of proven identity was built up. These resulting collections of each species contained individuals which were anatomically and morphologically quite diverse, yet were proven to be compatible within each species.

These collections were subsequently analyzed to determine variation in morphology, anatomy and cultural characteristics so that accurate species descriptions could be prepared.

The morphology or macrostructure of basidiocarps has been primarily used in the past for species identification and is consequently considered first in this thesis (Chapter II). The greatest problem met with results from the difficulty of describing such characters as degree of zonation, colour, etc., in terms which will convey exactly the same meaning to other workers. Terms such as 'hirsute', 'pubescent' or 'isabelline' mean different things to different workers, since they can not accurately be defined.

In such cases, photographs are an excellent addition to a species description.

An analysis of the collections of basidiocarps of *C. hirsutus* and *C. pubescens* has enabled specific characters to be determined and has also resulted in an extension of the size range for these basidiocarps. Large basidiocarps, well outside the accepted range for *C. pubescens*, have been shown by interfertility tests to be compatible with the typical ones. It has also been possible to determine which basidiocarp characteristics are species specific. These characteristics are consequently of considerable importance and are emphasized since they are reliable taxonomic criteria.

The anatomy or microstructure is considered in Chapter III. Corner (1932a, 1932b, 1953) has described the microstructure of basidiocarps of several species of polypore in great detail. He reported that basidiocarp hyphae may be modified to form skeletal or binding hyphae, or may remain unmodified as generative hyphae and that basidiocarps of different species can be characterized by having: 1. generative hyphae alone, 2. generative hyphae and skeletal or binding hyphae, or 3. generative, skeletal and binding hyphae. Cunningham (1947, 1954, 1965) continued the work originated by Corner and erected a classification system based largely on microstructural characteristics. The works of Corner and Cunningham provided a basis for the study of *C. hirsutus* and *C. pubescens*. It was found,

however, that there were severe limitations to the application of Corner's original principles to the species under study and it was found necessary to develop a new concept of microstructure by considering the development of discrete end cells into specialized structural components. This could not be achieved until the development of the various types of specialized basidiocarp components had been elucidated and was thoroughly understood.

The results of the interfertility studies are given in Chapter IV. The tetrapolarity of both species had been previously reported and has been confirmed. Evidence that the two species *C. hirsutus* and *C. pubescens* are intersterile is presented. A common phenomenon observed in test pairing was the development of pigment lines in the contact zone between cultures grown in the same petri dish. These pigment lines occurred with such regularity that records were kept in an attempt to discover a basis for this phenomenon.

Cultural characteristics have been considered by Nobles (1958b, 1965) to have considerable taxonomic significance and the cultural studies of *C. hirsutus* and *C. pubescens* are presented in Chapter V. The microstructure of the mycelium in culture had not been satisfactorily described and it has been found possible to consider the separate differentiated components of a mycelium as discrete units which have developed from single cells. A study of the microstructural detail of cultures of *C. hirsutus* and

C. pubescens has allowed the taxonomic significance of these characters to be assessed.

Although the five taxa *C. hirsutus*, *C. pubescens*, *C. versicolor*, *C. velutinus* and *C. zonatus* have all been reported from Alberta, the results of the interfertility tests have shown that only the two species *C. hirsutus* and *C. pubescens* have been found to occur in this area. The study of these two species is presented in this thesis. The taxon *C. versicolor* occurs in British Columbia and Eastern Canada and, since some data on it was obtained, it is occasionally mentioned in this thesis to illustrate differences between it and the Albertan taxa.

CHAPTER II

BASIDIOCARP MACROSTRUCTURE

INTRODUCTION

The traditional approach to identification and classification of wood decay fungi has emphasized easily observable macroscopic characters of the basidiocarp. Macroscopic characters are, unfortunately, very variable and differing environmental conditions of growth have a profound effect on the expression of various macroscopic characters. In addition, the difficulty of accurately describing such subjective characters as degree of pubescence or colouration of zones in a manner that can be used by other workers to identify unknown specimens has resulted in ill-defined species limits. The macroscopic characters of the five taxa, *C. hirsutus*, *C. pubescens*, *C. versicolor*, *C. velutinus* and *C. zonatus* have been described by a number of workers, yet universal agreement on the number of good species in this group and the limits of each species is far from a reality. Each worker has had his own ideas of the limits of each species and the lines between the separate species have been arbitrarily drawn, not always in the same place, by different workers.

Murrill (1905) was responsible for the first major work on the Polyporaceae of North America. This work appeared later in revised form (1907) and from it the

following descriptions are taken.

Coriolus pubescens.

"Pileus rather thick, imbricate, laterally connate, sessile, dimidiate or flabelliform, conchate, 3-5 x 4-8 x 0.2-0.4 cm; surface white, zonate, hirsute-villose to nearly glabrous, finely radiate-lineate in front at times, often radiately-furrowed or slightly plicate; margin at times thin, but usually obtuse, somewhat inflexed; context thin, white, fibrous, 1-2 mm thick; tubes white, 2-4 mm long, mouths angular, regular, 2-3 to a mm, edges very thin, entire to denticulate, white to discoloured;...."

Coriolus nigromarginatus (Schw.) Murrill. (=*C. hirsutus*).

"Pileus confluent-effused, more or less imbricate, sessile, dimidiate, applanate, corky-leathery, rather thick, flexible or rigid, 3-5 x 5-8 x 0.3-0.8 cm; surface conspicuously hirsute, isabelline to cinereous, concentrically furrowed and zoned, margin at length thin, often fuliginous, sterile, finely strigose-tomentose, entire or undulate; context white, thin, fibrous, spongy above, 1-4 mm thick; tubes white, 1-2 mm long, mouths circular to angular, 4 to a mm, quite regular, edges thin, firm, tough, entire, white to yellowish or umbrinous;...."

More recently, L.O. Overholts (1953) has published the following descriptions of *C. pubescens* and *C. hirsutus*.

Polyporus pubescens Schum. ex Fries (=*C. pubescens*).

"Sporophore sessile, or in circular clusters attached to the center, often imbricate, coriaceous or

tough and watery when fresh, drying rigid, reviving; pileus 1.5-5 x 2.5-8 x 0.3-1 cm, white or grayish yellow when fresh, often grayish or yellowish (rarely umber) in herbarium specimens, villose-tomentose (or occasionally almost hirsute at the base) to finely appressed tomentose or becoming subglabrous, often radiate-lineate toward the margin; context white, 1-5 mm thick; pore surface white when fresh, drying yellowish or umber at times, the tubes 1-4(-6) mm long, the mouths angular, averaging 3-4 mm, the walls thin (except in immature plants), often dentate;. ..."

Polyporus hirsutus Wulf. ex Fries. (=*C. hirsutus*).

"Sporophore sessile or effused-reflexed, coriaceous when fresh, drying rather rigid, reviving; pileus 1.5-6 x 1.5-10 x 0.15-1 cm, grayish to yellowish or brownish but nearly unicolorous in any one specimen except perhaps for one or more marginal darker zones, hirsute or tomentose, usually rather strongly zoned or furrowed, sometimes with the margin brown and the center blackish; context white, 1-6 mm thick; pore surface white to yellowish or smoky, the tubes 1-4 mm long, the mouths subcircular, the walls typically thick and entire but occasionally thin and somewhat sinuous or lacerate, averaging 3-4 per mm;...."

In this study, identification was done by means of interfertility tests, described in detail in Chapter IV, which allow positive confirmation of conspecificity so that it was possible to build up a collection of specimens of

proven identity. By study of these collections, the characteristics of each species and the range of variation to be expected in macroscopic characters were determined. It was then possible to examine and identify herbarium material with a high degree of confidence.

It should be noted that an estimate of species variation based on positively identified collections had not been obtained prior to this study. An estimate based on interfertility tests is essential to determine accurate data on species variation.

In this chapter, the variation in macroscopic characters for the two species, *C. hirsutus* and *C. pubescens*, will be considered together with information resulting from studies of herbarium material.

MATERIALS AND METHODS

Fresh basidiocarps of *C. hirsutus* and *C. pubescens* were collected in Alberta and typical specimens of each species were identified. Specimens of uncertain affinity were positively identified by interfertility tests. A few additional collections of *C. hirsutus* and *C. pubescens* were made in British Columbia and Saskatchewan, while several collections of *C. versicolor* were made in Ontario. Four collections of *C. hirsutus* were received from Sweden. A complete list of all collections used in this study is given in Appendix I.

The cryptogamic herbarium of the Department of Botany, University of Alberta, Edmonton and the herbarium of the Northern Forest Research Centre, Canadian Forestry Service, Edmonton provided dried material for this study. A complete list of herbarium specimens examined with collection data is provided in Appendix II. Measurements of basidiocarp size were made for many collections and the photographs were taken with a 35 mm Nikkormat camera fitted with a micro-Nikkor lens.

OBSERVATIONS

a. General basidiocarp shape.

Basidiocarps of both *C. hirsutus* and *C. pubescens* generally develop in groups of three to ten, but as many as fifty or more basidiocarps may be found developing in close proximity on a single piece of wood. Each basidiocarp may exist as a discrete structure or several basidiocarps may become laterally united and confluent. Often a number of imbricate basidiocarps may be formed in close proximity. Typical specimens of *C. pubescens* (Figure 1) are effused-reflexed or sessile and applanate. They are coriaceous when fresh and moist, but become rigid on drying. They are usually found in a shaded habitat on stumps or fallen branches within approximately one meter of the ground. Maximum width of a single basidiocarp may reach ten centimeters. Resupinate basidiocarps may be found on the underside of fallen logs which are supported just above ground

level, but they always have a pileate edge to the basidiocarp. In more exposed locations, basidiocarps of *C. pubescens* tend to become thicker and less applanate (Figure 2). Young specimens have a wide, blunt sterile margin to the basidiocarp (Figure 2), but as the basidiocarp attains full development towards the end of the growing season, the margin becomes thin and acute (Figure 3). Imbricate basidiocarps in exposed habitats may become confluent (Figure 4). Basidiocarps which develop in very exposed locations, such as three to five meters above ground on a dead Birch tree, are ungulate in shape (Figure 5).

Basidiocarps of *C. hirsutus* are typically effused-reflexed or sessile and applanate (Figure 6). Large numbers of basidiocarps may develop in close proximity and consequently become imbricate and confluent (Figure 7). Basidiocarps which develop in more exposed habitats tend to become ungulate in shape, but much less so than those of *C. pubescens*. The width of individual basidiocarps of *C. hirsutus* ranges up to eight centimeters but confluent basidiocarps can become twelve centimeters or more wide. Resupinate basidiocarps may be formed on the undersides of fallen logs supported just above the ground, but, as in *C. pubescens*, a narrow pileus can be observed surrounding the basidiocarp.

b. The upper surface.

The upper surface of basidiocarps of *C. pubescens* is

pallid to pale yellow, azonate (Figure 9) to faintly zonate (Figure 10) and occasionally radiate-striate (Figure 11). Ungulate forms (Figure 5) are pale yellow to golden in colour. All basidiocarps in heavily shaded habitats are almost white. The upper surface of basidiocarps of *C. hirsutus* range from pale yellow-brown in heavily shaded conditions, through golden-brown to a dark grey-brown (umber) in more exposed habitats. Zonation is variable and basidiocarps may be almost azonate (Figure 12) or zones of different colours may be present (Figures 13, 14, 15). Zonation may be faint on dark coloured specimens (Figure 16). For comparison, the upper surface of a Swedish basidiocarp (Figure 17) is deeply furrowed (sulcate) and the zones are broader than those of the Canadian specimens. Basidiocarps of *C. versicolor* (Figure 18) characteristically have broad zones of varying shades and a pale margin is usually present.

The degree of pubescence on the upper surface of basidiocarps of *C. pubescens* and *C. hirsutus* is variable but, in general, that of *C. pubescens* can best be described as being velutinous or villose-tomentose to appressed-tomentose on growing specimens. The upper surface of basidiocarps of *C. hirsutus* is velutinous to minutely pubescent (puberulent) but, occasionally, small hirsute patches may be found at the base of the pileus in some specimens. It has a velvety feel imparted by the fine pubescence, in contrast to that of *C. pubescens* which is

rougher due to the surface becoming tomentose. The Swedish basidiocarps of *C. hirsutus* are strongly hirsute to hispid (Figures 17, 36), while basidiocarps of *C. versicolor* are silky and have a characteristic glossy sheen.

c. The lower surface.

The pore surface of basidiocarps of *C. pubescens* is even and plane and varies in colour from pale creamy to creamy yellow (Figure 19). Pores average 3-4 per mm and are angular in young basidiocarps with thin dissepiments (Figure 20). Ungulate basidiocarps also have 3-4 pores per mm, but the pores may be irregular in shape and the dissepiments thicker (Figure 21).

As basidiocarps reach full development at the end of the growing season the pore surface breaks up into teeth and the individual pores become difficult to detect (Figure 22). Actively growing basidiocarps have a sterile margin around the pore field (Figure 20) which is particularly noticeable in ungulate forms (Figure 21). Once full development of the basidiocarp has been reached, the sterile margin disappears (Figure 22). The pore surface of young basidiocarps of *C. hirsutus* is even and plane (Figures 23, 24) and pores average 3-4 per mm. Initially, pores are round and dissepiments thick (Figure 23). Actively growing basidiocarps usually have a sterile margin (Figure 24). With increased growth of the basidiocarp the dissepiments become thinner and the sterile margin becomes less noticeable (Figure 25). The pore surface of a fully developed

basidiocarp remains even while the pores become angular and irregular, the dissepiments become thin and the sterile margin disappears (Figure 26). The colour of the pore surface varies from creamy-grey in specimens growing in a shaded habitat to a dark grey-brown in specimens growing in exposed locations. The pore surface of a Swedish basidiocarp of *C. hirsutus* (Figure 27) is similar in most respects to that of a Canadian specimen of comparable age. The pore surface of *C. versicolor* (Figure 28) is even, plane and has 5-6 pores per mm, while the pores are round and shallow and the colour of the pore surface is pale creamy-grey.

d. The context and dissepiments.

The context of basidiocarps of *C. pubescens* is white, tough and fibrous and, in applanate forms, may range up to 15 mm thick (Figures 29, 30, 31). It tapers in thickness towards the growing margin. The tubes range up to 10 mm in depth and are shortest at the margin. Ungulate forms may have a context several centimeters thick, but the tube length is usually less than 10 mm. The context of basidiocarps of *C. hirsutus* ranges in thickness from a maximum of 14 mm (Figure 32) to a minimum of 1.5 mm (Figure 33), but in typical forms it is 2-5 mm thick (Figures 34, 35) and the tubes are 1-2 mm long. For comparison, the context of the Swedish basidiocarps of *C. hirsutus* (Figure 36) is 2.5 mm thick while the tubes are 2.5 mm long, measurements being taken at a point one third in from the margin.

e. Herbarium material.

Studies of herbarium material provided some information on the macrostructure of basidiocarps and also yielded information on both type of substrate and geographic distribution of these fungi. The herbarium collections were examined at a stage in this study when a good knowledge of the characteristics of each species had been gained and herbarium material could consequently be identified with confidence. It was discovered that many of the herbarium specimens had been incorrectly identified and both the listed and correct names are given in Appendix I.

f. Distribution and substrate preference.

Information regarding substrates utilized by *C. hirsutus*, *C. pubescens* and *C. versicolor* is summarized in Table 1, while the geographic distribution of the examined collections of these species is given in Table 2. Of particular interest is the fact that none of the collections of *C. pubescens* occurred on *Populus*, the most common substrate for this species being *Betula*. *C. hirsutus* was most commonly recorded on *Populus*, but was also often found on *Betula*. *C. versicolor* appears to utilize *Fagus* and *Acer* most frequently, but also occurs on *Betula* and *Populus*. With regard to geographic distribution, it is important to note that *C. versicolor* was not found in Alberta or Saskatchewan, although it has been noted in Ontario, Manitoba and British Columbia.

g. Basidiocarp descriptions.

The following descriptions of macroscopic characters of *C. pubescens* and *C. hirsutus* have been compiled from studies of fresh collections.

C. pubescens.

Basidiocarp coriaceous and watery to tough and rigid, sessile or effused-reflexed; pileus 2.5-5.5 x 4-10 x 0.5-1.7(3.5) cm, dimidiate, applanate to ungulate, often imbricate; surface azonate, pallid to golden yellow, velutinous or villose-tomentose to appressed tomentose, often radiate striate; margin entire, at first broadly blunt, later acute; pore surface even and plane, pallid to creamy yellow; pores 3-4 per mm, angular; dissepiments thin, later becoming irpiciform; tubes 2-10 mm deep.

C. hirsutus.

Basidiocarp coriaceous, tough, drying rigid, sessile or effused-reflexed; pileus 1.5-4 x 3-8 x 0.2-0.5 (1.6) cm, dimidiate, applanate, occasionally rosulate or somewhat ungulate, often imbricate; surface concentrically zonate, golden brown to umber, velutinous to minutely pubescent with occasional hirsute patches at base; margin entire, at first blunt and sterile, later acute; pore surface even, plane, initially pallid but quickly becoming light smoky to dark smoky, pores 3-4 per mm, initially round, later angular; dissepiments thick, becoming thinner with age; tubes 1-2 mm deep.

DISCUSSION

The macroscopic descriptions for *C. pubescens* and *C. hirsutus*, formulated by this study, differ in a number of respects from other published descriptions. This is particularly noticeable in measurements of basidiocarp size. Most authors quote two figures for each dimension, these being the minimum and maximum size for the particular species. The minimum size, of course, depends greatly on the age of the basidiocarp and will therefore reflect the length of time it has been growing. It is possible, by collecting early in the growing season, which in Alberta begins in late June, to find basidiocarps of very small size. The minimum figures given in this thesis represent the smallest size of basidiocarp which can be readily and confidently identified. The maximum figure should indicate the largest size normally attained by a particular species. Collections made towards the end of the growing season will likely approach this maximum dimension.

The results from this study show that the range of basidiocarp thickness for both *C. pubescens* and *C. hirsutus* is much greater than previous work would indicate. The tissues of context and dissepiments constitute the bulk of a basidiocarp and it is their combined measurement which is taken as basidiocarp thickness. The ungulate type of basidiocarp of *C. pubescens*, with its thick context, has not been previously described and consequently the range of

basidiocarp size of this species must be extended to include it. Since the ungulate type occurs in the same area as the applanate type, and especially, as they have been shown to be interfertile (see Chapter IV), the ungulate type should not be given the subspecific rank of variety or form. It should be included within the normal range of variation of this species. Variation in basidiocarp macrostructure results from an interaction of the developing basidiocarp with its environment. Plunkett (1956) has shown that, in pure culture, the development and final shape of basidiocarps of *Collybia velutipes* and *Polyporus brumalis* depend upon the interaction of aeration, humidity and light. In the study area, field observations have shown that applanate basidiocarps of *C. pubescens* are invariably found under shaded conditions where radiation intensity is low and humidity is high, whereas ungulate basidiocarps are always found in exposed habitats where radiation intensity is high and humidity low. There is a need for critical studies under natural conditions to correlate basidiocarp development and habitat to resolve this problem.

Ungulate basidiocarps of *C. hirsutus* have also been found, but they are much less pronounced than those of *C. pubescens*. Two reasons are responsible for this difference. Firstly, basidiocarps of *C. pubescens* are inherently larger than those of *C. hirsutus*, particularly in relation to thickness. Secondly, basidiocarps of *C. hirsutus* are found in habitats less exposed to drying

than those of *C. pubescens*. Basidiocarps of *C. hirsutus* are seldom found more than two meters above ground level and are usually in somewhat shaded habitats. Those of *C. pubescens* are frequently found five to six meters above ground level on an isolated dead tree where they are very exposed. In the study area, *C. pubescens* was primarily found on *Betula papyrifera*, while *C. hirsutus* was primarily found on decaying trees of *Populus tremuloides* and *P. balsamifera*. There are important differences between *Betula* and *Populus* which are reflected in the ability of wood decay fungi to colonize them. The bark of dead *Populus* trees becomes cracked and detached with the result that standing dead trees dry out and consequently the upper portions of these trees become an unsuitable habitat for the development of wood decay fungi. The lower portions of these trees remain moist, since they are in contact with the soil, and they can be utilized by wood decay fungi. In contrast, the bark of *Betula papyrifera* is extremely durable and is retained on dead trees, where it acts as a moisture barrier, preventing the upper portions of these trees from drying out. This situation, coupled with the fact that *Betula* trees are frequently rooted in areas with a high water table, means that there is ample moisture in the upper portions of these trees for the development of wood decay fungi. Consequently, *C. pubescens* is frequently found fruiting in these exposed habitats. This species appears to prefer substrates with a high moisture content, a fact expressed in the watery

condition of its basidiocarp, and probably is unable to develop on substrates such as dead *Populus*, which becomes low in moisture. *C. hirsutus* appears from field observations to have the ability to develop and decay wood of lower moisture content than *C. pubescens* and is therefore able to utilize wood of *Populus* spp. The significant fact that no specimen of *C. pubescens* was found on dead *Populus*, appears due to the substrate moisture requirements of this species.

Some characters used in descriptions of basidiocarp macrostructure change with increasing development of the basidiocarp. The pores of *C. hirsutus* are initially round, but as the dissepiments grow downwards, becoming thinner as they do so, the pores become correspondingly larger and irregular. The pore surface of a basidiocarp collected at the end of the growing season will consequently differ considerably from one collected early in the year. Similarly, the pores of *C. pubescens* are initially subround to angular, but, as the basidiocarp reaches full development, the dissepiments break up into teeth (irpiciform). Dissepiments of *C. hirsutus* do not become irpiciform, an important point of difference between it and *Cerrena unicolor*, with which it is confused. The sterile margin around the pore field is another character which depends on the degree of development of the basidiocarp. Young basidiocarps have a wide, sterile and blunt margin, since this is the area where growth by cell elongation is taking place (see Chapter III). As full development is reached the margin

becomes acute and the pores develop to the edge of the pore field. The coloration of the pore surface is in part dependent on basidiocarp age. Well-developed basidiocarps of *C. hirsutus* always have a smoky-colored pore field, a character of great importance in separating this species from *C. pubescens* which never has a smoky-colored pore field. Very young basidiocarps of *C. hirsutus*, particularly if growing in shaded conditions, may have a pallid pore surface similar to that of *C. pubescens*. It is of utmost importance to take the degree of development of a basidiocarp into account when making decisions as to its identity.

One other point worth noting is the too frequent occurrence of weathered basidiocarps in a herbarium. Full grown specimens are found in herbaria with a collection date of May or June given. These specimens have invariably developed during the preceeding summer and have withstood the ravages of at least one winter before being collected. They are virtually impossible to identify with any degree of confidence and only serve to confuse the taxonomy of this group. Such weathered and bleached specimens are better not collected.

With regard to geographic distribution, no specimens of *C. versicolor* have been found in Alberta. Schrenk and Spaulding (1909) have earlier noted that *C. versicolor* is widespread in areas of humid climate. This species is abundant in British Columbia and Ontario and is found also in Manitoba. Although reported to occur in Alberta, it

appears that collections listed as *C. versicolor* from this province have been incorrectly identified. This species may well be confined to areas of high humidity and be unable to establish itself in dryer areas. Factors other than humidity may be involved, the extreme winter temperatures experienced in Alberta may play a part in controlling the distribution of *C. versicolor*.

The herbarium studies have shown that the names *C. zonatus* and *C. velutinus* have been applied by other workers to collections which are undoubtedly of *C. hirsutus*. No collection of either *C. zonatus* or *C. velutinus* has been examined which does not fall within the range of variation of *C. hirsutus*. The evidence suggests therefore that these three taxa are one and the same.

TABLE 1. Substrates utilized by collections of *C. pubescens*, *C. hirsutus* and *C. versicolor*.

Substrate	*C. pubescens*	*C. hirsutus*	*C. versicolor*
Betula	111	28	2
Populus	0	110	1
Acer	5	5	4
Fagus	0	2	7
Alnus	4	0	0
Carya	0	2	0
Quercus	1	1	0
Pyrus	0	1	0
Prunus	1	1	0
Malus	1	1	0
Fraxinus	0	0	1
Sorbus	0	1	0
Ulmus	0	0	2
Salix	0	1	0
Picea	0	1	1

TABLE 2. The Location of Examined Collections of *C. pubescens*, *C. hirsutus* and *C. versicolor*.

Area	*C. pubescens*	*C. hirsutus*	*C. versicolor*
Alberta	107	121	0
Saskatchewan	8	9	0
British Columbia	0	3	3
Manitoba	2	11	2
Ontario	4	1	4
Quebec	0	0	1
Nova Scotia	0	3	9
Massachusetts	0	9	4
Iowa	0	3	3
Minnesota	0	1	1
Arizona	0	2	0
New York	3	1	1
Colorado	0	0	1
Texas	0	0	1
Hawaii	0	0	3
China	0	0	1

FIGURES 1 - 4. *C. pubescens.*

Figure 1. Applanate, effused-reflexed basidiocarps. #300, X 0.75.

Figure 2. Developing basidiocarps. Note blunt round margin. #122, X 1.0.

Figure 3. Fully developed basidiocarp. Note acute margin. #156, X 0.75.

Figure 4. Developing imbricate basidiocarps which have become confluent. #131, X 1.0.

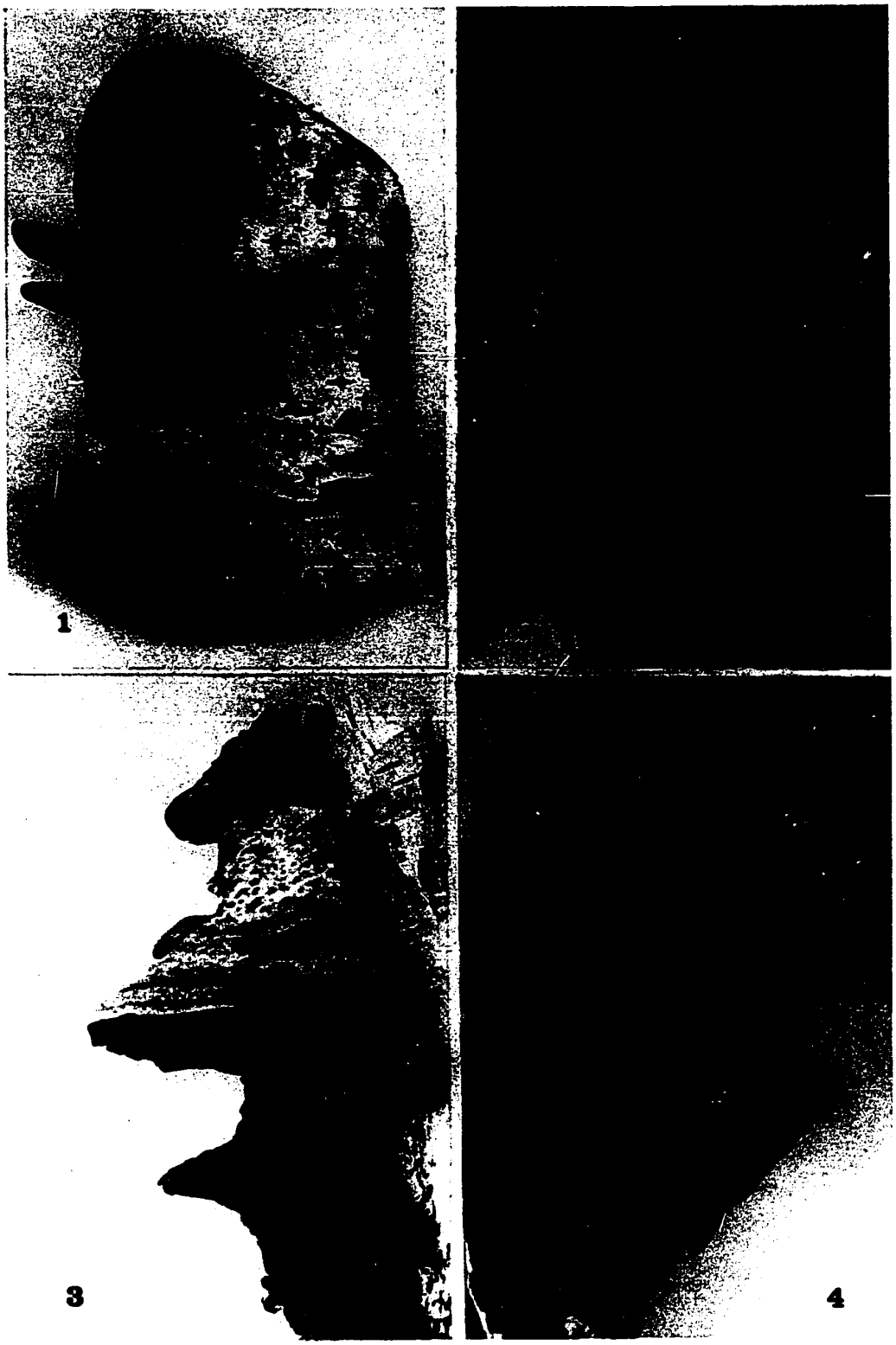
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FIGURE 5. *C. pubescens*.

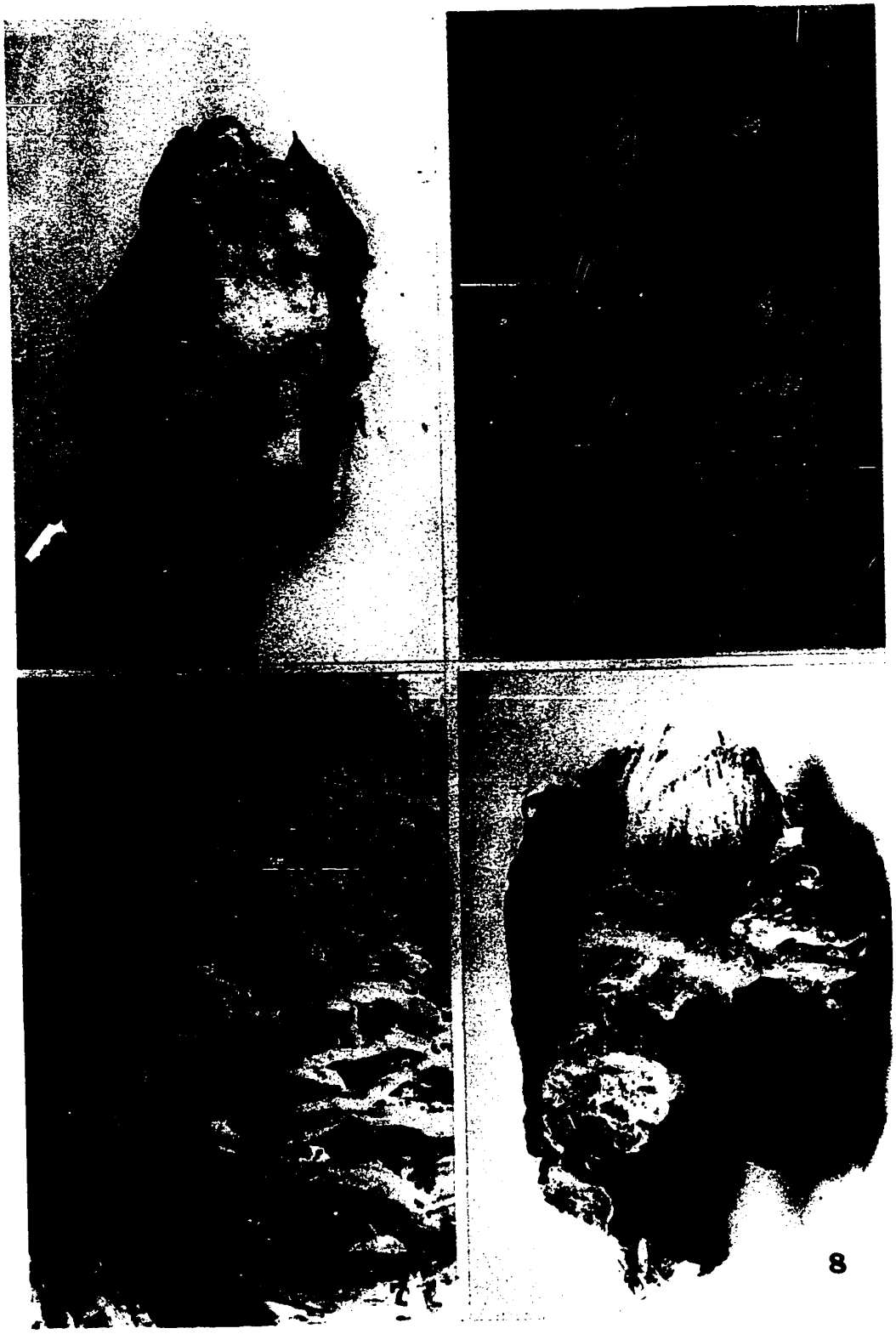
FIGURES 6 - 8. *C. hirsutus*.

Figure 5. Ungulate basidiocarp. #237, X 1.3.

Figure 6. Developing applanate basidiocarps. #389, X 1.0.

Figure 7. Imbricate basidiocarps. #157, X 0.9.

Figure 8. Thickened basidiocarps on *Populus*. #207, X 0.75.



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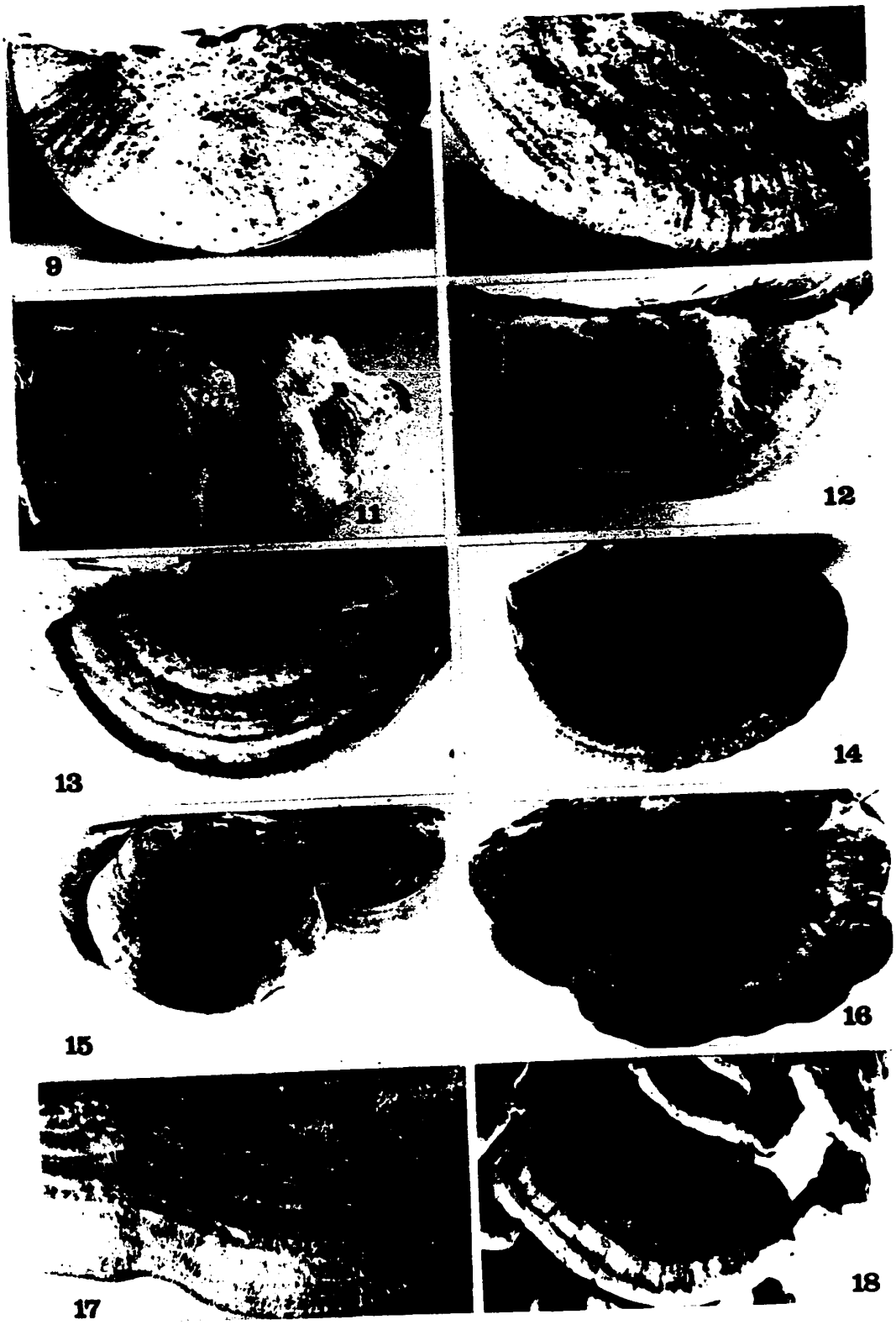
FIGURES 9 - 11. Variation in upper surface characters of applanate basidiocarps of *C. pubescens*. Figure 9, #300, X 1.25. Figure 10, #156, X 1.0. Figure 11, #261, X 1.4.

FIGURES 12 - 16. Variation in upper surface characters of basidiocarps of *C. hirsutus*. Figure 12, #209, X 1.4. Figure 13, #271, X 1.5. Figure 14, #194, X 1.5. Figure 15, # 290, X 1.7. Figure 16, #208, X 1.8.

FIGURE 17. Upper surface of Swedish basidiocarp of *C. hirsutus*. Note degree of pubescence. #422, X 1.1.

FIGURE 18. Upper surface of basidiocarps of *C. versicolor*. #415, X 2.0.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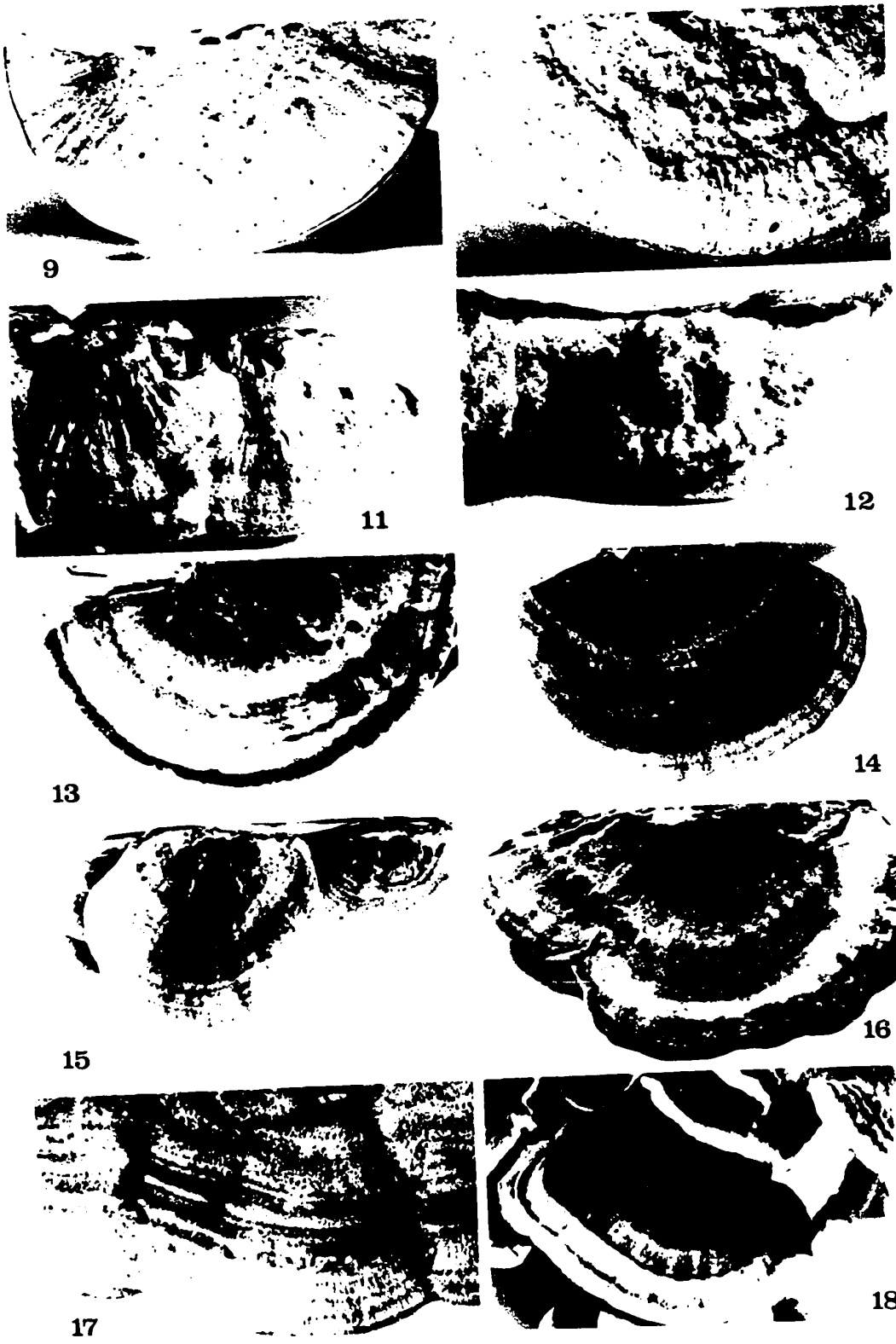
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FIGURES 19 - 22. Variation in pore surface of basidiocarps of *C. pubescens*.

Figure 19. Developing basidiocarp. #261, X 1.4.

Figure 20. Developing basidiocarp. Note sterile margin. #300, X 2.0.

Figure 21. Developing ungulate basidiocarp. Note sterile margin. #237, X 2.0.

Figure 22. Fully developed basidiocarp. Note acute margin and irpiciform dissepiments. #156, X 1.3.

FIGURES 23 - 26. Variation in pore surface of basidiocarps of *C. hirsutus*.

Figure 23. Young basidiocarp. Note round pores. #290, X 2.8.

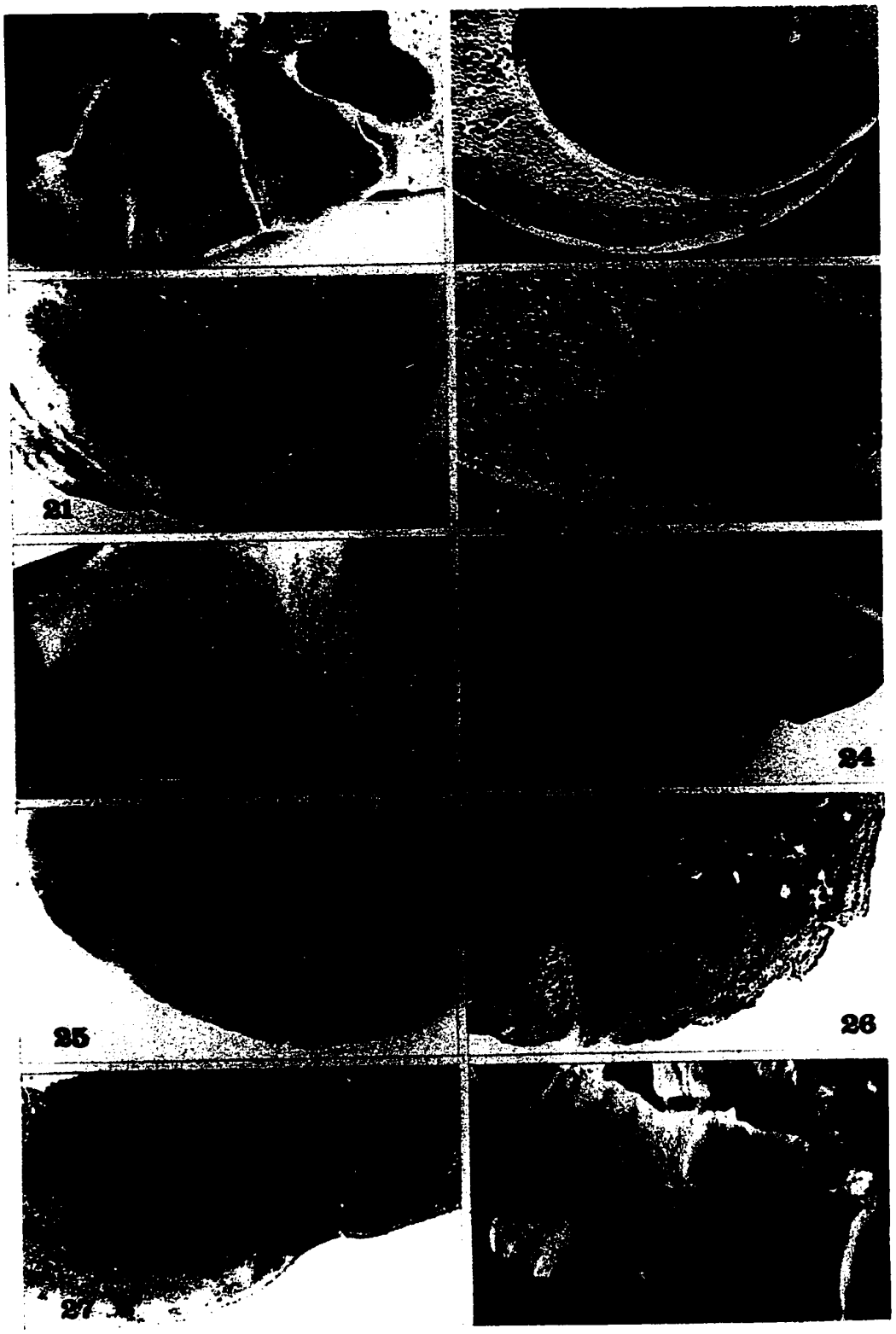
Figure 24. Developing basidiocarp. Note sterile margin. #208, X 1.9.

Figure 25. Developing basidiocarp. Note pores larger and sterile margin narrower. #209, X 1.3.

Figure 26. Fully developed basidiocarp. Note irregular pores and acute margin. #299, X 1.8.

FIGURE 27. Pore surface of Swedish basidiocarp of *C. hirsutus*. #422, X 1.2.

FIGURE 28. Pore surface of basidiocarp of *C. versicolor*. #415, X 2.5.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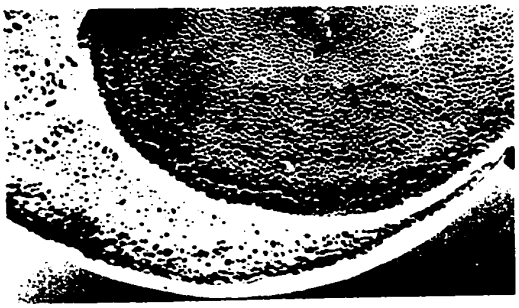

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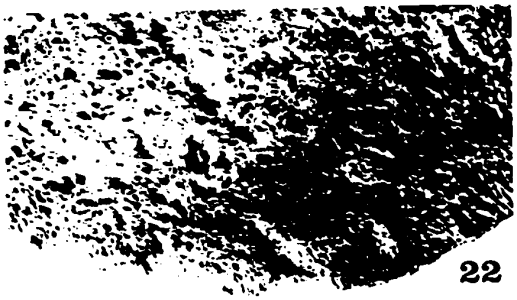

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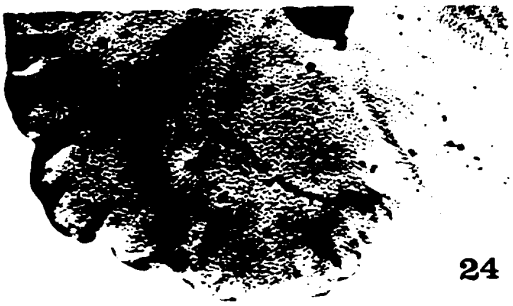

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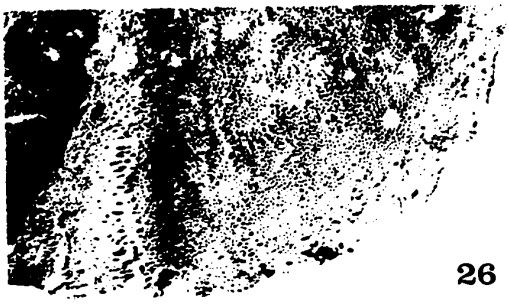

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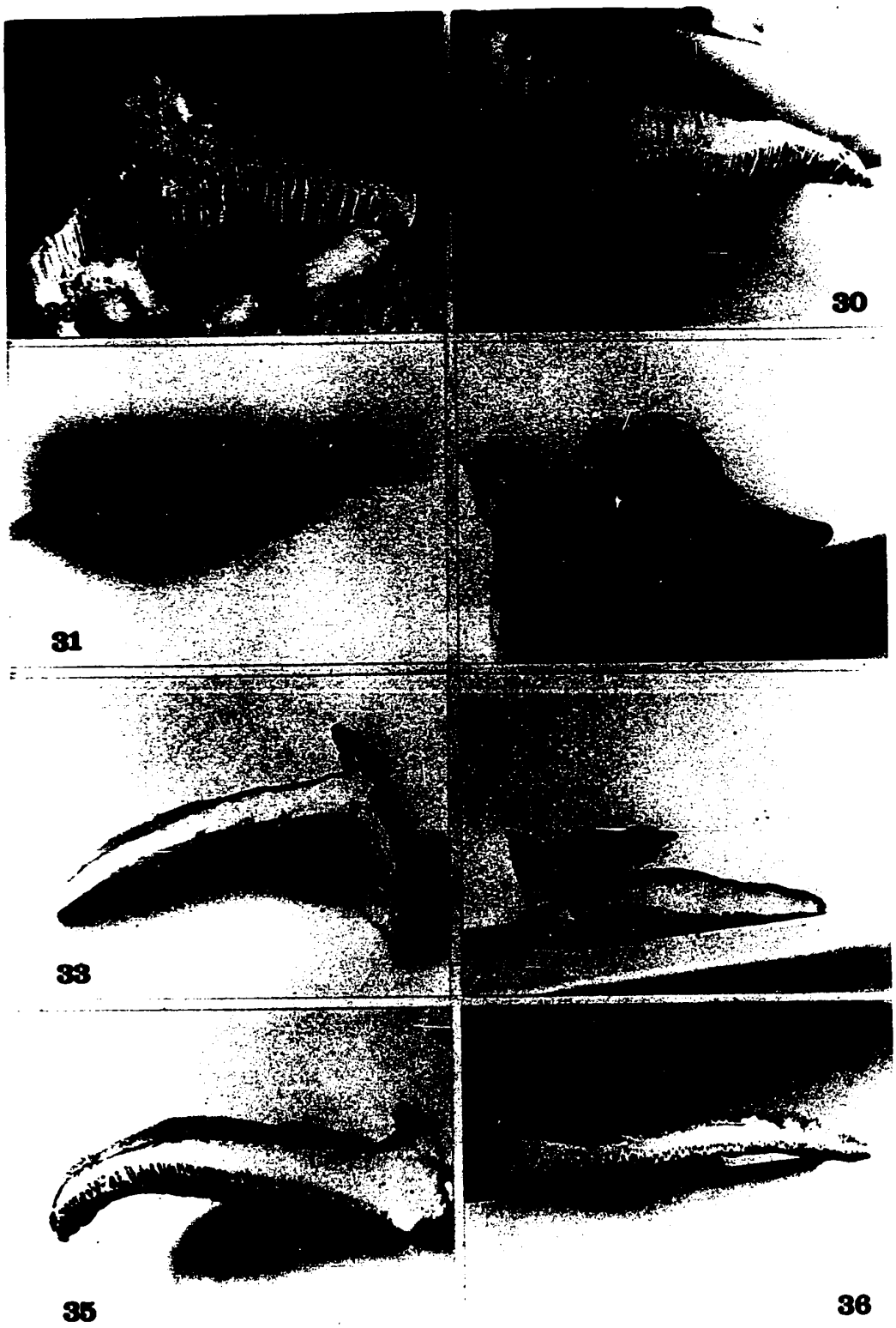
FIGURES 29 - 31. Vertical radial section through basidiocarps of *C. pubescens*. Note variation in context thickness.
Figure 29, #261, X 2.3. Figure 30, #156, X 1.5. Figure 31, #261, X 2.0.

FIGURES 32 - 35. Vertical radial section through basidiocarps of *C. hirsutus*. Note variation in context thickness and dissepiment depth.
Figure 32, #209, X 1.8. Figure 33, #290, X 3.1. Figure 34, #296, X 2.0. Figure 35, #299, X 2.6.

FIGURE 36. Vertical radial section through Swedish basidiocarp of *C. hirsutus*. Note trichoderm. #422, X 1.3.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CHAPTER III

BASIDIOCARP MICROSTRUCTURE

INTRODUCTION

Few detailed descriptions of the microstructure of the basidiocarps of species of the Polyporaceae based on the examination of whole basidiocarp elements have been published. Most descriptions are illustrated with fragments of hyphae which provide no information on the ontogeny or mature form of the hyphal elements. The difficult and time consuming work involved in determining the sequence of hyphal development is responsible for this lack of information. The problem of extracting complete unbroken elements from the tissue is almost insurmountable because of the complex structure of the basidiocarp which is usually described in taxonomic works as "composed of interwoven hyphae".

The first thorough investigation into polypore microstructure was made by Corner who, in 1932, published two classical papers wherein he described in detail the anatomy of two species, *Polystictus xanthopus* and *Fomes levigatus*. In his first paper Corner (1932a) demonstrated that the basidiocarp of *P. xanthopus* was characteristically composed of three series of hyphae which he termed generative hyphae, skeletal hyphae and binding hyphae. He described generative hyphae as follows, "thin-walled, branched, septate, longitudinal or interwoven, 1.5-2.5 μ wide, rarely 3 μ, with a clamp at each septum and abundant protoplasmic contents

throughout, the cells 30-80 μ long with 0-4 branches from the distal end, occasionally from the middle or proximal end; H-connections scarce". Skeletal hyphae were described as follows, "thick-walled, unbranched, aseptate, straight or slightly flexuous, longitudinal, 2-5 μ wide, with the lumen more or less obliterated in mature parts, but the apices thin-walled with dense contents". Binding hyphae were described as follows, "thick-walled, much branched, aseptate, interwoven, narrow, 1-2.5 μ wide, rarely 3 μ, with the lumen linear or obliterated in mature parts, often coralloid and flattened, kinked, nodulose or spiculiferous, as if from mutual pressure and abortive branching, with the apices thin-walled or thick-walled, aseptate, and with scant contents in mature parts like the skeletal hyphae; H-connections scarce". In addition, Corner described a fourth series termed mediate hyphae as follows, "slightly thick-walled, sparingly or frequently branched, aseptate, flexuous, longitudinal or somewhat interwoven, 1.5-3 μ wide, with the walls 0.5-1 μ thick, with scant contents like the skeletal hyphae and the apices thin-walled and aseptate". He reported that the generative hyphae gave rise to the skeletal hyphae, which built the framework, and to the binding hyphae which tied the structure together. In his second paper Corner (1932b) proposed the terms 'trimitic' to describe a basidiocarp with generative, skeletal and binding hyphae; 'dimitic' to describe one with generative and either skeletal or binding hyphae and 'monomitic' to

describe one with only generative hyphae.

This work was confirmed by Cunningham (1947, 1954, 1965) who, after extensive research, proposed a classification system for polypores based on this concept of mitic systems. This classification was revised and published in its final form in 1965 in "The Polyporaceae of New Zealand" (1965). In this publication *Coriolus hirsutus* (as *Trametes hirsuta*) is described as being trimitic. The closely related species *Coriolus pubescens* is not included in this work as it apparently does not occur in New Zealand, although the related taxa *C. versicolor*, *C. zonatus* and *C. velutinus*, all of which are trimitic, are included.

The polymorphic nature of basidiocarps of the Polyporaceae has prevented the development of a stable classification for this group. The usual macroscopic characters such as the degree of pubescence, zonation and colour are not reliable taxonomic criteria. They are subjective and their expression in any basidiocarp is influenced by the environmental conditions under which the basidiocarp is developing.

The five taxa, *C. hirsutus*, *C. pubescens*, *C. versicolor*, *C. zonatus* and *C. velutinus*, constitute a species complex, each taxon being given species rank by some authors. Each taxon has a typical form to which the specific epithet is attached. Since the macroscopic variation in each taxon is considerable, one taxon grades into another and the limits of each are poorly defined. Each author has arbitrarily

drawn a line between these taxa according to his own convictions. Consequently there has been little agreement as to the number of species, varieties and forms in this complex.

This type of problem can best be resolved by interfertility tests between taxa to determine the number of distinct species in this group and, at the same time, provide information on the macroscopic variation within each species. Since interfertility tests can be used to prove or disprove the conspecificity of two taxa, the results of this type of study are unequivocal and not subject to different interpretation by other workers.

As all five taxa have been reported from Alberta (Lowe and Gilbertson, 1961), there is a plentiful local supply of material for a study to determine the number of distinct species in this area.

The microstructure of basidiocarps of *C. hirsutus* *C. pubescens* is poorly described in current taxonomic works and has not been investigated in detail. A study of the microstructure was therefore undertaken to provide needed taxonomic criteria which are more stable than the usual macroscopic ones. The development of various hyphal elements in trimitic basidiocarps has been described previously for only one species, *Polystictus xanthopus*. This species has a stipitate basidiocarp and is restricted to the tropics. Both *C. hirsutus* and *C. pubescens* are trimitic, but they have sessile basidiocarps and their

distribution is considered to be cosmopolitan. Their distribution and abundance provided an opportunity to work out the development of this trimitic type of basidiocarp and to assess the stability of microscopic characters. It was also possible to compare their development with that of trimitic species from different genera.

MATERIALS AND METHODS

The collections of basidiocarps used in this study were gathered during 1968-1971 and are listed in the appendix. In addition, herbarium material from several sources was examined. Many of the Alberta collections could not be readily identified and appeared to be intermediate between *C. hirsutus* and *C. pubescens*. These intermediate types could only be identified by compatible mating experiments using single spore cultures. The single spore cultures were made from typical basidiocarps of each species by gluing a small piece of fresh basidiocarp with a rubber based cement to the underside of the lid of a sterile petri plate containing Nobles malt agar (Nobles, 1965). After the cement had dried (1-2 hours), the basidiocarp tissue was moistened with sterile distilled water and left. Spores were discharged onto the agar and, by periodically turning the lid, the basidiocarp tissue, which had not been glued centrally to it, could be moved, thus spreading the spore deposit over the agar and not confining it to one small area. The spores germinated immediately and sporelings

were picked out with a needle honed to a flat chisel point under a Bausch and Lomb Stereo-Zoom microscope fitted with a 2X supplementary lens (total magnification X60). The sporelings were transferred to plates of Nobles agar which was the standard medium used throughout this study. This medium contained 12.5 gm Difco Bacto malt extract and 20.0 gm Difco Bacto-Agar per litre of distilled water.

Intermediate basidiocarps and basidiocarps of uncertain affinity were identified by growing single spore cultures from them and pairing these with the single spore cultures obtained from typical basidiocarps. The formation of a dikaryotic mycelium, easily recognized by the formation of clamp connections, was considered to prove conspecificity of the test culture with the known culture. This concept has been used by other workers (Nobles, 1943; Mounce and Macrae, 1938; Denyer, 1960) and allowed the positive identification of the materials used for basidiocarp analysis. Many pairings were made between monospore cultures of *C. hirsutus* and monospore cultures of *C. pubescens* and, in no case, was a dikaryotic mycelium formed. These pairings will be discussed in more detail in another chapter.

Several techniques for basidiocarp analysis were tried (Cunningham, 1947; Teixeira, 1960, 1962), but it was found that the following method yielded the greatest amount of information. The basidiocarp was first cut vertically from the growing margin to the point of attachment in order to

divide it into two halves. Thin sections of about 100 μ thickness were sliced freehand from the exposed surface. Small pieces of tissue, approximately 1-2 mm square, were cut from various positions in the section. Since the relative position of each square of tissue was known, it was possible, by starting at the growing margin and working back into the context, to gain an understanding of the ontogeny of the structural elements of the basidiocarp.

Each square of tissue was placed on a separate slide and a drop of 5% aqueous KOH solution added. Two methods were used to separate the basidiocarp tissue. The tissue was teased apart with fine needles under the binocular dissecting microscope or it was gently tapped with the blunt end of a glass rod which caused the hyphae to separate. The second method was preferred since the first one resulted in the hyphae becoming clumped. The advantage of carrying out this procedure in the KOH solution is that, in this solution, the hyphae swell and regain their normal size and also the lubricative quality of the KOH solution helps in disentangling and separating the hyphae. It may be noted here that a novel method of separating basidiocarp hyphae had been reported by Fidalgo (1967) who, by exposing the tissue to an ultrasonic generator, was able to extract intact skeletal elements which measured from 1.5-2 mm in length.

A drop of 1% filtered aqueous phloxine and a drop of 0.5% Congo red were added to the slide with the dissected

tissue in KOH and mixed. After ten minutes, a coverslip was added and the preparation pressed between paper towels and examined. The ten minute waiting period may be replaced by gently heating the slide over an alcohol flame. The Congo red stain consisted of 0.1 gm Congo red in 20 ml distilled water with 4-5 drops of 95% alcohol added to dissolve the stain. This combination of KOH-phloxine-Congo red was developed independently, although it has since been discovered that Wells (1969) used a modified combination to stain basidiocarp tissue of the Tremellales. The KOH-phloxine method was first reported by Martin (1934). Preparations made by the method described above are temporary, but some success was achieved in converting them to permanent mounts by a rather drastic process which involved rinsing the dissected and stained tissue in absolute alcohol, clearing them in xylene and mounting in Canada balsam. These permanent preparations were not as good as the temporary ones as many of the thin-walled hyphae had collapsed. Freehand sections of basidiocarp tissue were studied by staining with KOH-phloxine-Congo red and using as temporary preparations or by staining with safranin-picroaniline blue and using as permanent preparations. These freehand sections yielded little information since it was impossible to find complete skeletal or binding elements in them.

All line drawings shown at the end of this chapter were made with the aid of a camera lucida.

OBSERVATIONS

Microstructure of *C. hirsutus*

1. The primordium

The vegetative mycelium develops within the substrate and is responsible for the degradation of the wood. The first indication of fruiting is the appearance of small, white, hemispherical, mycelial pads which develop in small cracks and fissures in the bark or on exposed wood surfaces. These mycelial pads are primordia or basidiocarp initials. Each primordium increases uniformly in size, retaining its hemispherical shape until it is 2-4 mm in diameter. At this stage differentiation of upper and lower surfaces has not occurred and the whole surface may become golden brown in colour. The generative hyphae of the very young primordium develop from the vegetative mycelium and are continuous with it. The actual point of change from the vegetative hyphae to the generative hyphae cannot be determined by observation. The trimitic hyphal system which characterizes the basidiocarps of this species can be observed in the primordium. The generative hyphae are thin-walled, 2.5-3.5 μ in diameter, branched and bear clamp connections at each septum. The skeletal hyphae are 1-2 mm long, 4-6 μ in diameter, thick-walled, aseptate elements with two or three branches at their distal ends. The binding hyphae are short, much branched, contorted, thick-walled, aseptate elements, 3-4 μ in diameter. In the

primordium the generative and skeletal hyphae are radially arranged, appearing to fan out from a central point.

2. The young basidiocarp

The primordium continues to grow in a horizontal plane with the result that a flattened semi-circular structure is formed (Figure 37). Growth continues to be fastest in the horizontal plane and consequently the flattened shape is maintained. The pore field develops by the initiation and downward growth of the dissepiments, *i.e.* the walls between the pores. New dissepiments are continually being formed beneath and behind the horizontal margin as it extends. Little growth takes place in an upward direction from the basidiocarp surface. The primordium develops into a mature, sporulating basidiocarp which continues to increase in size until the end of the growing season.

3. The mature basidiocarp

a. The marginal region.

Growth of the basidiocarp in a horizontal plane takes place by elongation of marginal hyphae. The growing margin consists of tightly packed, thin-walled, hyphal tips, 4-8 μ in diameter (Figure 38). These hyphal tips are the growing ends of marginal cells which are destined to become skeletal elements. Some marginal cells are quite short, being no more than 50 μ long, while other marginal cells can be traced back from the margin for 1-2 mm before a clamp connection is reached. The margin therefore,

consists of cylindrical, unbranched cells of different lengths, each with a thin-walled growing region at its distal end. Although these short marginal cells are thin-walled, the walls of the longer marginal cells are partially thickened by a process which takes place in a particular manner. As a marginal cell grows by apical extension, the cell wall remains thin until the cell has reached a length of 200-500 μ. The cell wall in a region about one third of this distance from the tip begins to thicken and this thickening progresses towards the growing cell tip at approximately the same rate as the tip is extending. This progressive thickening never catches up with the growing tip but always lags some distance behind. Cell wall thickening has not been observed at the proximal ends of these elongate marginal cells. A typical elongate marginal cell is shown in Figure 39. It has a total length of about 700 μ, a distal thin-walled growing tip and a proximal thin-walled region. The remainder of the cell has a wall of variable thickness, the wall tapering in thickness towards each end of the cell. The cell wall in the middle of the cell may become so thickened that the lumen of the cell becomes capillary.

The marginal cells are of limited growth and, as their maximum length, which may be in excess of 2 mm is reached, their apical growth rate slows down and the cell tip falls behind into the submarginal region. This slowing down of apical growth rate may be due to the restriction of

nutrient flow to the apical region as a result of the thickened walls and capillary lumen which develop in the central region of the cell. One or two branch initials appear near the cell apex (Figure 38) and develop into branches. These branches continue to grow, but at a slower rate, and as they are in a submarginal position, they have to grow intrusively between other cells. This intrusive growth of the branches results in an uneven appearance. As the branches grow, they gradually become narrower in diameter and the wall thickening process begins to catch up with the branch apices. Finally, the branches cease growth and wall thickening continues until the lumina become capillary right to the branch tips (Figure 41). The cell wall at the proximal end of the cell does not become thickened.

This differentiated, single cell structure which originates as a terminal cell at the margin of the basidiocarp is a skeletal element. Each skeletal element has a definite and limited type of growth and the growing apices of developing skeletal elements are the sole constituents of the growing margin. At their proximal ends they are attached to a thin-walled, generative cell by a clamp connection. The proximal regions of the skeletal elements always remain thin-walled and may be several hundred microns long, while the overall length of a skeletal element is 2 mm or more. The diameter of a skeletal element is least at its proximal end, where it is the same diameter as the

generative cell from which it has developed. Its diameter increases to a maximum of 6-8 μ at the point of branching at the distal end. The term skeletal element is used in preference to skeletal hypha since each skeletal element is formed by differentiation of a single terminal cell and is therefore aseptate.

The generative cells from which the skeletal elements develop are in a subterminal position. New hyphal tips which arise from the subterminal, generative cells are destined to become skeletal elements. New generative cells may be delimited before the skeletal elements are differentiated. This process is illustrated in Figure 40, where Figure 40A shows a hyphal tip dissected from the growing margin. This new tip will become the distal end of a skeletal element. A clamp connection which will delimit a subterminal, generative cell (arrow) is being initiated and is shown fully developed in Figure 40B. The terminal cell has elongated and the subterminal, generative cell has initiated a new growing apex (Figure 40C). This new apex will grow into the marginal area and eventually become a skeletal element. As it grows, a clamp connection is formed to cut off the new terminal cell from the original generative cell. A second clamp connection may be formed later delimiting a new generative cell as shown in Figure 40D. The new hyphal tip shows rapid growth at first, catching up with the other hyphal tips of the margin. When fully differentiated, the distal end of a typical skeletal element

usually has two branches, each less than 100 μ long (Figure 41). The branches are often unequal, one being the original cell apex and the other branch arising as a new growing point just behind the original apex (Figure 38).

b. The context.

The binding elements develop deeper in the context at a distance of at least 500 μ from the basidiocarp margin. Here, generative cells, which were formed earlier, are producing branches by initiation of new growing points. These new growing points develop into apices which grow forward towards the margin. Clamped septa form close to the parent generative cells, cutting off the new terminal cells with their growing apices. Each terminal cell continues to grow and begins to branch by initiating more new growing points (Figure 44). It is these terminal cells which differentiate into binding elements. Since each binding element is developing intrusively between the maturing skeletal elements, its branches have to grow by weaving around and between these skeletal elements, consequently they become contorted. Before each binding element is fully developed, cell wall thickening begins at its proximal end and progresses towards the branch apices which eventually become completely thick-walled with capillary lumina. Binding elements may be initiated at any point in the context, with the exception of the marginal region, but they are predominately formed in the region 0.5-2 mm from the margin. Their position in the context during development

has a great influence on their final form. New terminal cells develop from generative cells at various distances from the growing margin, those that develop close to the margin become a part of the margin and eventually become skeletal elements. Those that develop a little further from the margin may be unable to grow fast enough to become part of the margin as their growth in this position is somewhat restricted. Since they are growing intrusively between the submarginal cells, they will branch and become binding elements. This type of binding element is characterized by a relatively long, unbranched, proximal region and a sparsely branched, distal region (Figure 45). In many cases they look like short versions of skeletal elements but, unlike the skeletal elements, they usually become completely thick-walled at their proximal ends. Binding elements which develop deeper in the context are much more restricted in their growth and consequently have a short unbranched proximal region (Figure 43). Their branches are short, contorted and enlarged at the ends. This type of binding element gives the context a dense and more compact quality and appears characteristic of *C. hirsutus*.

The Congo red stain has a particular affinity for the walls of binding elements which readily take up the stain at all stages of growth. Skeletal elements are weakly stained by Congo red unless exposed to the stain for an excessive period of time. The phloxine stain is taken up by the cytoplasm of all cells.

The generative cells in the submarginal region are thin-walled but, in older parts of the context, wall thickening does take place and may even progress to the point where the cell lumen becomes capillary. The clamp connections also become thick-walled and frequently irregular swellings appear in these generative cells as their walls become thickened. These swellings readily stain with Congo red and a capillary lumen is usually visible through them (Figure 52).

c. The trichoderm.

The growing margin of the basidiocarp consists of the thin-walled ends of developing skeletal elements. Since newly formed apices of immature skeletal elements appear just within the margin and rapidly grow into the marginal zone, the total numbers of elongating cell tips at the margin is continually increasing. Consequently, cell tips above and below the horizontal centre line of growth tend to be displaced upwards and downwards respectively. Only a small proportion are exactly horizontal and on the centre line of growth. These central, horizontal cell tips have the fastest rate of elongation, while the displaced cell tips, which are directed upwards or downwards to varying degrees, depending on their position, grow at a slower rate. The marginal cells which are displaced and directed upwards form the upper surface or trichoderm of the basidiocarp. These upward displaced cell tips grow at a slower rate and do not branch at their distal ends. The walls at the apices

of these cells become partially thickened and the apical contents become coloured with a brown substance which is responsible for the brown pigmentation of the trichoderm. Pigmented cell apices are 5-7 μ in diameter and frequently become cemented together in small groups which protrude up to 400 μ and give the trichoderm its characteristic appearance. Pigmented cell tips can also be found just beneath the surface in the upper region of the context.

d. The dissepiments.

The dissepiments or walls between the pores are composed of an inner layer, the trama, on which the spore-bearing hymenium is borne. Marginal cells which are displaced downwards become vertically aligned to form the dissepiments. The growing edge of a dissepiment is sterile and consists of thin-walled cell apices which will develop into skeletal elements in the same way as the skeletal elements of the basidiocarp margin were formed (Figure 53). Similarly, the binding elements of the dissepiments develop some distance from its growing edge.

The skeletal and binding elements of the dissepiments differ in their mature form from their counterparts in the context. This difference is related to the downward rate of growth of the dissepiments which is considerably less than the horizontal rate of growth of the basidiocarp. Skeletal elements of the dissepiments are shorter in overall length with a much reduced, thin-walled, proximal region and have two or three short branches (Figure 49). Binding elements

which have developed at some distance from the dissepiment edge have swollen branch ends (Figure 47) while those that develop closer to the growing edge of the dissepiments are longer and less branched (Figure 46).

Most of the basidiocarps examined have a smoky-coloured pore surface. This colouration is due to the presence of specialized, pigmented cell apices near the surface of the dissepiments. The pigment is located in the cytoplasm of the apical region which remains thin-walled while the remainder of the cell, with the exception of the extreme proximal region, becomes thick-walled (Figure 50). These pigmented elements appear to be similar to those found in the upper context and trichoderm.

Branches from generative cells in the dissepiments grow outward and form, by repeated branching, a network over the vertical sides of the dissepiments. This network is the subhymenium and consists of short, thin-walled, branched, generative cells. From this subhymenium the basidia develop as a palisade layer of terminal cells. The basidia are clavate, hyaline, 10-15 x 4-5 μ and bear four sterigmata up to 5 μ long, each bearing a spore (Figure 42). The spores are hyaline, cylindrical, with smooth walls and measure 5-7(8)x2-3(3.5) μ. They are one-celled at maturity and uninucleate (Figure 42). The hymenium forms a continuous cylindrical lining to each pore but, as the growing edges of the dissepiments are sterile, the hymenium is discontinuous over the lower surface of the basidiocarp.

SUMMARY

Figure 61 is a simplified, foreshortened, semi-diagramatic representation of the growing margin of the basidiocarp. Terminal cells at the margin form skeletal elements which may become 1-2 mm long before branching distally. The distal, branched ends of two fully-developed, thick-walled skeletal elements are shown in the context and a branching skeletal element is shown in the submarginal region. End cells in the context have developed into thick-walled, binding elements with short proximal regions and contorted branches which have enlarged at their ends. End cells in the context, but closer to the margin, are developing into binding elements which, in this position, have a longer, unbranched, proximal region and are less contorted. Generative cells are shown throughout the context where they form a branched network. They are never found in a terminal position.

Figure 62 represents the growing edge of part of a dissepiment. Again, terminal cells at the growing edge will develop into skeletal elements while end cells in the context develop into binding elements. Similarly, binding cells which develop deeper in the trama are more contorted with thicker branch ends than those that develop closer to the growing edge. The basidia develop as end cells from the generative hyphae which form the subhymenium.

These two figures (61,62) serve to emphasize the point that the basidiocarp is basically constructed of individual

cells. The generative cells are interconnected and form a continuous system. All other specialized cells, binding elements, skeletal elements and basidia, develop as discrete structures from end cells which have been delimited by the generative system.

Microstructure of *C. pubescens*

In the terminology of Corner, the hyphal system in the basidiocarp of *C. pubescens* is trimitic, consisting of a branched generative series, with abundant clamp connections, and discrete skeletal and binding elements. The development of the skeletal and binding elements appears to be essentially similar to that of their counterparts previously described for the basidiocarp of *C. hirsutus*. There are, however, certain differences in microstructure between these two species.

The primordia of *C. pubescens* develop in exactly the same way as those of *C. hirsutus* and appear indistinguishable from them. Similarly, both the pore field and the trichoderm develop in the same manner as those of *C. hirsutus*.

The mature basidiocarp

a. The marginal region.

The growing margin of the basidiocarp of *C. pubescens* is similar to that of *C. hirsutus*, consisting of tightly packed, thin-walled cell tips which are the growing ends of

developing skeletal elements. As the skeletal elements elongate, they become thick-walled and branch at their distal ends. Usually there are at least three branches at the distal ends of the skeletal elements and often four or five branches are formed (Figure 54). The skeletal elements are 5-7(9) μ in diameter and their length often exceeds 2 mm. The proximal end of each skeletal element remains thin-walled (Figure 51), while the branched, distal end becomes thick-walled with a capillary lumen. The remainder of the cell has a wall tapering in thickness to each end. Skeletal elements develop as end cells which are delimited from sub-marginal generative cells and which become part of the growing margin. Generative cells are not found in the marginal zone.

b. The context.

The context consists, in part, of a branched network of generative cells, 2.5-4 μ in diameter which bear clamp connections at each septum. End cells which originate as new growing apices in the context develop into binding elements. The binding elements can be stained readily with Congo red and do not develop within 500 μ of the growing margin. As each binding element develops, many new growing apices are formed and consequently the cell becomes very much branched. Since it is growing around and between other hyphae of the context, it becomes contorted. Wall thickening may begin at the proximal end of the element or it may take place evenly throughout the cell once its full size

and final shape have been attained. Eventually, the whole cell becomes thick-walled and the lumen becomes capillary. The branch ends of the binding elements do not become swollen or enlarged as they do in *C. hirsutus*, but remain isodiametric throughout (Figure 55).

As the growing margin of the basidiocarp consists of end cells which develop into skeletal elements, there must be a process for providing new end cells at the margin, otherwise the number of marginal end cells would become depleted. New marginal cells arise as branches from generative cells which are themselves in a subterminal position to the shorter marginal cells. These branches form end cells which penetrate the marginal zone and become a part of it. Generative cells a little further from the margin also produce branches which become delimited as end cells. These new end cells are too far from the marginal zone to catch up with it, yet they are not deep enough in the context to become true binding elements. Since they are surrounded by cells that have only slightly thickened walls, their growth is little restricted and they develop into an intermediate type of binding element (Figure 48). Typically, they are branched along their length, which may be 200-300 μ, have a completely thickened cell wall at maturity, have a capillary lumen and are 4-5 μ in diameter. Stages of development of these intermediate binding elements can be found 300-500 μ from the margin and mature intermediate binding elements occur throughout the context and dissepiments. They are

especially common in the thicker basidiocarps of this species.

A peculiarity of the skeletal elements, often noticed in the thicker basidiocarps of this species, is the formation of a constriction of the unbranched shaft of the element (Figure 59). This structure appears to be formed as the result of a pause in the growth of the basidiocarp. If the basidiocarp temporarily stops growing due to desiccation, the elongation of the marginal apices of the skeletal elements will cease, but the wall thickening process may not stop and may catch up with the stationary apices. In this way, the walls of the apices of the young skeletal elements may become slightly thickened. When growth resumes, only the very tip of the cell elongates so that the extending cell is now narrower in diameter than previously (Figure 60). This feature appears characteristic of the thicker basidiocarps of this species which are only found in dry microhabitats. The thinner basidiocarps are invariably found in moist microhabitats where they are unlikely to become desiccated.

In the older parts of the context which are more than 1 cm from the margin, generative cells frequently become thick-walled. Blister-like swellings, which readily stain with Congo red, may be observed occasionally on these thick-walled cells.

c. The trichoderm.

The trichoderm develops from upward deflected tips of

the skeletal elements from the marginal region in the same manner as described for *C. hirsutus*. The surface is pubescent and consists of the short, upright, unbranched, distal ends of these modified elements. The surface often has a pale creamy colour which is located in the thickened walls of the cells of the trichoderm and is only apparent when they are viewed collectively. The colour is not seen when individual cells are viewed under the microscope.

d. The dissepiments.

The dissepiments develop by growth from the marginal region of downward deflected hyphae. The growing edge of a dissepiment is essentially similar to the growing margin of the basidiocarp and consists of tightly packed, thin-walled, elongating cells which are generally narrower than those at the basidiocarp margin, measuring 4-5 μ in diameter. The cells which comprise the dissepiment edge develop into skeletal elements which are of slightly smaller magnitude than those of the context, although a similar pattern of branching is found. Binding elements, including the more elongate, intermediate type of binding element, are present in the dissepiments and appear to be indistinguishable from their counterparts in the context (Figures 57, 58). Cell apices with pigmented contents have not been observed in the dissepiments of this species, consequently the pore surface is creamy in colour.

The subhymenium develops as a network of thin-walled, short, branched cells over the sides of the dissepiments

and bears the basidia as a palisade layer. Basidia are clavate, 8-16x4-5 μ and bear four spores (Figure 56). The sterigmata are curved and project up to 5 μ into the pore cavity. The uninucleate spores are similar in shape and size to those of *C. hirsutus*. They are cylindrical, hyaline, with smooth walls and measure 6-8x2.5-3 μ.

SUMMARY

The basic construction of *C. pubescens* is similar to that of *C. hirsutus*, although there are some differences in the structural elements. The generative cells form a branched network which gives rise to discrete end cells which become differentiated into skeletal elements, binding elements or basidia, depending on their location in the basidiocarp.

The skeletal elements of *C. pubescens* are more branched with longer branches than those of *C. hirsutus*, while the binding elements of *C. pubescens* have a greater number of branches and remain isodiametric throughout. The binding elements of *C. hirsutus* have fewer branches which become enlarged at their ends. Specialized elements, which have pigmented contents at their distal apices, are not found in the trichoderm or pore surface of *C. pubescens*. The presence of these structures in corresponding regions of *C. hirsutus* is responsible for the brown colour of the trichoderm and the smoky colour of the pore surface of this species.

DISCUSSION

In order to appreciate the taxonomic value of this study, published information on the microstructure of basidiocarps of *C. hirsutus* and *C. pubescens* must be considered. Overholts (1953) has described the microstructure of *C. hirsutus* as follows, "hyphae mostly a mixture of thin-walled and thick-walled ones, nearly simple, with no cross walls or clamps, 3-6(8) μ in diameter, a few hyphae somewhat smaller and much branched, but well developed hyphal complexes apparently not formed; spores cylindrical or allantoid, smooth, hyaline, 4.5-7x2-2.5 μ". Similarly, Overholts has described the anatomical characters of *C. pubescens* as follows, "hyphae generally simple, straight, with entirely thickened walls, with no (or extremely few) cross walls or clamps, 4-8.5 μ in diameter; hyphal complexes present and distinct, the hyphae 3-4 μ in diameter; spores cylindrical or allantoid, smooth, hyaline, 5-8x2-2.5 μ".

For comparison, Bondartsev (1953) has described the anatomical characters of *C. hirsutus* as follows, "hyphae of tissue hyaline, thick-walled, often even devoid of lumen, sinuous, elastic, looser toward the surface, (1.5)2-5(6) μ thick; subhymenial hyphae thin-walled, 1.5-2 μ in diameter; hyphae of the tube trama colourless, 2-3(3.5) μ thick, thick-walled, rarely thin-walled, with sparse septa; hymenial layer dense, basidia 12-15x4-5 μ; spores smooth, colourless, almost cylindrical, straight or slightly curved, at the base slightly drawn out, 6-8x2-3 μ". Similarly his description

of *C. pubescens* includes, "in pileus trama, hyphae sinuous, sparsely branched, colourless or almost colourless, entwined, thick-walled, with narrow lumen, sometimes altogether solid, but thin-walled, ribbon-shaped hyphae also occur, occasionally with collapsed walls, 3-6.5 μ thick, in tubes hyphae somewhat thinner, 2.5-3.5 μ thick; basidia 12-15x4-5 μ; spores colourless, cylindrical, flattened on one side, or slightly curved, rostellate at the base, 5-8x2-2.5(3) μ, guttulate".

From my observations, the following descriptions of the microstructure of *C. hirsutus* and *C. pubescens* have been prepared.

C. hirsutus. Tissue of context and trama of dissepiments composed of an interconnected and branched system of generative hyphae from which end cells are delimited and differentiate into binding and skeletal elements; generative hyphae hyaline, 2-4 μ in diameter, at first thin-walled but walls eventually becoming thickened with occasional blister-like swellings in older parts, with clamp connections from which branches frequently arise, cells of generative hyphae of context up to 300 μ long, shorter in dissepiments; skeletal elements discrete, hyaline cells up to 2 mm long, 4-6(8) μ in diameter, with 2(3) thick-walled branches to 120 μ long at distal end, proximal region to 300 μ long, thin-walled, with wall increasing in thickness to distal end where the lumen becomes capillary; binding elements discrete, hyaline, thick-walled cells, 3-4 μ in diameter, with 3-15

short contorted branches which are frequently enlarged to 8-10 μ in diameter at the branch ends; specialized elements in dissepiments and trichoderm similar to skeletal elements except distal region unbranched, with only partially thickened walls and containing pigment in cytoplasm; subhymenium consisting of short, branched, generative cells, 2-3 μ in diameter; basidia clavate, hyaline, 10-15x4-5 μ; spores hyaline, cylindrical, with smooth walls, 5-7(8)x2-3 μ.

C. pubescens. Tissue of context and trama of dissepiments composed of an interconnected and branched system of generative hyphae from which end cells are delimited and differentiate into binding and skeletal elements; generative hyphae hyaline, 2-4(5) μ in diameter, at first thin-walled but walls eventually becoming thickened with occasional blister-like swellings in older parts, with clamp connections from which branches frequently arise, cells of generative hyphae up to 400 μ long, shorter in dissepiments; skeletal elements discrete, hyaline cells, up to 2.5 mm long, 4-6(8) μ in diameter, with 3-5(6) thick-walled branches up to 300 μ long at the distal end, proximal region up to 500 μ long, thin-walled, with wall increasing in thickness to distal end where lumen becomes capillary; binding elements discrete, hyaline, thick-walled, isodiametric cells, 3-4 μ in diameter, with 6-40 short, contorted branches; subhymenium of short, branched, generative cells, 2-3.5 μ in diameter; basidia clavate, hyaline, 8-16x4-5 μ; spores hyaline, cylindrical, with smooth walls, 6-8x2,5-3 μ.

From these descriptions, it can be seen that basidiocarps of *C. hirsutus* have distinctly different skeletal and binding elements to those of *C. pubescens*, and in addition, *C. hirsutus* has pigmented elements in the dissepiments and trichoderm which are not present in basidiocarps of *C. pubescens*.

In comparing the descriptions which I have provided with those given by Overholts and Bondartsev, there are a number of correlations and discrepancies which are apparent. A noteable omission in the earlier descriptions is the clamp connections which are easily demonstrable in the submarginal regions of both species. Since clamp connections are an obvious character found in certain species of the Polyporaceae, they should be included in formal descriptions of species which form them. The "generally simple, straight hyphae with entirely thickened walls" and the "distinct hyphal complexes" of *C. pubescens* to which Overholts refers, correspond to the long, unbranched regions of skeletal elements and the binding elements respectively. Similarly, the "hyaline, thick-walled, sinuous hyphae" of *C. hirsutus* and the "sinuous, sparsely branched, entwined, thick-walled hyphae" of *C. pubescens*, referred to by Bondartsev, are skeletal elements, his descriptions reflecting the more numberous and longer branches found in *C. pubescens*. The "thin-walled, ribbon-shaped hyphae" described for *C. pubescens* by Bondartsev, correspond to the proximal regions of the skeletal elements which remain thin-walled.

This study has enabled a more accurate picture of the microstructure of the basidiocarps of each species to be gained and has provided additional stable taxonomic criteria which may be used to separate the two species.

Both species have a similar trimitic microstructure which can be compared with that described for *Polystictus xanthopus*. Corner (1932b) reported that only the skeletal, binding and generative systems are characteristic of *P. xanthopus* and that they are the result of division of labour. In this species, the unbranched, thick-walled, skeletal hyphae are held together by branched, binding hyphae, consequently it is reasonable to assume that there is a division of labour and the hyphae can be classified according to their function. In proposing the terms skeletal hypha and binding hypha, Corner gave a functional role to structural components. His definitions of hyphal types were based on function rather than structure. Such a system will work only if each structure has a clearly defined function.

For monomitic basidiocarps, which are composed solely of generative hyphae, this concept may be applied, since there are no specialized structural components and no division of labour. For dimitic basidiocarps with generative and either skeletal or binding hyphae, this concept may again be applied as the division of labour between generative hyphae and the structural components is at once apparent. It is more difficult to apply this concept to

trimitic systems such as those of *C. hirsutus* and *C. pubescens*. The skeletal elements of the basidiocarps of these species are branched at their distal ends and the branches grow in between and weave around other skeletal elements. In so doing, they must act as binding hyphae as they help to tie the context hyphae together to some extent. This type of skeletal element is referred to as 'arboriform' by Teixeira (1962) because of its resemblance to the branches of a tree. Binding elements of *C. hirsutus* and *C. pubescens* frequently have a proximal, unbranched region which, when thick-walled and mature, is unable to bind but is itself bound by the branches of other binding elements. In this instance a part of a binding element is assuming the function of a skeletal element. Clearly, the intermediate types of binding element carry out both functions to a greater or lesser degree, depending on the exact location in which they develop. Generative cells in the older context of the two species studied often become thick-walled and, in doing so, contribute to the structural rigidity of the basidiocarp. Here, the function of a particular cell changes as the basidiocarp ages. This wall thickening of older generative cells occurs in many species of the Polyporaceae. Corner (1953) has stated that "the monomitic hyphae of *Polyporus adustus*, and many others such as *Polystictus microcyclus* described in this paper, become thick-walled".

The concept of mitic systems was further developed by Cunningham (1947) who subdivided the skeletal series into

two general types termed 'bovista' and 'long' types. The 'bovista' type "consists of a main stem, 3-10 μ in diameter, producing several lateral branches, which in turn may be branched and tapered towards the ends". He further subdivided the bovista type into thin-walled and thick-walled types. The 'long' type was said to ramify in the context for several millimeters. Binding hyphae were also divided into 'bovista' types and 'long' types. Corner (1953) considered the bovista type of skeletal hypha described by Cunningham to be a binding hypha. It must be emphasized that for *C. hirsutus* and *C. pubescens*, neither the skeletal elements nor the binding elements form a continuous series since they are discrete units. The generative series is continuous, consisting of similar cells joined end to end. The concept of mitic systems is therefore limited and, while it may be applied successfully to some polypores, especially monomitic and dimitic ones, there are difficulties in applying it to trimitic species such as *C. hirsutus* and *C. pubescens*.

In the two species studied there is a range of structural elements. At one end are the skeletal elements which merge through the intermediate binding elements with the true binding elements at the opposite end of the range. I consider that the type of structural element formed is directly related to its position in the developing basidiocarp, a point not made by other authors. The growing margin of the basidiocarps of these two species is composed

of the elongating apices of end cells which will develop into skeletal elements and I believe that every marginal cell is of limited growth and will eventually become a skeletal element. This concept is not in agreement with Corner's observations as he reported that both skeletal and generative hyphae occurred at the growing margin of *P. xanthopus*, and that they were indistinguishable. The results of the present study show that generative cells do not occupy a terminal position on a hypha, since end cells at the margin become skeletal elements, end cells in the context become binding elements and end cells in the hymenium become basidia.

As there is a range of discrete structural components in the basidiocarps of these species, it would be more meaningful to regard them as one series rather than two. This concept has been suggested by Pinto-Lopes (1952) who classified all differentiated hyphae as tertiary hyphae and all undifferentiated generative hyphae of the basidiocarp together with the dikaryotic mycelium in the substrate as secondary hyphae. He applied the term primary hyphae to the monokaryotic mycelium which develops from the germination of a single spore. If taken no further, this concept does not distinguish between terminal or intercalary structural components and also fails to consider the position in the basidiocarp where the structural components are being formed. There is also the problem of the generative cells which become partly thick-walled and which must change from

secondary to tertiary hyphae. Other problems which must be considered in this concept are the fact that thick-walled 'tertiary' hyphae may be formed in monokaryotic 'primary' cultures without the necessity of a 'secondary' stage and the fact that Pinto-Lopes (1952) reported cases of 'tertiary' to 'secondary' and 'secondary' to 'primary' regression. It should be noted here that Pinto-Lopes considered the character 'type of secondary hyphae' to be of first importance; the character 'type of tertiary hyphae' to be of secondary importance; and the characters of the secondary and the tertiary hyphae 'taken together' to possess a taxonomic value of first importance, 'for they allow the division of the family into subfamilies'.

The concepts of Corner and of Pinto-Lopes do not take into account the distinction between specialized elements which develop in a terminal or in an intercalary position. In the species I have described, all skeletal and binding elements are terminal structures, but in the species *Polyporus sulphureus* and *P. squamosus*, all binding elements are intercalary. These binding elements are formed from a generative cell from which a number of long whip-like processes develop and grow intrusively through the context, the whole cell finally becoming thick-walled. I believe that it is necessary to make this fundamental distinction between intercalary and terminal positions for specialized structural components. In the investigations of polypore microstructure, the emphasis must be on both the

developmental position of specialized elements, including their relation to the basic generative series, and the cellular construction of the basidiocarp with respect to the differentiation of single cells into specialized structures. In the past little attention has been given to the fact that basidiocarps are composed of individual elongated cells. It should be stressed that the cell is the basic unit and, once this is realized, it follows that in the generative series, which is continuous and composed of similar cells joined end to end, septa will always be found. In specialized elements which have been formed by differentiation of a single end cell, there can be no septa.

The term hypha has become meaningless in its application to the higher basidiomycetes, since it has been applied to groups of cells joined end to end, to single cells and even to parts of a single cell. I interpret the term hypha to mean a branch of the mycelium composed of several elongate cells. It is therefore acceptable to refer to the generative series as generative hyphae, but incorrect to refer to discrete structural units which develop from single cells, such as the skeletal and binding elements of *C. hirsutus* and *C. pubescens*, as hyphae.

It is worthwhile to note that, although binding hyphae were so named because they appear to have the function of binding the context tissue together, it may be that they have to grow intrusively between and around the other hyphae of the context. In other words, they may be merely filling

spaces in the context rather than be specifically developed for a specialized purpose.

Reference must be made to the species *Cerrena unicolor* (Fries) Murr., since it has been described as trimitic by Cunningham (1950) and by Teston (1953) who placed it in the genus *Coriolus* Quel. Van der Westhuizen (1963), who studied this species, suggested that it is in fact monomitic as he could discover no distinct morphological differences between thick-walled and thin-walled hyphae. He concluded that "thick-walled hyphae develop by interseptal elongation and thickening of the walls of nodose-septate hyphae after their formation at the margin", a process difficult to visualize. In the species I have studied, elongation of all basidiocarp elements takes place by apical extension.

It is apparent that neither the concept of mitic systems nor the concept of secondary or tertiary hyphae is a workable basis for polypore classification. As Smith (1966) says in reference to the mitic system, "its greatest value was psychological in that it finally brought mycologists face to face with the real problem, namely, the degree to which the hyphae of the basidiocarp become specialized to further the processes of spore production and dispersal, directly or indirectly".

Before we can make full use of basidiocarp microstructure, and there is no doubt that it will provide invaluable taxonomic evidence, we must redefine our terminology, even for simple words like hypha, and

redescribe the microstructure of polypores with special emphasis on the development of their various specialized and differentiated elements. Only then will it be possible to get a clear picture of relationships within this group and to develop a classification which will reflect this knowledge.

FIGURE 37. A diagramatic vertical section through a typical basidiocarp of *C. hirsutus*.

A - substrate.

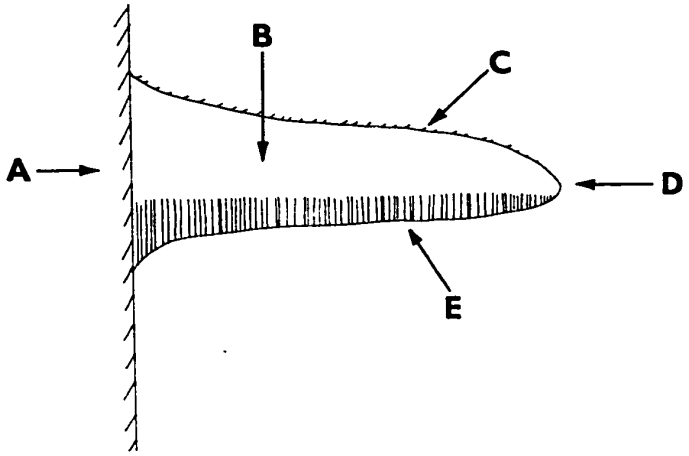
B - context.

C - trichoderm.

D - growing margin.

E - dissepiments (pore field).

FIGURE 38. Tightly packed, thin-walled, hyphal tips at the growing margin of the basidiocarp. The distal end of a skeletal element is becoming branched (arrow). *C. hirsutus*.



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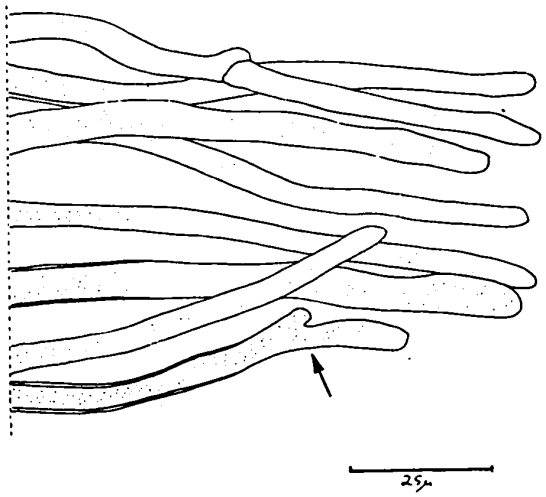
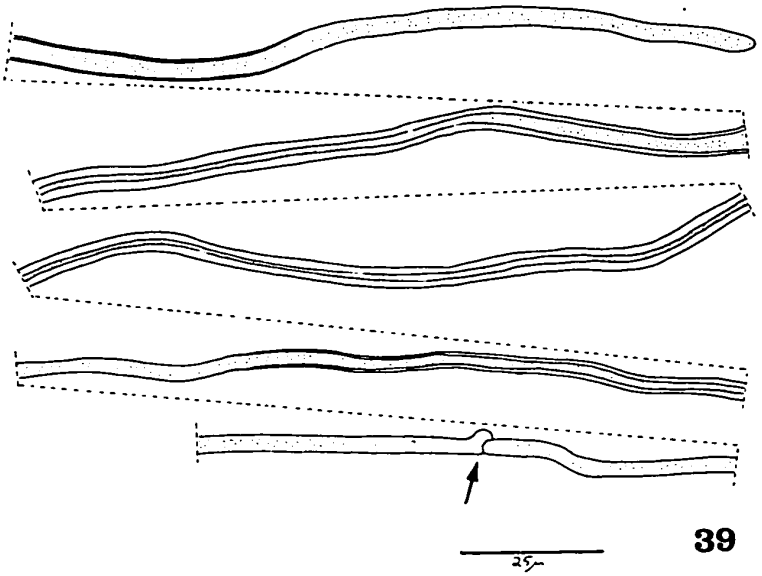


38

FIGURE 39. A typical elongate marginal cell which has developed from a clamp connection (arrow). Note thickening of wall in central region of cell. *C. hirsutus*.

FIGURE 40. Development of new generative and terminal cells.
A - initiation of clamp connection (arrow).
B - terminal cell delimited by clamp connection.
C - initiation of new growing point (arrow) from subterminal cell.
D - development of new growing point with subsequent formation of new subterminal generative cell and terminal elongating cell.
All *C. hirsutus*.



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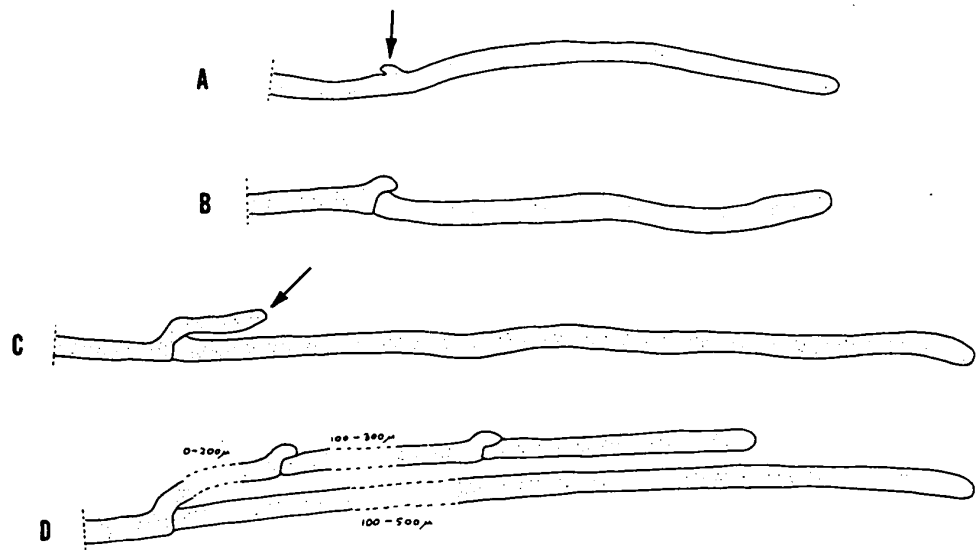


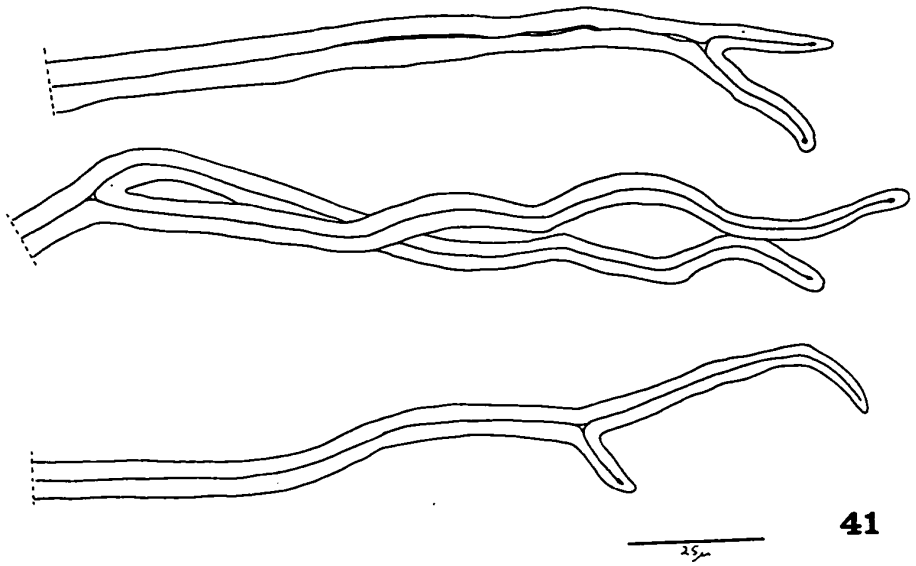
40

FIGURE 41. Branched distal ends of skeletal elements. *C. hirsutus*.

FIGURE 42. Basidial development and basidiospores. *C. hirsutus*.

FIGURE 43. Binding elements from context of *C. hirsutus*. Note short proximal region and short contorted branches enlarged at ends.

FIGURE 44. Early stage of development of binding element as a terminal cell joined to a subterminal cell by a clamp connection (arrow). *C. hirsutus*.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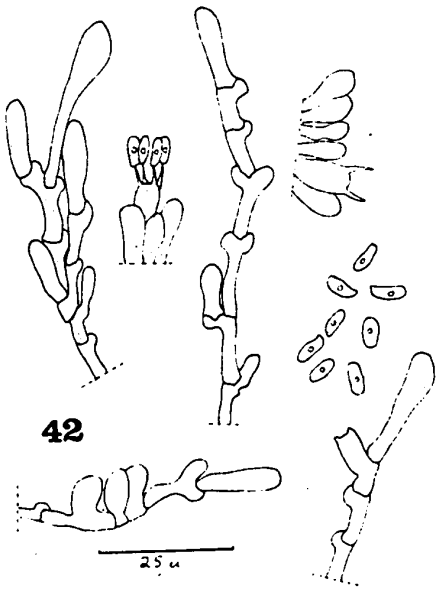
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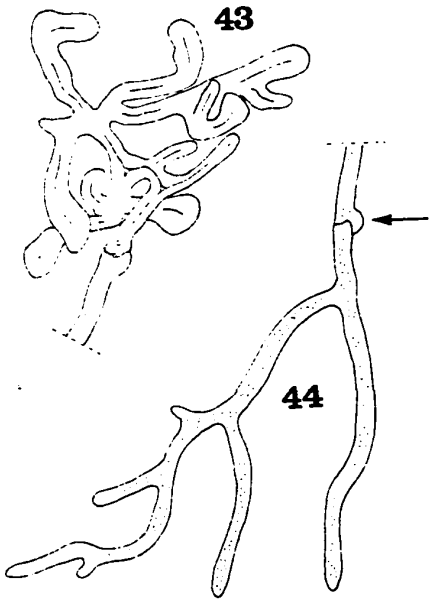
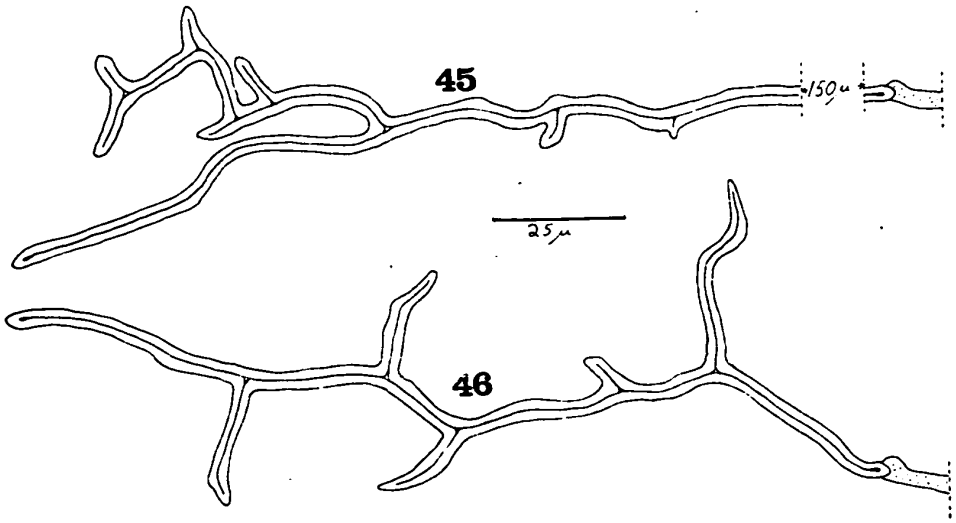

43
44

FIGURE 45. Intermediate type of binding element produced near the growing margin and characterized by a relatively long, unbranched, proximal region and a sparsely branched distal region. *C. hirsutus*.

FIGURE 46. Binding element from near the edge of a growing dissepiment. Note absence of enlargement of branch ends. *C. hirsutus*.

FIGURE 47. Binding elements from trama of dissepiments. Note enlarged branch ends. *C. hirsutus*.


45
150 μ
25 μ
46

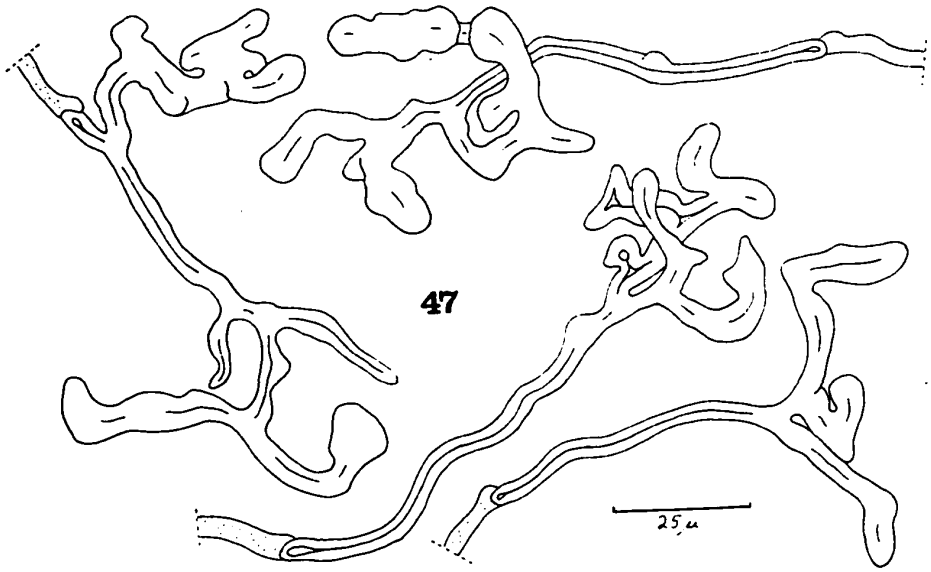

47
25 μ

FIGURE 48. Intermediate type of binding elements. The thin-walled, proximal regions (arrows) will eventually become thick-walled. *C. pubescens*.

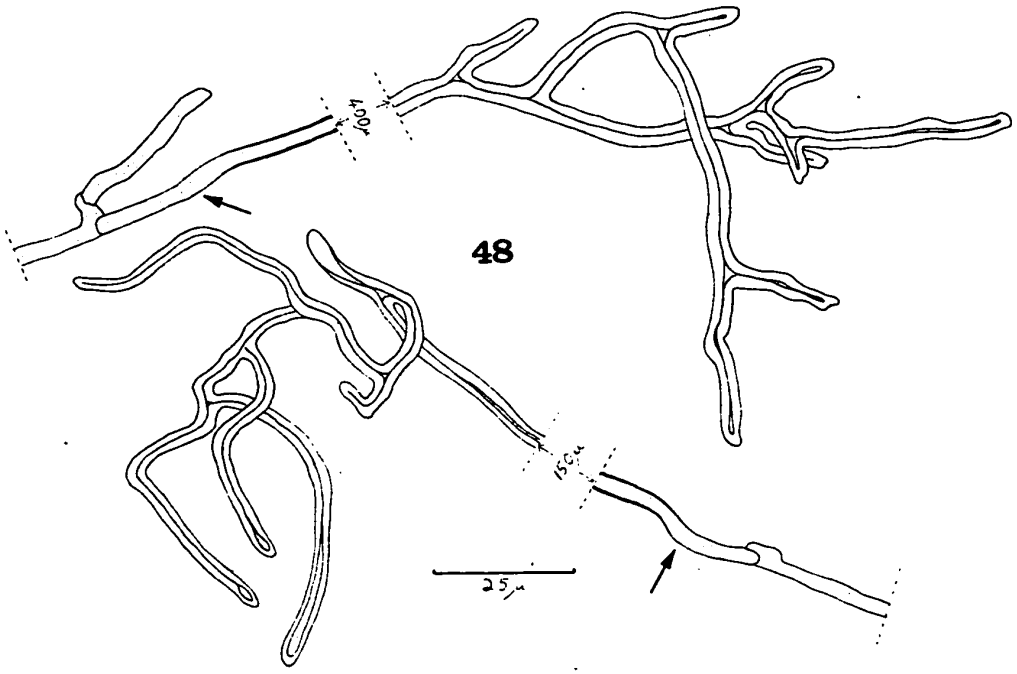
FIGURE 49. Distal end of a thick-walled, skeletal element from dissepiments. *C. hirsutus*.

FIGURE 50. Thin-walled, apical regions (arrows) of specialized cells containing the pigment material responsible for the smoky colouration of the pore surface. *C. hirsutus*.

FIGURE 51. Thin-walled, proximal regions (arrows) of two skeletal elements which have developed from a generative cell. *C. pubescens*.

FIGURE 52. Swellings (arrows) which have developed on an intercalary, thick-walled, generative cell. *C. hirsutus*.

FIGURE 53. Thin-walled, hyphal tips from the growing edge of a dissepiment. *C. hirsutus*.



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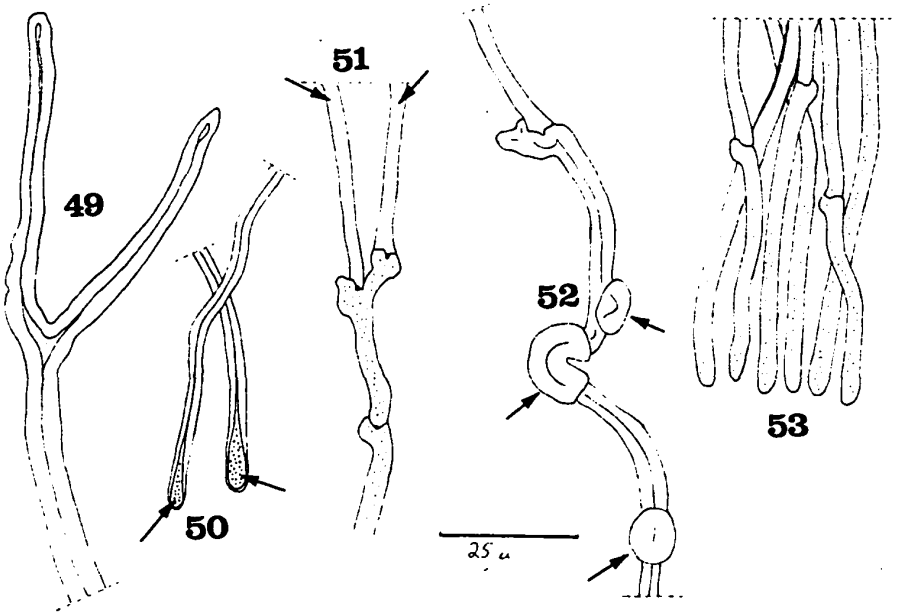

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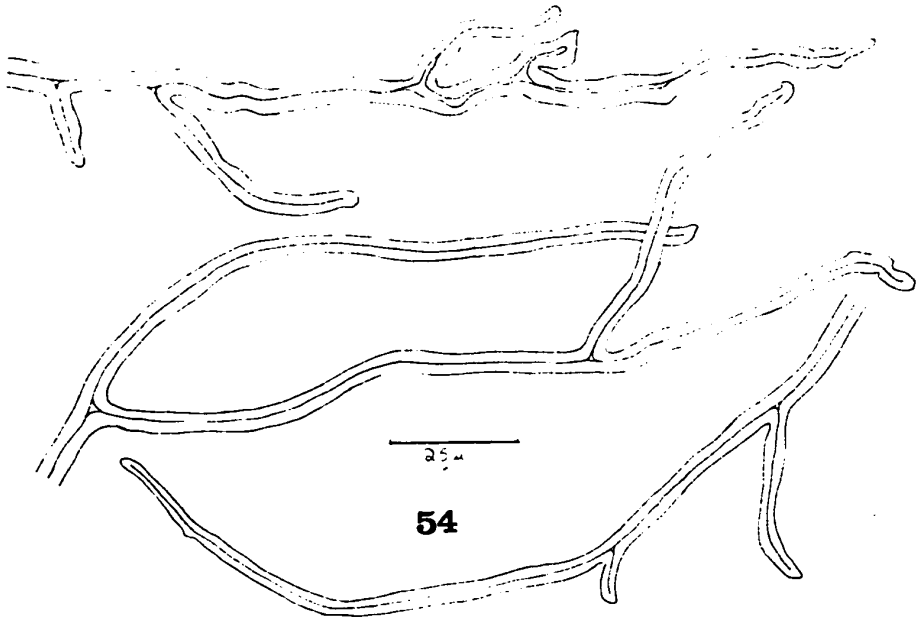
FIGURE 54. Various, branched, distal ends of mature, skeletal elements from context of *C. pubescens*.



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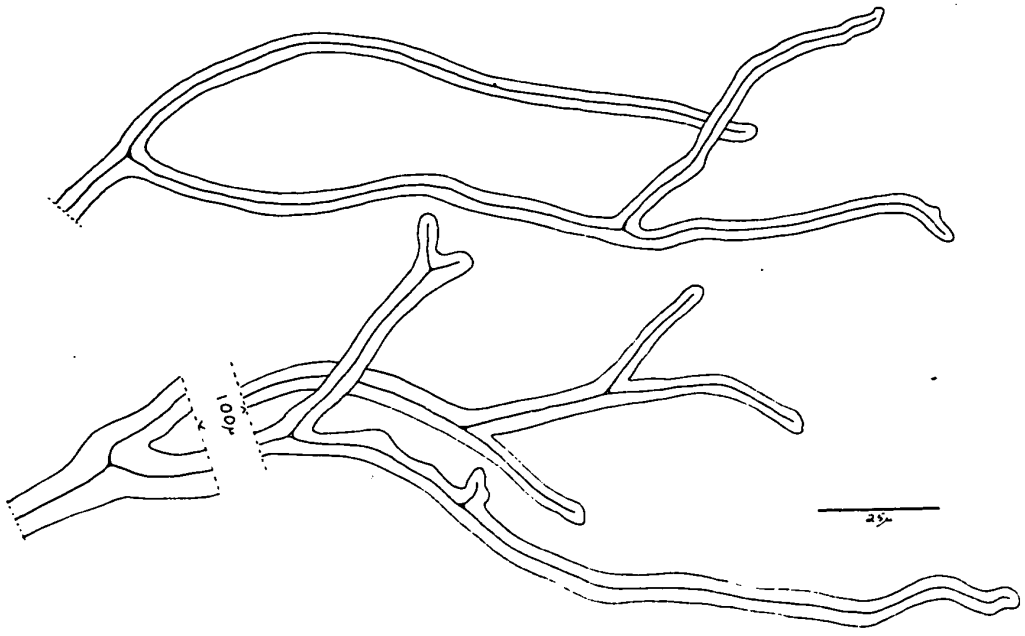
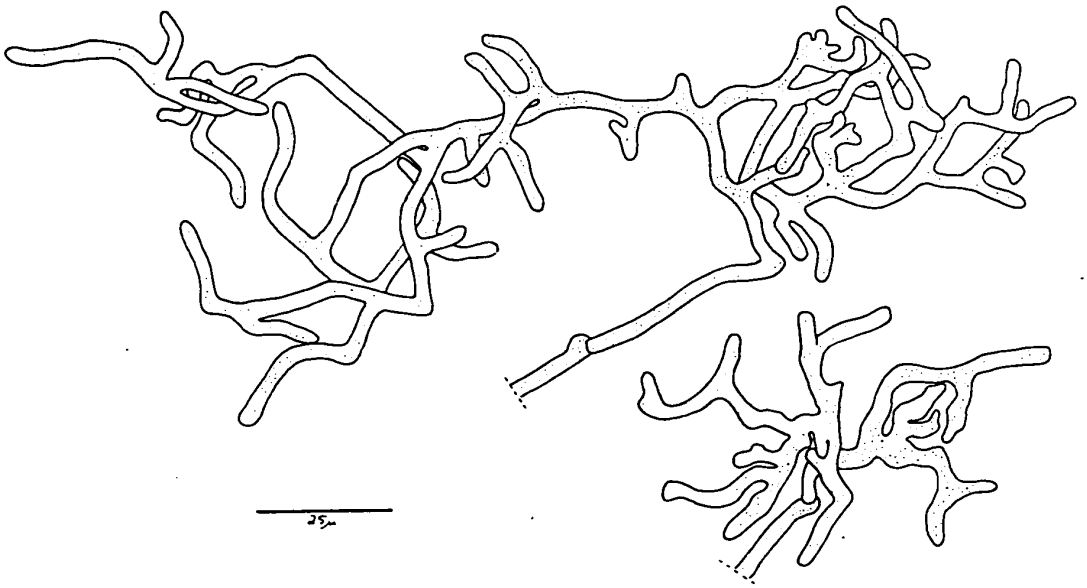


FIGURE 55. Developing binding elements from context. Each arises as a branched, terminal cell. *C. pubescens*.



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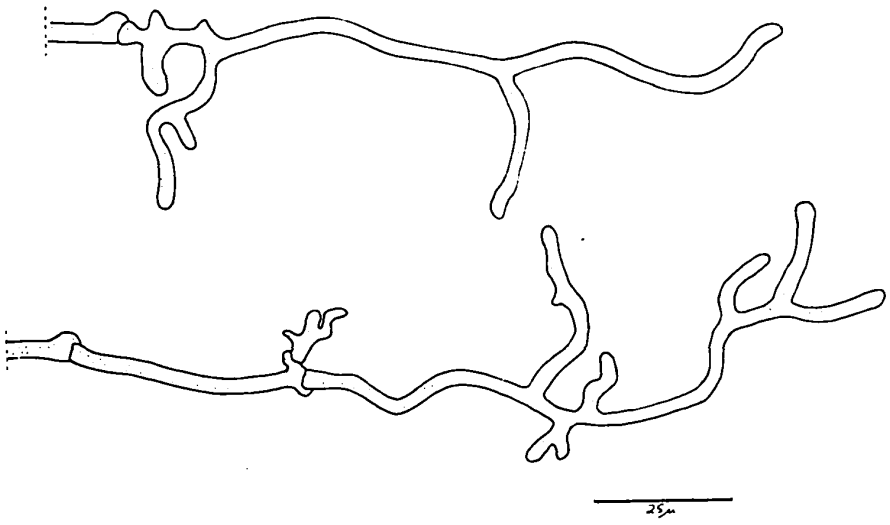


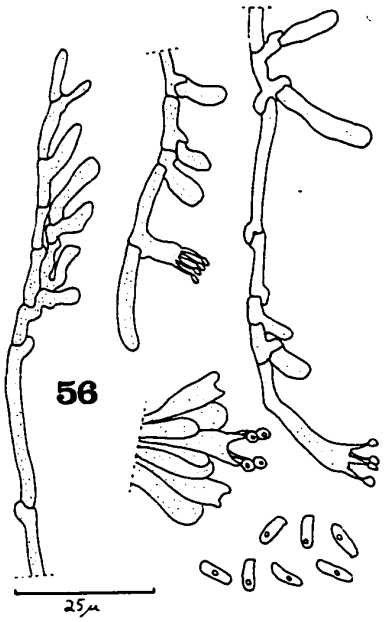
FIGURE 56. Examples of basidial development and basidiospores. *C. pubescens*.

FIGURE 57. Mature, binding element from dissepiment. *C. pubescens*.

FIGURE 58. Mature, binding element from dissepiment. *C. pubescens*.

FIGURE 59. Constriction (arrow) in unbranched shaft of skeletal element caused by a growth check. *C. pubescens*.

FIGURE 60. Resumption of growth by the apex of a developing skeletal element after a growth check. *C. pubescens*.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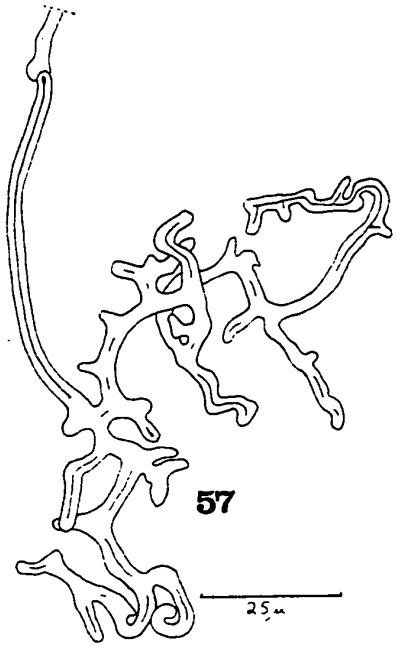
25μ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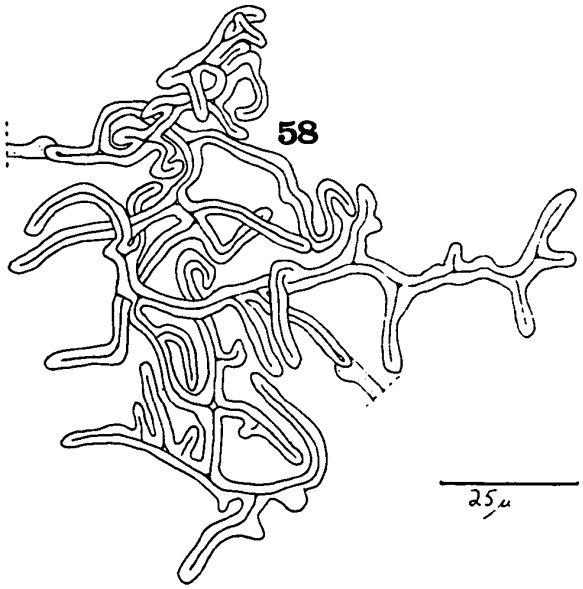
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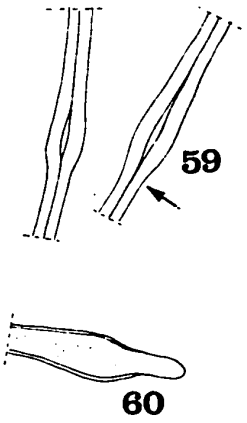
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FIGURE 61. Simplified, fore-shortened, semi-diagramatic representation of a vertical section through the growing margin of a basidiocarp of *C. hirsutus*.

A - generative cell.

B - distal end of mature skeletal element.

C - upward deflected cell apex which will become part of the trichoderm.

D - new growing apex arising from generative cell.

E - thick-walled, contorted, binding element deep in context.

F - developing intermediate type of binding element, nearer to margin.

G - branching distal end of developing skeletal element.

H - thin-walled cell apex which will eventually become a skeletal element.

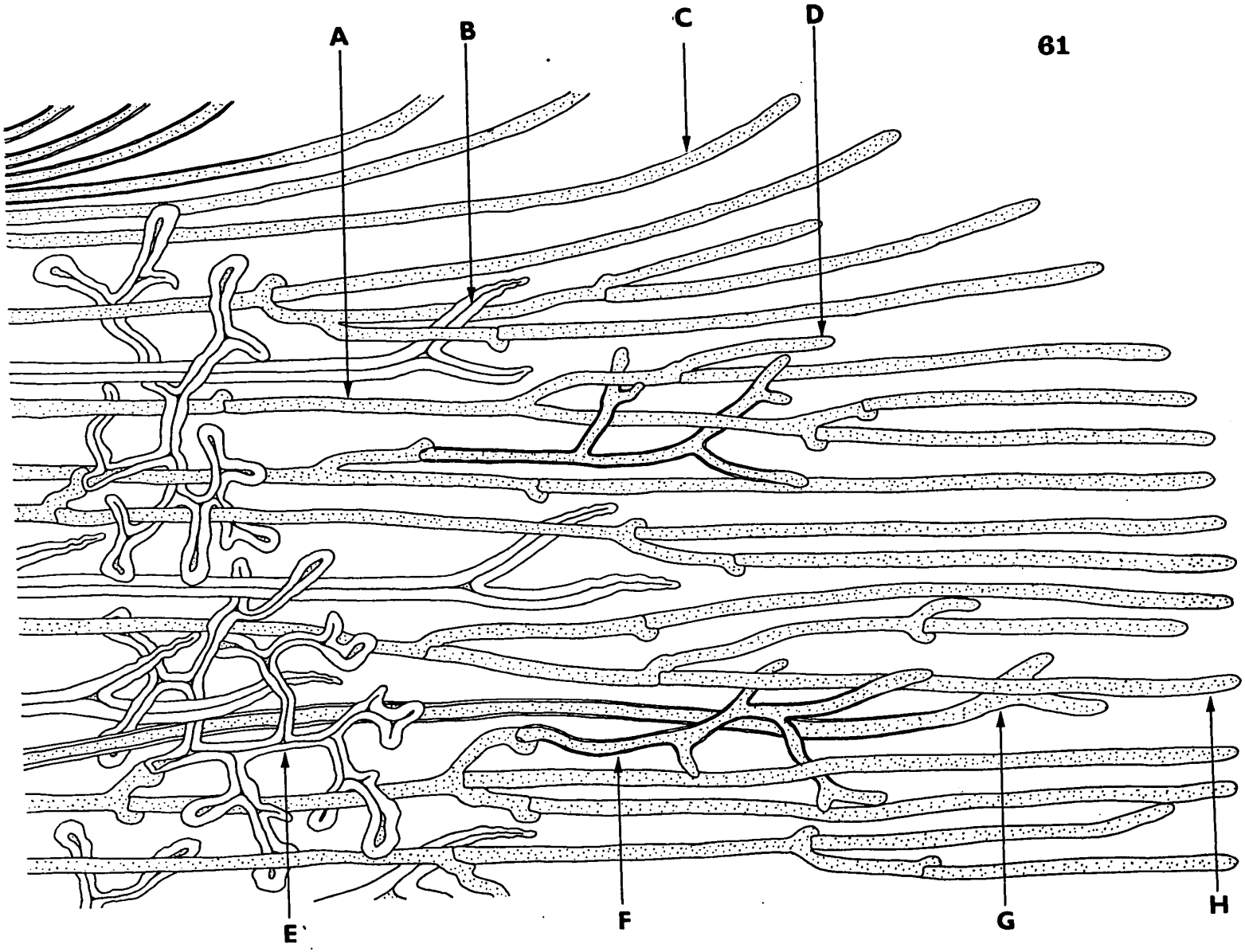

A
B
C
D
E
F
G
H

FIGURE 62. Simplified, fore-shortened, semi-diagramatic representation of a vertical section through part of a dissepiment of *C. hirsutus*.

A - mature, thick-walled, binding element developed in trama.

B - distal end of mature, thick-walled, skeletal element.

C - developing intermediate type of binding element near to dissepiment edge.

D - generative cell.

E - new growing apex developing from a generative cell.

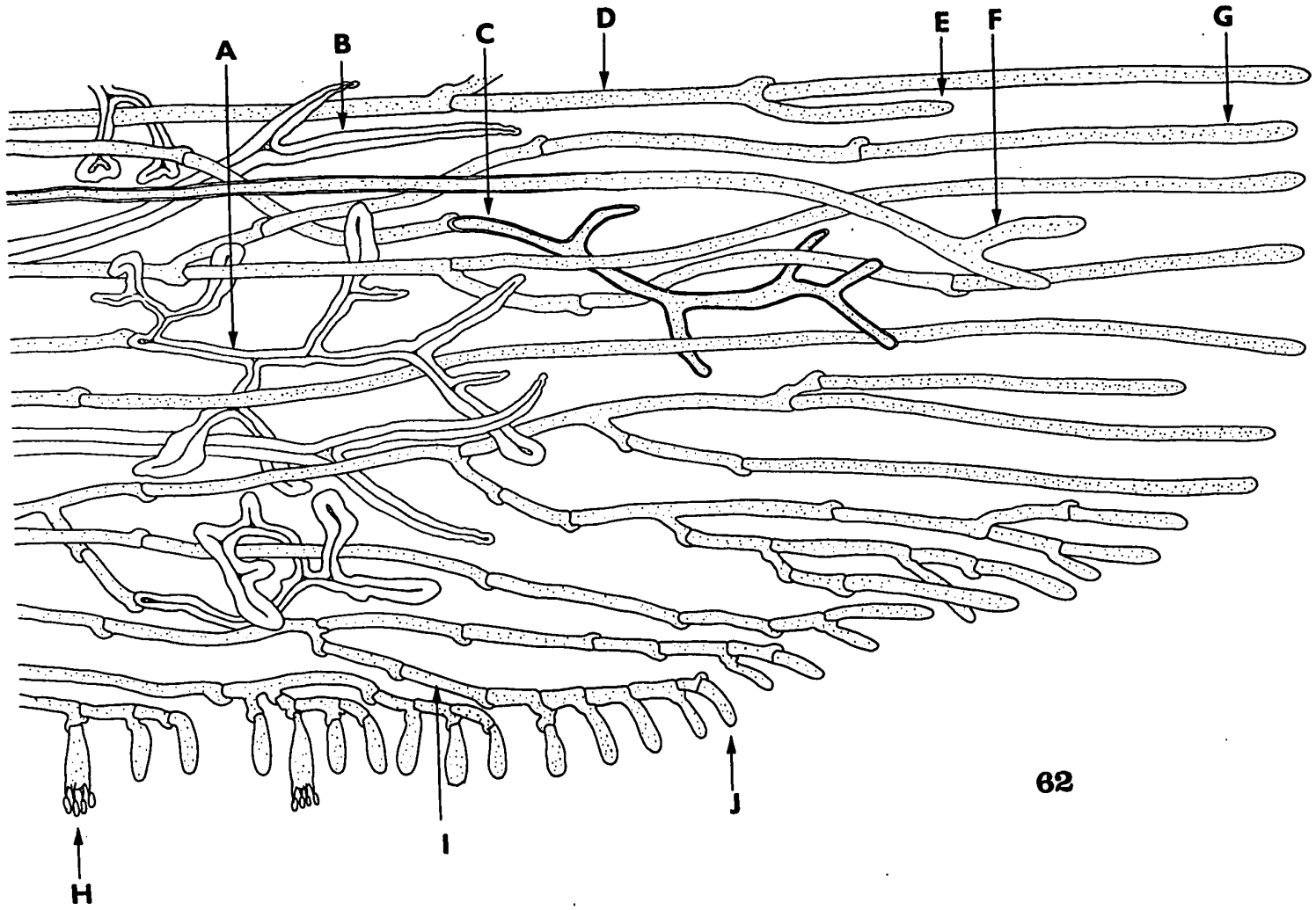
F - branched distal end of a developing skeletal element.

G - thin-walled cell apex at the growing edge which will eventually become a skeletal element.

H - basidiospores developing from a basidium.

I - short cells of the subhymenium.

J - developing basidium.



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CHAPTER IV

INTERFERTILITY STUDIES

INTRODUCTION

Interfertility tests have been frequently employed by other workers to resolve taxonomic problems and confirm the identity of Basidiomycete species. Kniep (1920) is credited with first establishing that monosporous cultures of *Schizophyllum commune* lacked clamp connections and that when these monosporous cultures were paired, clamped dikaryotic cultures developed in certain pairings. Kniep suggested that two segregating pairs of alleles, Aa and Bb, were the cause of this type of mating reaction and he further hypothesized that the dikaryon was only established when strains having different alleles of each pair (*i.e.* AB X ab or Ab X aB) were mated. Consequently, if two unlinked loci were involved then the progeny of each dikaryotic basidiocarp should contain individuals of four mating types. Although Kniep was unable to prove this hypothesis at that time by mating a number of monospore isolates from the same basidiocarp in all combinations, he was able to mate two monospore isolates and bring the resultant dikaryon to fruit (*e.g.* AB X ab). Of the 28 monospore isolates made from this fruiting, 10 (AB) were compatible with one parent, 5 (ab) with the other parent and 13 (Ab and aB) with neither parent. Kniep also mated ten monospore isolates from one basidiocarp with

eleven monospore isolates from a basidiocarp from another locality and found that, in every case, a dikaryotic mycelium was established.

Later, Kniep (1922) was able to isolate tetrads of spores from *Aleurodiscus polygonis*. By pairing the culture derived from each spore in a tetrad with the other monospore cultures of the same tetrad, he was able to prove conclusively that the mating system was controlled by the independent segregation of two pairs of factors, one pair at locus A and the other at locus B, resulting in the formation of four possible mating types. He also showed that an extensive series of factors could be present at each locus and that a dikaryotic mycelium was only formed when there was no common factor at either locus. This type of interfertility became known as tetrapolar sexuality to denote the occurrence of the four mating types.

In 1923-24 other workers (Vandendries, 1923, 1924; Brunswick, 1923, 1924) demonstrated the occurrence of bipolar sexuality, a term first used by Burgeff (1920) to describe the two-class mating pattern of the Mucorales. Bipolar sexuality was characterized by two mating types formed by segregation of a number of factors at a single locus. Again it was demonstrated that in most cases the progeny of basidiocarps from different localities were interfertile and that to form a dikaryon different factors were necessary at the locus. In the following years the type of interfertility for many species of Basidiomycetes was

established.

Studies with some fungi have demonstrated that the two loci of the tetrapolar system are responsible for different physiological effects. The A locus is now understood to control the formation of clamp connections while the B locus is responsible for nuclear migration. If paired cultures have common A factors then clamp connections will not be formed, and if paired cultures have common B factors then clamp connections may form at the immediate contact zone but will not spread throughout each monospore culture. Raper (1966) has suggested that different A factors are necessary for nuclear association, conjugate division, hook cell formation and hook cell septation, while different B factors are required for nuclear migration and hook cell fusion.

Buller (1930) has demonstrated that a monokaryotic mycelium could be dikaryotized by a dikaryotic mycelium, a process which has become known as the Buller phenomenon. This procedure has subsequently been used by many workers to identify unknown cultures by mating either a known dikaryon with an unknown monokaryon or a known monokaryon with an unknown dikaryon. In either case the dikaryotization of the monospore culture is taken as proof that the cultures are conspecific.

Another interesting phenomenon associated with the pairing of monospore cultures is the formation of a barrage zone at the contact line between the two cultures. The barrage zone was first described by Vandendries and Brodie

(1933) as a region between the two cultures which was sparsely penetrated by hyphae. Other types of interaction such as lines of demarcation and lines of aversion have been described by other workers. Dark lines between paired cultures of *Fomes cajanderi* have been reported to be similar to those found in nature in decaying wood and described by Adams and Roth (1967) as zone lines. The formation of demarcation lines between paired cultures of wood decay basidiomycetes is considered by several workers (Adams and Roth, 1967; Childs, 1937; Mounce, 1929) to be evidence that the cultures are separate biological entities. In the present study the occurrence of dark lines between pairings was recorded in an attempt to determine the basis for this interaction.

In 1934 Bose (1934), reporting his studies on the sexuality of *Coriolus hirsutus*, referred to as *Polystictus hirsutus* (Fries), said that this species is heterothallic and bisexual (bipolar). He paired 15 monospore cultures together in all combinations and his observations showed that, of the 56 pairings which produced clamp connections, 15 were homogeneous, 38 formed white lines of aversion, and 3 showed spaces of aversion. Out of the 49 pairings which did not form clamps, 8 were homogeneous, 41 formed white lines of aversion and none formed spaces of aversion. He did not report the formation of dark lines although he was using a similar type of medium to the one used in this study. It must be mentioned that the specimens Bose used in his

study were collected near Calcutta, India, and are not necessarily conspecific with the North American type. Kimura (1954) paired 20 monospore isolates in all combinations from a single basidiocarp of *C. hirsutus* and his results show this species to be heterothallic and tetrapolar. Nobles, Macrae and Tomlin (1957) reported that most isolates of *C. hirsutus* show the tetrapolar type of interfertility, but that certain isolates seem to be bipolar. Burnett (1956) comments that aversion phenomena are not normally developed by the bipolar *C. hirsutus* in incompatible matings, but in the taxonomically closely related tetrapolar *C. versicolor* aversion reactions are associated with matings where one allelomorph is common to the two reacting mycelia, *i.e.* where factors at the B locus are identical.

Only one report of the interfertility for *C. pubescens* has been discovered. Nobles, Macrae and Tomlin (1957) report it to be tetrapolar. No information on aversion line or dark line formation has been published for this species. It was apparent that the type of interfertility for *C. hirsutus* was still in doubt and that the formation of dark lines in pairing cultures of monospore isolates had not been reported or described for either species.

METHOD

The standard method for determining interfertility has been described previously (Nobles, Macrae and Tomlin, 1957) and was used with slight modifications. Single spore

cultures were derived from a number of basidiocarps of *C. hirsutus* and *C. pubescens* and maintained on slants of Nobles malt agar (Nobles, 1965). Identification numbers for the monospore isolates were suffixed to the identification number of the basidiocarp from which they had been derived. For example, #240-2 is monospore isolate number 2, grown from a spore produced by basidiocarp number 240. To determine the interfertility for each species, a number of monospore cultures from a single basidiocarp were paired in all combinations. The pairing was done by inoculating one side of a 9 cm plastic petri plate with a piece of mycelium from one monospore culture and placing a piece from another culture at the opposite side of the plate. The plates were set aside for 4 weeks. At the end of this time, small pieces of the mycelium taken from a point at least 1 cm from the contact line where the two cultures met were examined microscopically, by staining with KOH-phloxine, to determine the presence or absence of clamp connections. Mycelium from both sides of the contact line and from the contact line itself was examined in this way.

OBSERVATIONS

The results of pairing, in all combinations, monospore cultures from 3 basidiocarps of *C. hirsutus* and from 3 basidiocarps of *C. pubescens* have been arranged in tetrapolar tables (Figures 63-68). It is customary to represent the four mating types as A1B1, A1B2, A2B1 and A2B2.

Therefore, the mating types for Figures 63-68 can be expressed as shown in Table 3. Figures 63-67 clearly indicate that both *C. hirsutus* and *C. pubescens* have a tetrapolar type of interfertility. In Figure 68 only 6 monospore cultures were used and only three of the four mating types appeared.

In all cases, complete interfertility occurred when both A factors and both B factors were different. In Figures 63, 64, 65, 67, 68 the mating types A1B1 and A2B1 are arbitrarily assigned, the usual procedure. Figure 66 allows the possibility of assigning the A1B1 and A2B1 factors on a firmer basis for, in this test, incomplete clamp connections or hooks were noted on the contact line and are indicated in Figure 66 by 'h'. It is probable that the paired cultures which formed these hooks had identical B factors and consequently the mating types A1B1 and A2B1 can be assigned in such a way as to show this interaction. Hooks were formed when certain isolates of types A1B1 and A2B1 were paired and again when certain isolates of types A2B2 and A1B2 were paired. The significance of the hook formation when #300-4 and #300-7 were paired must be emphasized, since both isolates are of the same mating type (A2B2). This behaviour can be taken as evidence that common B factors allow hook formation in the contact zone.

When the type of interfertility for *C. hirsutus* and *C. pubescens* was established, the next step was to determine if they were separate and distinct species by pairing

monospore isolates of each species together. Initially, 10 monospore isolates of #240 (*C. hirsutus*) were paired with 10 monospore isolates of #237 (*C. pubescens*) (Figure 69). The resulting 100 plates from this test were checked and in no case were either clamp connections or hooks formed. Subsequently, a large number of tests using groups of 4 monospore isolates from basidiocarps of each species were made and the results showed an almost complete lack of fertility between the two species (Figures 71, 73, 75, 78, 80, 81, 83, 86, 94, 96). There was one exception which should be mentioned. In one of the 16 pairings made between monospore isolates of #237 (*C. pubescens*) and #303 (*C. hirsutus*) a few hooks were found in the contact line (Figure 98).

When monospore isolates derived from different basidiocarps of the same species were paired together, the result was almost complete interfertility and bilateral formation of clamp connections. Completely fertile pairings between monospore isolates of *C. hirsutus* (Figures 70, 74, 76, 82, 84, 89, 95, 97, 100, 101) resulted in the formation of a dikaryotic mycelium. Similarly, a dikaryotic mycelium was formed when monospore isolates of *C. pubescens* were paired (Figures 73, 77, 79, 85, 87, 88, 90, 91, 93, 102). One exception was found when #290-2 was paired with #240-4. Clamp connections formed throughout the mycelium on the #290 side of the contact zone and not on the #240 side. This type of reaction is known as unilateral dikaryotization. A

second exception was discovered when monospore isolates of #303 and #240 were paired. #240-2 formed hooks when paired with #303-2 and #303-3, suggesting that these three isolates share a common B factor. The failure of #240-4 to form clamp connections when paired with #303-1, #303-2, #303-3 or #303-4 could be explained by the presence of identical A factors, identical B factors or identical A and B factors. Since there is a limited number of factors which may be present at the A or B locus, this type of result must be expected. Partington (1959) has estimated that, in *C. versicolor*, there may be 25 factors at the A locus and a further 25 factors at the B locus.

Many monospore isolates were taken from intermediate types of basidiocarps and paired with those derived from typical basidiocarps of *C. hirsutus* and *C. pubescens*. Isolates of these intermediate types were found to be completely compatible with either the typical *C. hirsutus* or the typical *C. pubescens*, but never with both. #237 is one intermediate type which should be given special mention since it was originally identified by Lowe (1968) as a thick form of *C. hirsutus*. Interfertility tests have shown that it is completely compatible with the typical *C. pubescens* cultures (Figures 73, 79, 88, 93, 102). The collection #300 contained several basidiocarps of the typical, thin type and one basidiocarp which was thicker, and much like those of collection #237. Monospore isolates #300-1 to #300-12 were taken from a thin basidiocarp, while

monospore isolates #300-13 to #300-16 were taken from a thick basidiocarp of this collection. All monospore isolates of #300 were found to be completely compatible with those of #237 (Figure 73). The four monospore isolates from the thick basidiocarp, #300-13 to #300-16, were paired with four others from a thin basidiocarp of the same collection, #300-1 to #300-4 (Figure 103). By comparing Figure 103 and Figure 66, where #300-1 is A1B1 type, #300-13 must be A2B2 type, since it is compatible with #300-1. Similarly, both #300-2 and #300-3 are A1B2 types and both are compatible with #300-15 which therefore must be A2B1 type. #300-4, type A2B2, was incompatible with the four isolates #300-13 to #300-16 indicating that type A1B1 is not found in these four isolates. By elimination, the remaining two isolates, #300-14 and #300-16 must be A1B2 type. It is important to realize that the #300 basidiocarps which were used have the same pair of factors at each of the two incompatibility loci, *i.e.* monospore isolates of the two basidiocarps when paired together behave as if they had originated from a single basidiocarp (Figure 103). In other words, both basidiocarps are genetically identical. Burnett and Partington (1957) have shown that the individual basidiocarps of a group of basidiocarps of *C. versicolor*, growing together on a single log, may or may not have the same genetic constitution of mating type factors.

Although the reasons for the mating experiments were

to determine the type of interfertility of *C. hirsutus* and *C. pubescens*, to show that they were separate taxa, and to positively identify intermediate types, the formation of pigmented or dark lines between paired cultures appeared so frequently that records were kept on the occurrence of this interaction. In the figures representing the mating experiments (Figures 63-117), the letter 'p' indicates the formation of a dark pigmented line at some point between the paired cultures. There was a considerable variation in the intensity and extent of the dark line. In some pairings it appeared very faint, while in others it was very striking. The extent of the dark line was also variable, usually it formed a continuous line completely dividing the plate into two regions. Occasionally the dark line was incomplete and only appeared in certain places.

A dark line was observed to form in 96% of the 276 pairings made between monospore isolates of *C. hirsutus* and those of *C. pubescens*, although in some cases the dark line was incomplete and in others it was faint. The exceptions occurred when certain isolates of #240 were paired with those of #233 (Figure 86). None of the 176 pairings made between monospore isolates from different basidiocarps of *C. pubescens* developed dark lines, while 30% of the 224 pairings made between monospore isolates from different basidiocarps of *C. hirsutus* developed dark lines. When monospore isolates from the same basidiocarp were paired together, it was found that, for *C. pubescens*, 27% of paired

cultures of #300 formed dark lines, while none was recorded for basidiocarp #237. For *C. hirsutus*, 5% of paired cultures of #207, 14% of paired cultures of #301 and 13% of paired cultures of #303 formed dark lines. Dark lines formed by paired cultures of *C. hirsutus* were narrow, seldom more than 2 mm wide, and usually dark brown to almost black in colour. Dark lines formed between paired cultures of *C. pubescens* were quite broad, being 4-6 mm wide, and yellow to light brown in colour.

Some of the reactions involving dark line formation are shown in Figures 118-131. When #300-5 was paired with 4 different monospore isolates of #301, the dark line formed showed considerable variation in intensity (Figure 118). The same variable reaction occurred when #216-3 was paired with the four different mating types of #240 (Figure 119), and when two monospore isolates of #303 were paired with three different mating types of #237 (Figure 120). When #271-2 was paired with the four mating types of #240, a complete dark line formed between all pairings (Figure 121), but when #271-6 was paired with the same four isolates of #240, only one pairing resulted in the formation of an incomplete dark line (Figure 122). In one instance, a double dark line formed between paired monospore isolates of *C. hirsutus* (Figure 125).

Although the pairings made between monospore isolates of #271 and #240 were compatible and resulted in reciprocal dikaryotization, dark lines developed in some of the paired

cultures (Figure 89). A similar phenomenon was observed in other compatible pairings of *C. hirsutus* isolates (Figures 74, 76, 92, 95, 97, 99, 100, 101). Since the four mating types of #240 were used in most of these pairings, the frequency of dark line formation for each mating type can be calculated and is 61% for #240-4 (A2B1), 39% for #240-2 (A1B1), 25% for #240-3 (A1B2) and 19% for #240-13 (A2B2). As the monospore isolates which were paired with the four mating types of #240 were randomly selected, the formation of a dark line should occur with equal frequency. An equal frequency was not obtained which suggests that isolates vary in their ability to form dark lines in paired cultures. In several cases a dark line developed between compatible isolates from the same basidiocarp (Figures 63, 66, 68, 123).

In order to study the formation of dark lines in compatible matings further, dikaryotized cultures were isolated from each side of the dark line which had formed. For example, when #271-2 was paired with #240-13, bilateral dikaryotization took place and subsequently a dark line formed between the two original cultures (Figure 121). Initially, the two monospore isolates of this pairing had nuclei of different genetic constitution since each monospore culture was derived from a single uninucleate spore taken from separate basidiocarps. During the process of dikaryotization, an exchange of nuclei took place between the paired cultures resulting in the cells of each original culture becoming binucleate and each containing, in addition

to the original nucleus, a nucleus derived from the opposite culture of the pairing. After the process of dikaryotization had been completed, both cultures of the pairing had the same nuclear constitution. Pieces of dikaryotic mycelium were removed from each side of the dark line which had formed in this and other pairings and grown separately on malt agar plates. After two weeks, a piece of mycelium was removed from the leading edge of each dikaryotic culture and placed at opposite sides of a malt agar plate. After the two mycelia had grown into contact, a dark line formed in the contact zone (Figure 124). Since the two opposing cultures on each plate had the same nuclear composition, it was expected that they would intermingle in the contact zone with little interaction and develop into a uniform mycelium. This test was carried out with pairings of different isolates and in each case an interaction occurred which resulted in the formation of dark areas in the vicinity of the contact zone. This result suggests that there may be a cytoplasmic factor present which is causing the reaction. An examination of these cultures showed that the pigment responsible for the darkening developed within specialized vesicles which arose as outgrowths or swellings on individual cells of the mycelium. The pigment was not retained in these vesicles but diffused into the surrounding agar.

At a later stage of this study, an opportunity arose to prepare monospore isolates from fresh basidiocarps of

C. versicolor which had been collected in Ontario. A number of pairings were made between these monospore isolates and monospore isolates derived from local basidiocarps of *C. hirsutus* and *C. pubescens*. It was anticipated that *C. versicolor* would prove to be completely incompatible with the local isolates. The first set of pairings (Figures 104, 105) revealed that two of the twelve pairings made between *C. versicolor* and *C. pubescens* resulted in the development of sparse clamp connections in the *C. versicolor* culture but not in the opposing *C. pubescens* culture. Similarly, eleven of the twelve pairings between monospore isolates of *C. versicolor* and *C. hirsutus* resulted in the more abundant development of clamp connections throughout the *C. versicolor* culture (Figure 133), but again there were no clamps to be found in the *C. hirsutus* culture. Subsequently, monospore isolates from the three *C. versicolor* basidiocarps were paired with monospore isolates derived from three different basidiocarps of *C. hirsutus* (Figures 106, 107, 108). Thirty-four of the forty-eight pairings resulted in the unilateral dikaryotization of the *C. versicolor* cultures, and in no case were clamp connections observed in the *C. hirsutus* cultures. Dark lines were again observed in many of these pairings and frequently, dark pigmented areas appeared in the dikaryotized culture (Figures 126, 127, 128, 129). Some of these 'hybrid' dikaryotic cultures were transferred to malt agar plates and later examined. In some of them there were many incompletely formed clamp connections which

appeared to be similar to the hooks previously described for some of the common B matings. The hooks appeared to contain a trapped nucleus and stained deeply with KOH-phloxine (Figure 132). Simple septa as well as normal clamp connections were observed in some of these 'hybrid' cultures, particularly at the growing edge of the mycelium.

It was considered desirable in this study to attempt pairings between monospore isolates of Alberta collections of *C. hirsutus* and *C. pubescens* and European cultures of these species. Four fresh basidiocarps of *C. hirsutus* were received from Sweden and monospore isolates were prepared from each of the basidiocarps. These monospore isolates were paired with monospore isolates derived from three, local basidiocarps of *C. hirsutus* (Figures 109, 110, 111). Clamp connections were not observed to form in any of the pairings and almost all pairings formed a strong dark line between the two cultures (Figures 130, 131). In addition, the Swedish monospore isolates were paired with monospore isolates of *C. pubescens* from Alberta and *C. versicolor* from Ontario (Figures 112, 113). In no case were clamp connections observed in the paired cultures, but dark lines were formed, especially in the pairings with *C. versicolor*.

Cultures of *C. hirsutus* and *C. pubescens*, together with their respective voucher collections, were obtained from the Plant Research Institute, Ottawa. These cultures were dikaryotic and an attempt was made to use the Buller phenomenon to prove the conspecificity of local isolates

with the Ottawa ones. The first set of cultures received from the Plant Research Institute was paired with monospore cultures derived from local basidiocarps of *C. hirsutus* and *C. pubescens*. The paired cultures were examined after 4 weeks to determine if the monospore cultures had become dikaryotic and had developed clamp connections (Figures 114, 115). None of the monospore cultures of *C. hirsutus* became dikaryotic, and only one of the Ottawa cultures of *C. pubescens* was found to have dikaryotized the local monospore isolates. Culture # DAOM 17540, reputed to be *C. pubescens*, was found on closer examination to be *Trametes suaveolens* Fries. Of the six cultures originally received from the Plant Research Institute, two were now eliminated from further tests. The four remaining cultures, together with a further six cultures received from the same source, were paired with monospore isolates derived from other local basidiocarps (Figures 116, 117). Three of the dikaryotic cultures of *C. pubescens* proved able to dikaryotize the local monospore isolates of the same species.

It should be noted that the condition of many of the dikaryotic cultures was very poor in that they grew slowly and the mycelium was thin, sparse and developed flat on the agar. This degenerate condition probably reflects the length of time the cultures had been in storage, 41 years for DAOM F1304. More details of the isolation dates of these cultures together with data on their respective voucher collections are given in Appendix III.

DISCUSSION

The results of my studies confirm those of Kimura (1954) who reported that *C. hirsutus* is tetrapolar. The failure of other workers to recognize this fact is, in part, due to the method they employed to culture the paired isolates. Frequently, monospore isolates have been paired in narrow culture tubes and only one sample of mycelium from the contact line of each pairing has been examined for clamp connections. As has been indicated earlier, in certain pairings involving common B factors, there is a very real possibility that incompletely formed clamp connections or hooks will be produced in the contact zone. In the past, these have been mistaken for normal clamps with consequent incorrect interpretation of results. Macrae (1941, 1966), for example, originally concluded that *Hirschioporus abietinus* was bipolar, but later reported it was tetrapolar and that her original studies had erred in recording common B matings as completely fertile. In order to avoid this kind of error, the monospore isolates used in this study were paired in 9 cm petri dishes and at least three samples of mycelium from each pairing were checked for the presence of clamp connections or hooks. One sample was taken from the contact line and the other two from each side of the contact line at a point at least one centimeter from it. By this technique, mating reactions which resulted in the formation of hooks in the contact line and not throughout

the rest of the mycelium could be detected. From the results of this study, both *C. hirsutus* and *C. pubescens* can confidently be reported to have the tetrapolar type of sexuality.

The four mating types may not be detected when only small samples of spores are paired. From these and other observations, it appears that at least ten and preferably twelve spores should be paired in all combinations in order that the four mating types be recovered. A theoretical mathematical formula for determining the probability of the four mating types being present in variously sized samples of spores has been derived and is given in Appendix IV. The probability that a sample of 15 spores will contain the four mating types is 0.95, while for a sample of 13 spores, the probability is 0.91.

The formation of hooks in certain pairings involving common B matings confirms the separate functions of the two loci of the tetrapolar system and lends support to the conclusions of Raper (1966) regarding the function of each locus. Since it is now well established that different A factors are necessary for the formation of the hook cell, the formation of hooks in the contact line when #300-4 was paired with #300-7 is difficult to explain. Both of these monospore isolates are of the same mating type and when paired have both common A and common B factors. One possible explanation is that the A factor is composed of two very close loci and that a mutation or recombination occurred to change one of these loci, thus effectively

changing the mating type. Compound loci have been shown to occur in *Schizophyllum commune* and *Coprinus lagopus* (Day, 1965).

The work reported in this thesis has demonstrated that, in the study area, *C. hirsutus* and *C. pubescens* can be regarded as separate, distinct and intersterile species. Each species is composed of an interbreeding population which is genetically isolated from the other species. There is a prezygotic barrier to the hybridization between these species in the form of an inability to conjugate and develop a stable dikaryon. The isolation of each species is thus maintained and they are able to share the same habitat.

The philosophy underlying this study is based on the biological or natural species concept which considers that all members of a species should possess some ability to interbreed. The complete sterility which was observed to occur between the Canadian and the Swedish isolates of *C. hirsutus* was entirely unexpected. Nobles and Frew (1962) have shown that European and Canadian isolates of *Pycnoporus cinnabarinus* are completely interfertile, while Macrae (1966) has reported partial compatibility between Canadian and European isolates of *Hirschioporus abietinus*. On the basis of the forty-eight pairings made in this study between Swedish and Canadian isolates of *C. hirsutus* and the failure of each pairing to form a dikaryon, it is apparent that they are genetically isolated. The inescapable conclusion is that the Swedish taxon named *C. hirsutus* is a

different species from the Canadian taxon named *C. hirsutus*. Although many more pairings must be made between isolates taken from different geographic areas of Europe and Canada to determine if there is, indeed, any interbreeding, the evidence from the forty-eight pairings made and the evidence of the different macroscopic appearance of respective Swedish and Canadian basidiocarps (Chapter II), strongly suggest that they are separate species. The complete failure to form clamp connections or even hooks in the contact zone between the paired monospore isolates and the strong pigmentation which developed in the contact zone of these pairings suggest that the two taxa are not even closely related.

The unilateral dikaryotization which occurred when monospore isolates of *C. versicolor* and *C. hirsutus* were paired was also completely unexpected. Burnett (1956) has reported that attempts made by himself to hybridize *C. versicolor* and *C. hirsutus* were unsuccessful. Many of the dikaryotic cultures which developed from the pairings between *C. hirsutus* and *C. versicolor* in this study appeared to be unstable and were reverting to the monokaryotic condition. This reversion results when incomplete clamp connections are formed during cell division. Cell division only occurs in the terminal cells of the mycelium and the formation of a clamp connection provides a means of passing a nucleus back from the terminal to the subterminal cell. It has been observed in these 'hybrid' cultures that, in

many instances, the clamp connection had failed to fuse with the subterminal cell and had formed a hook containing a trapped nucleus. The subterminal cell will consequently only contain one nucleus and subsequent branches which arise from this cell will be monokaryotic and will develop into monokaryotic hyphae with simple septa. It is possible that the monokaryotic hyphae may have a faster growth than the dikaryotic ones and it is suggested that this is the reason why the growing edge of these cultures frequently have only simple septate hyphae.

It must be emphasized that the formation of an interspecific dikaryon is not to be taken as evidence of complete hybridization between the two species. It is only the first step in the process of sexual reproduction and must be followed by nuclear fusion, meiosis and the production of viable spores before it can be claimed as complete hybridization.

The occasional formation of clamp connections between species has been taken by Nobles and Frew (1962) to indicate the species are closely related. The formation of a dikaryon must therefore be considered as evidence that the two species, *C. hirsutus* and *C. versicolor*, are very closely related, but, it must be remembered that these two species have separate ranges and their possible interbreeding under natural conditions may be prevented by geographic separation.

The formation of dark lines between paired monospore

cultures has been almost totally ignored by other workers. Adams and Roth (1967) paired dikaryons of *Fomes cajanderi* and reported that "the dark lines of demarcation which frequently form at the interface of paired colonies constitutes a reliable basis for distinguishing genetically distinct mycelia. They also stated that when cultures of the pair were distantly related demarcation lines formed 95-100% of the time and when cultures were of closest relationship lines formed approximately 50% of the time". The results of this study conflict with the statements reported above. The formation of a dark line between sexually compatible, monospore isolates from the same basidiocarp has been observed in several instances. It has been demonstrated also that two dikaryotic cultures with the same nuclear composition can interact and form dark pigmented areas in the contact zone (Figure 62). In other words, closely related cultures can interact to produce dark lines. The significance of this observation is that factors other than the nuclear composition of the dikaryon play a part in inducing the formation of pigment areas. Cytoplasmic factors seem to be implicated although the nature or location of these factors is as yet unknown. The formation of dark lines between paired monospore isolates of the same species appears to be unrelated to mating type, since monospore isolates of the same mating type have shown different reactions when paired with another monospore isolate. Monospore isolates also appear to differ in their

ability to form dark lines, some almost always forming them, while others rarely form them. Dark lines do not prevent anastomosis, nor do they prohibit nuclear exchange. They do not develop until after anastomosis has taken place and cannot therefore be considered a barrier to conjugation. Obviously, much more work remains to be done in this area before the basis for pigment formation is completely understood.

To conclude this chapter, it is proposed to devote some space in defence of the biological species concept and in evaluating the biological significance of the dikaryon.

The true biological significance of the dikaryon and the advantages it bestows on the Basidiomycetes have so far eluded the minds of mycologists. Mather (1965) has stated, "the association of two haploid nuclei in the dikaryon of a Basidiomycete offers no advantage for the development of the fungus that could not equally well be secured by their fusion, yet they are kept apart while maintained in step by the elaborate mechanisms of synchronous division and clamp connections. And the moment they fuse, meiosis sets in. This suggests that these fungi have not, so to speak, mastered the problem of managing a diploid nucleus, that they do not possess the refined genetic balance to secure the accurate timing in relation to one another of the cycles of chromosome division and nuclear division necessary for diploid mitosis to follow a normal path, undisturbed by chance partial and uncontrolled pairing of homologues with

an irregular mixture of mitotic and quasi-meiotic behaviour as the result". Raper (1966) has stressed the importance of the dikaryon as a locale for somatic recombination and nuclear selection, and its consequent increased capability of adaptive alterations in response to environmental change.

The Basidiomycetes possess two unique features. The first is the formation of a dikaryotic mycelium composed of binucleate cells and the concomitant development of clamp connections to facilitate cell division. The second is the ability of individual mycelia of the same species to anastomose and form one mycelium which acts as a single physiological unit. The significance of anastomosis seems to have escaped the attention it deserves and a new concept will be presented to account for the significance of the dikaryon and of anastomosis, for the two are related.

It is a well established fact that multiple infections commonly occur with wood decay fungi and that the freshly exposed surface of a tree branch, for example, will be infected with a large number of spores. These spores germinate and give rise to haploid mycelia which will become dikaryotic by mutual dikaryotization or by anastomosis with a dikaryotic mycelium through the Buller phenomenon, the latter being, according to Raper (1966), "the more prevalent and perhaps predominant means in nature". The end result is a unified mycelium which is genetically a mosaic containing nuclei of many types, yet the whole mycelium is a single physiological unit which will

eventually produce the basidiocarps.

In order to appreciate the significance of the dikaryon, it is necessary to hypothetically consider what might happen if, like most other organisms, conjugation was immediately followed by nuclear fusion. In this case, two haploid mycelia coming into contact would anastomose and the two nuclei in each fusion cell would join and form a single diploid nucleus. Diploidization would be confined to individual cells which had formed by anastomosis along the line of contact of the two haploid mycelia. Haploid nuclei from one mycelium would be unable to migrate through the other mycelium since they would not pass through the diploid cells joing the two mycelia. Eventually, by anastomosis of the two mycelia, many of the cells would become diploid. Supposing a cell of a third haploid mycelium now came into contact with a diploid cell. If anastomosis took place, an exchange of nuclei would be impossible and the haploid mycelium would remain in its haploid condition. The end result would be the establishment of separate, individual diploid and haploid mycelia which would be in direct competition with one another. It must be stressed firstly that the substrate on which a wood decay fungus is growing is limited by the size of the wood and the proportion of it which the mycelium is able to colonize. Secondly, considerable resources are needed to produce a basidiocarp. In this hypothetical case where diploid and haploid mycelia existed as discrete units, there would be direct competition

between each mycelium for the substrate and each would lack the resources necessary for the production of a basidiocarp.

An appreciation of the significance of the dikaryon and the role it plays, may also be gained by considering the life cycle of these fungi. The haploid mycelium, resulting as it does from the germination of a single spore, is the equivalent of the gametophyte generation. The diploid sporophyte generation is represented by a short stage in the development of the basidium. The haploid mycelium, being the gametophyte generation produces the gametes. There are, however, no specialized gametangia formed by these fungi. The hyphal tips are the structures which contain the haploid nuclei and act in the role of gametangia. By their fusion somatogamy is achieved and a dikaryon is formed. Karyogamy is delayed with the result that the dikaryon becomes a long intermediate stage in the life cycle. The dikaryon can be regarded as a third phase which has retained the ability to act as a gametophyte while being the physiological equivalent of a diploid.

The fundamental biological significance of the dikaryon then, is firstly, to enable haploid and dikaryotic mycelia to link up and join forces, so to speak, in order to utilize all of the substrate and produce the optimum number of basidiocarps at the most suitable locations. Secondly, the formation of the dikaryon eliminates intraspecific competition while maintaining the ability to compete with other species. The elimination of intraspecific competition is a

unique feature of the higher fungi and is unknown in any other organism. It gives them a tremendous advantage in the struggle for existence and its importance cannot be too strongly stressed. The development of the dikaryotic phase of the life cycle has provided the Basidiomycetes with a mechanism which has all the advantages of the usual diploid system and, in addition, has the added benefit of eliminating competition between mycelia of the same species.

The problem of answering the question "what is a species" is one with which biologists have grappled for many years. It is a particularly difficult problem for the mycologist since the study of populations of individuals is prevented by the ability of the higher fungi to anastomose so that the identity of individuals becomes lost. Traditional studies of Basidiomycetes have been based on the taxonomic species concept. This concept groups together individuals which share certain characters of morphology and anatomy of their basidiocarps. Since there is considerable variation in the appearance of genetically related basidiocarps, species descriptions have been based on particular types of basidiocarps and every mycologist has had his own opinion of the acceptable deviation from a particular type. The biological species concept allows for the wide morphological variation which can exist between individual basidiocarps of the same species. Mayr (1940) has defined the biological species as "actually or potentially interbreeding populations which are reproductively

isolated from other such groups". In other words, instead of arbitrarilly drawing the line between the taxonomic species, we should let nature draw it for us by the use of interfertility tests. For the large group of polypores which form clamp connections, interfertility tests are a simple procedure. The advantage is that interfertility tests will always give the same result, no matter who is carrying them out. The results are in most cases indisputable. It is therefore of utmost importance that interfertility tests be carried out and used to determine the extent of variation within an interbreeding population, so that the variation can be described and incorporated in the species description.

TABLE 3. Distribution of Mating Type Factors

Figure	Basidiocarp	Mating type	Isolate
63	207	A1B1	1,6,8.
		A2B2	2,13,14,15.
		A1B2	9,10,12.
		A2B1	3.7.
64	240	A1B1	2,5.
		A2B2	13,22,23,33,35.
		A1B2	3,14,34.
		A2B1	4,19.
65	237	A1B1	36,52,63.
		A2B2	13,45,68,69,70.
		A1B2	40,42,48.
		A2B1	67.
66	300	A1B1	1,9,11,12.
		A2B2	4,7,13.
		A1B2	2,3,6.
		A2B1	5,10,15.
67	301	A1B1	1,3,4,6.
		A2B2	2,5.
		A1B2	7.
		A2B1	8.
68	303	A1B1	1,2,3.
		A2B2	5,6.
		A1B2 or A2B1	4.

In Figures 63-117 the following symbols are used:-

+ indicates bilateral dikaryotization.

- indicates intersterility.

* indicates unilateral dikaryotization.

h indicates formation of incomplete clamps in contact zone.

p indicates formation of dark line in contact zone.

o indicates pairing not done.

FIGURE 63. Monospore isolates of #207 *C. hirsutus* paired in all combinations.

FIGURE 64. Monospore isolates of #240 *C. hirsutus* paired in all combinations.

63 #207

#207	1	6	8	3	7	9	10	12	2	13	14	15
1		−	−	−	−	−	−	−	+	+	+	+
6	−		−	−	−	−	−	−	+	+	+	+
8	−	−		−	−	−	−	−	+	+	+	p+
3	−	−	−		−	+	+	+	−	−	−	−
7	−	−	−	−		+	+	+	−	−	−	−
9	−	−	−	+	+		−	−	−	−	−	−
10	−	−	−	+	+	−		−	−	−	−	p−
12	−	−	−	+	+	−	−		−	−	−	−
2	+	+	+	−	−	−	−	−		−	−	p−
13	+	+	+	−	−	−	−	−	−		−	−
14	+	+	+	−	−	−	−	−	−	−		−
15	+	+	p+	−	−	−	p−	−	p−	−	−	

64 #240

#240	2	5	3	14	34	4	19	13	22	23	33	35
2		−	−	−	−	−	−	+	+	+	+	+
5	−		−	−	−	−	−	+	+	+	+	+
3	−	−		−	−	+	+	−	−	−	−	−
14	−	−	−		−	+	+	−	−	−	−	−
34	−	−	−	−		+	+	−	−	−	−	−
4	−	−	+	+	+		−	−	−	−	−	−
19	−	−	+	+	+	−		−	−	−	−	−
13	+	+	−	−	−	−	−		−	−	−	−
22	+	+	−	−	−	−	−	−		−	−	−
23	+	+	−	−	−	−	−	−	−		−	−
33	+	+	−	−	−	−	−	−	−	−		−
35	+	+	−	−	−	−	−	−	−	−	−	

FIGURE 65. Monospore isolates of #237 *C. pubescens* paired in all combinations.

FIGURE 66. Monospore isolates of #300 *C. pubescens* paired in all combinations.

65 #237

#237	36	52	63	40	42	48	67	13	45	68	69	70
36		−	−	−	−	−	−	+	+	+	+	+
52	−		−	−	−	−	−	+	+	+	+	+
63	−	−		−	−	−	−	+	+	+	+	+
40	−	−	−		−	−	+	−	−	−	−	−
42	−	−	−	−		−	+	−	−	−	−	−
48	−	−	−	−	−		+	−	−	−	−	−
67	−	−	−	+	+	+		−	−	−	−	−
13	+	+	+	−	−	−	−		−	−	−	−
45	+	+	+	−	−	−	−	−		−	−	−
68	+	+	+	−	−	−	−	−	−		−	−
69	+	+	+	−	−	−	−	−	−	−		−
70	+	+	+	−	−	−	−	−	−	−	−	

66 #300

#300	1	9	11	12	5	10	15	2	3	6	4	7	13
1		−	−	p−	−	p−	−	−	−	−	+	+	+
9	−		−	−	−	p−	p−	−	−	−	+	+	+
11	−	−		−	−	ph	p−	−	−	−	+	+	+
12	p−	−	−		−	ph	p−	−	p−	−	+	+	+
5	−	−	−	−		−	−	+	+	+	−	−	−
10	p−	p−	ph	ph	−		−	p+	p+	p+	−	−	−
15	−	p−	p−	p−	−	−		+	+	+	−	−	−
2	−	−	−	−	+	p+	+		−	−	p−	p−	p−
3	−	−	−	p−	+	p+	+	−		−	−	p−	p−
6	−	−	−	−	+	p+	+	−	−		h	p−	h
4	+	+	+	+	−	−	−	p−	−	h		ph	−
7	+	+	+	+	−	−	−	p−	p−	p−	h		−
13	+	+	+	+	−	−	−	p−	p−	h	−	−	

FIGURE 67. Monospore isolates of #301 *C. hirsutus* paired in all combinations.

FIGURE 68. Monospore isolates of #303 *C. hirsutus* paired in all combinations.

FIGURE 69. Monopore isolates of #240 *C. hirsutus* paired with those of #237 *C. pubescens*.

67 #301

#301	1	3	4	6	7	8	2	5
1		−	p−	−	−	−	+	+
3	−		−	−	−	p−	+	+
4	p−	−		−	−	−	+	+
6	−	−	−		−	p−	+	+
7	−	−	−	−		+	p−	p−
8	−	p−	−	p−	+		−	−
2	+	+	+	+	p−	−		−
5	+	+	+	+	p−	−	−	

68 #303

#303	1	2	3	4	5	6
1		−	−	p−	+	+
2	−		−	−	+	+
3	−	−		−	p+	+
4	p−	−	−		−	−
5	+	+	p+	−		−
6	+	+	+	−	−	

69 #240

#237	2	4	5	13	14	19	22	23	33	34
13	p−	p−	p−	p−	p−	p−	p−	p−	p−	p−
36	p−	p−	p−	p−	p−	p−	p−	p−	p−	p−
42	p−	p−	p−	p−	p−	p−	p−	p−	p−	p−
48	p−	p−	p−	p−	p−	p−	p−	p−	p−	p−
52	p−	p−	p−	p−	p−	p−	p−	p−	p−	p−
63	p−	p−	p−	p−	p−	p−	p−	p−	p−	p−
67	p−	p−	p−	p−	p−	p−	p−	p−	p−	p−
68	p−	p−	p−	p−	p−	p−	p−	p−	p−	p−
69	p−	p−	p−	p−	p−	p−	p−	p−	p−	p−
70	p−	p−	p−	p−	p−	p−	p−	p−	p−	p−

FIGURE 70. Monospore isolates of #207 *C. hirsutus* paired with those of #240 *C. hirsutus*.

FIGURE 71. Monospore isolates of #207 *C. hirsutus* paired with those of #237 *C. pubescens*.

FIGURE 72. Monospore isolates of #301 *C. hirsutus* paired with those of #300 *C. pubescens*.

FIGURE 73. Monospore isolates of #300 *C. pubescens* paired with those of #237 *C. pubescens*.

FIGURE 74. Monospore isolates of #301 *C. hirsutus* paired with those of #240 *C. hirsutus*.

FIGURE 75. Monospore isolates of #231 *C. hirsutus* paired with those of #237 *C. pubescens*.

FIGURE 76. Monospore isolates of #213 *C. hirsutus* paired with those of #240 *C. hirsutus*.

FIGURE 77. Monospore isolates of #214 *C. pubescens* paired with those of #237 *C pubescens*.

70 #207

#240

	1	7	12	15
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
13	+	+	+	+

71 #207

#237

	1	7	12	15
36	p−	p−	p−	p−
42	p−	p−	p−	p−
67	p−	p−	p−	p−
69	p−	p−	p−	p−

72 #301

#300

	1	2	3	4
1	p−	p−	p−	p−
2	p−	p−	p−	p−
4	p−	p−	p−	p−
5	p−	p−	p−	p−

73 #300

#237

	1	2	3	4	13	14	15	16
36	+	+	+	+	+	+	+	+
42	+	+	+	+	+	+	+	+
67	+	+	+	+	+	+	+	+
69	+	+	+	+	+	+	+	+

74 #301

#240

	1	2	3	4
2	+	+	+	p+
3	+	+	+	+
4	p+	+	+	p+
13	+	+	+	+

75 #213

#237

	10	14	16	19
36	p−	p−	p−	p−
42	p−	p−	p−	p−
67	p−	p−	p−	p−
69	p−	p−	p−	p−

76 #213

#240

	10	14	16	19
2	p+	+	p+	p+
3	p+	+	p+	+
4	p+	p+	p+	p+
13	p+	+	+	+

77 #214

#237

	1	4	7	10
36	+	+	+	+
40	+	+	+	+
67	+	+	+	+
69	+	+	+	+

FIGURE 78. Monospore isolates of #214 *C. pubescens* paired with those of #240 *C. hirsutus*.

FIGURE 79. Monospore isolates of #216 *C. pubescens* paired with those of #237 *C. pubescens*.

FIGURE 80. Monospore isolates of #216 *C. pubescens* paired with those of #240 *C. hirsutus*.

FIGURE 81. Monospore isolates of #222 *C. hirsutus* paired with those of #237 *C. pubescens*.

FIGURE 82. Monospore isolates of #222 *C. hirsutus* paired with those of #240 *C. hirsutus*.

FIGURE 83. Monospore isolates of #223 *C. hirsutus* paired with those of #237 *C. pubescens*

FIGURE 84. Monospore isolates of #223 *C. hirsutus* paired with those of #240 *C. hirsutus*

FIGURE 85. Monospore isolates of #233 *C. pubescens* paired with those of #237 *C. pubescens*.

78 #214

#240	1	4	7	10
2	p-	p-	p-	p-
3	p-	p-	p-	p-
4	p-	p-	p-	p-
13	p-	p-	p-	p-

79 #216

#237	3	6	7	14
36	+	+	+	+
40	+	+	+	+
67	+	+	+	+
69	+	+	+	+

80 #216

#240	3	6	7	14
2	p-	p-	p-	p-
3	p-	p-	p-	p-
4	p-	p-	p-	p-
13	p-	p-	p-	p-

81 #222

#237	2	6	11	14
36	p-	p-	p-	p-
40	p-	p-	p-	p-
67	p-	p-	p-	p-
69	p-	p-	p-	p-

82 #222

#240	2	6	11	14
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
13	+	+	+	+

83 #223

#237	4	7	9	11
36	p-	p-	p-	p-
40	p-	p-	p-	p-
67	p-	p-	p-	p-
69	p-	p-	p-	p-

84 #223

#240	4	7	9	11
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
13	+	+	+	+

85 #233

#237	3	8	10	12
36	+	+	+	+
42	+	+	+	+
67	+	+	+	+
69	+	+	+	+

FIGURE 86. Monospore isolates of #233 *C. pubescens* paired with those of #240 *C. hirsutus*.

FIGURE 87. Monospore isolates of #261 *C. pubescens* paired with those of #300 *C. pubescens*.

FIGURE 88. Monospore isolates of #266 *C. pubescens* paired with those of #237 *C. pubescens*.

FIGURE 89. Monospore isolates of #271 *C. hirsutus* paired with those of #240 *C. hirsutus*.

FIGURE 90. Monospore isolates of #272 *C. pubescens* paired with those of #237 *C. pubescens*.

FIGURE 91. Monospore isolates of #276 *C. pubescens* paired with those of #237 *C. pubescens*.

FIGURE 92. Monospore isolates of #290 *C. hirsutus* paired with those of #240 *C. hirsutus*.

FIGURE 93. Monospore isolates of #293 *C. pubescens* paired with those of #237 *C. pubescens*.

86 #233

#240

	3	8	10	12
2	–	–	–	–
3	–	p–	–	–
4	p–	p–	p–	–
13	–	p–	–	p–

87 #261

#300

	1	2	3	4
1	+	+	+	+
2	+	+	+	+
4	+	+	+	+
5	+	+	+	+

88 #266

#237

	1	2	3	4
36	+	+	+	+
42	+	+	+	+
67	+	+	+	+
69	+	+	+	+

89 #271

#240

	1	2	4	6
2	p+	p+	+	+
3	p+	p+	+	+
4	p+	p+	p+	p+
13	p+	p+	+	+

90 #272

#237

	4	5	6	7
36	+	+	+	+
42	+	+	+	+
67	+	+	+	+
69	+	+	+	+

91 #276

#237

	6	7	8	9
36	+	+	+	+
42	+	+	+	+
67	+	+	+	+
69	+	+	+	+

92 #290

#240

	1	2	3	4
2	p+	+	+	+
3	p+	p+	+	+
4	+	*	+	+
13	p+	+	p+	p+

93 #293

#237

	1	2	3	4
36	+	+	+	+
40	+	+	+	+
67	+	+	+	+
69	+	+	+	+

FIGURE 94. Monospore isolates of #293 *C. pubescens* paired with those of #240 *C. hirsutus*.

FIGURE 95. Monospore isolates of #299 *C. hirsutus* paired with those of #240 *C. hirsutus*.

FIGURE 96. Monospore isolates of #299 *C. hirsutus* paired with those of #300 *C. pubescens*.

FIGURE 97. Monospore isolates of #303 *C. hirsutus* paired with those of #207 *C. hirsutus*.

FIGURE 98. Monospore isolates of #303 *C. hirsutus* paired with those of #237 *C. pubescens*.

FIGURE 99. Monospore isolates of #303 *C. hirsutus* paired with those of #240 *C. hirsutus*.

FIGURE 100. Monospore isolates of #304 *C. hirsutus* paired with those of #240 *C. hirsutus*.

FIGURE 101. Monospore isolates of #306 *C. hirsutus* paired with those of #240 *C. hirsutus*

94 #293

#240

	1	2	3	4
2	p-	p-	p-	p-
3	p-	p-	p-	p-
4	p-	p-	p-	p-
13	p-	p-	p-	p-

95 #299

#240

	1	2	3	4
2	+	+	+	+
3	+	+	+	+
4	p+	p+	p+	p+
13	+	+	+	+

96 #299

#300

	1	2	3	4
1	p-	p-	p-	p-
2	p-	p-	p-	p-
4	p-	p-	p-	p-
5	p-	p-	p-	p-

97 #303

#207

	1	2	3	4
1	p+	p+	p+	p+
2	+	+	+	+
3	p+	p+	p+	p+
9	+	+	+	+

98 #303

#237

	1	2	3	4
36	p-	p-	p-	p-
42	p-	p-	p-	p-
67	p-	p-	p-	p-
69	p-	p-	p-	ph

99 #303

#240

	1	2	3	4	5	6	7	8
2	-	ph	p-	h	+	+	+	+
3	+	+	+	+	+	+	+	+
4	-	-	-	-	+	p+	p+	p+
13	p+	+	+	+	+	+	+	+

100 #304

#240

	1	2	3	4
2	+	p+	p+	p+
3	p+	+	+	+
4	+	+	+	p+
13	+	+	+	+

101 #306

#240

	1	2	3	4
2	p+	+	+	p+
3	p+	+	+	p+
4	p+	p+	p+	p+
13	+	+	+	+

FIGURE 102. Monospore isolates of #309 *C. pubescens* paired with those of #237 *C. pubescens*.

FIGURE 103. Monospore isolates of #300 *C. pubescens* paired with others from the same collection.

FIGURE 104. Monospore isolates of #300 *C. pubescens* paired with those of #414, #415 and #416 *C. versicolor*.

FIGURE 105. Monospore isolates of #301 *C. hirsutus* paired with those of #414, #415 and #416 *C. versicolor*.

FIGURE 106. Monospore isolates of #414 *C. versicolor* paired with those of #301 *C. hirsutus*.

FIGURE 107. Monospore isolates of #415 *C. versicolor* paired with those of #299 *C. hirsutus*

FIGURE 108. Monospore isolates of #416 *C. versicolor* paired with those of #306 *C. hirsutus*.

FIGURE 109. Monospore isolates of #421 *C. hirsutus* (from Sweden) paired with those of #301 *C. hirsutus*.

102 #309

#237	1	2	3	4
36	+	+	+	+
42	+	+	+	+
67	+	+	+	+
69	+	+	+	+

103 #300

#300	13	14	15	16
1	+	–	–	–
2	p–	–	+	–
3	p–	–	+	–
4	–	p–	–	p–

104 #300

	1	2	3	4
#414 – 1	p*	p–	o	o
#414 – 2	p–	p–	o	o
#415 – 1	o	o	p–	p–
#415 – 2	o	o	p–	*
#416 – 1	o	p–	p–	o
#416 – 2	o	p–	p–	o

105 #301

	1	2	3	4
#414 – 1	p*	p*	o	o
#414 – 2	p*	p*	o	o
#415 – 1	o	o	p*	–
#415 – 2	o	o	p*	p*
#416 – 1	o	p*	p*	o
#416 – 2	o	*	*	o

106 #414

#301	1	2	3	4
1	p*	p*	p*	p–
2	p*	p*	p*	p–
3	p*	p*	p*	p–
4	p*	p*	p*	ph

107 #415

#299	1	2	3	4
1	p–	*	p*	–
2	p–	p*	p–	–
3	p–	*	p*	–
4	p*	*	p*	–

108 #416

#306	1	2	3	4
1	*	–	*	p*
2	*	p*	–	p*
3	*	*	*	p*
4	*	*	p*	p*

109 #421

#301	1	2	3	4
1	p–	p–	p–	p–
2	p–	p–	p–	p–
3	p–	p–	p–	p–
4	p–	p–	p–	p–

FIGURE 110. Monospore isolates of #422 *C. hirsutus* (from Sweden) paired with those of #299 *C. hirsutus*.

FIGURE 111. Monospore isolates of #424 *C. hirsutus* (from Sweden) paired with those of #306 *C. hirsutus*.

FIGURE 112. Monospore isolates of #423 *C. hirsutus* (from Sweden) paired with those of #300 *C. pubescens*.

FIGURE 113. Monospore isolates of #414 *C. versicolor* paired with those of #422 *C. hirsutus*

FIGURE 114. Monospore isolates of #240 *C. hirsutus* paired with dikaryotic cultures from the Plant Research Institute, Ottawa.

FIGURE 115. Monospore isolates of #237 *C. pubescens* paired with dikaryotic cultures from the Plant Research Institute, Ottawa.

110 #422

#299

	1	2	3	4
1	p-	p-	p-	p-
2	p-	p-	-	p-
3	p-	p-	-	p-
4	p-	p-	p-	p-

111 #424

#306

	1	2	3	4
1	p-	p-	p-	p-
2	p-	p-	p-	p-
3	p-	p-	p-	p-
4	p-	p-	p-	p-

112 #423

#300

	1	2	3	4
1	-	-	-	-
2	-	p-	p-	-
3	-	p-	p-	-
4	-	-	p-	-

113 #414

#422

	1	2	3	4
1	p-	p-	p-	-
2	p-	p-	p-	-
3	p-	p-	p-	-
4	p-	p-	p-	-

114 #240

DAOM

	2	3	4	13
F9884	p-	p-	p-	p-
10240	p-	-	p-	p-
21158	p-	-	-	p-
17540	p-	p-	p-	p-
73309	p-	p-	p-	p-
94026	p-	p-	p-	p-

115 #237

DAOM

	36	42	67	69
F9884	-	p-	p-	p-
10240	-	p-	p-	p-
21158	p-	-	p-	p-
17540	p-	p-	p-	p-
73309	p-	+	p+	p+
94026	p-	p-	p-	p-

FIGURE 116. Monospore isolates of #301 *C. hirsutus* paired with dikaryotic cultures from the Plant Research Institute, Ottawa.

FIGURE 117. Monospore isolates of #300 *C. pubescens* paired with dikaryotic cultures from the Plant Research Institute, Ottawa.

116 #301

DAOM	1	2	3	4
F9884	-	-	p-	-
10240	p-	-	p-	-
21158	p-	-	-	p-
94026	p-	p-	p-	p-
94064	p-	-	-	p-
72381	-	-	-	-
F1304	-	p-	-	-
94039	p-	-	p-	p-
53503	p-	p-	p-	p-
94017	-	-	p-	p-

117 #300

DAOM	1	2	4	5
F9884	-	-	-	-
10240	-	-	-	-
21158	-	-	-	-
94026	p-	-	-	-
94064	-	-	-	-
72381	-	-	-	-
F1304	-	-	-	-
94039	+	+	+	+
53503	+	+	+	+
94017	+	+	+	+

FIGURE 118. Variation in dark line formation. #300-5 *C. pubescens* paired with four monospore isolates of #301 *C. hirsutus*.

#300-5 X #301-1 #300-5 X #301-2

#300-5 X #301-3 #300-5 X #301-4

FIGURE 119. Variation in dark line formation. #216-3 *C. pubescens* paired with four monospore isolates of #240 *C. hirsutus*.

#216-3 X #240-2 #216-3 X #240-3

#216-3 X #240-4 #216-3 X #240-13

FIGURE 120. Variation in dark line formation. Two monospore isolates of #303 *C. hirsutus* paired with three monospore isolates of #237 *C. pubescens*.

FIGURE 121. Dark line formation between monospore isolates of *C. hirsutus*.

#271-2 X #240-13 #271-2 X #240-2

#271-2 X #240-3 #271-2 X #240-4

FIGURE 122. Dark line formation between monospore isolates of *C. hirsutus*. Compare with Figure 59.

FIGURE 123. Dark line formation between compatible isolates from a single basidiocarp of *C. hirsutus*. #207-15 X #207-8

FIGURE 124. Reciprocal pairings of dikaryotic cultures. The two cultures on each plate have the same nuclear composition but differ in cytoplasmic

factors.

#271-1/#240-4

#271-2/#240-3

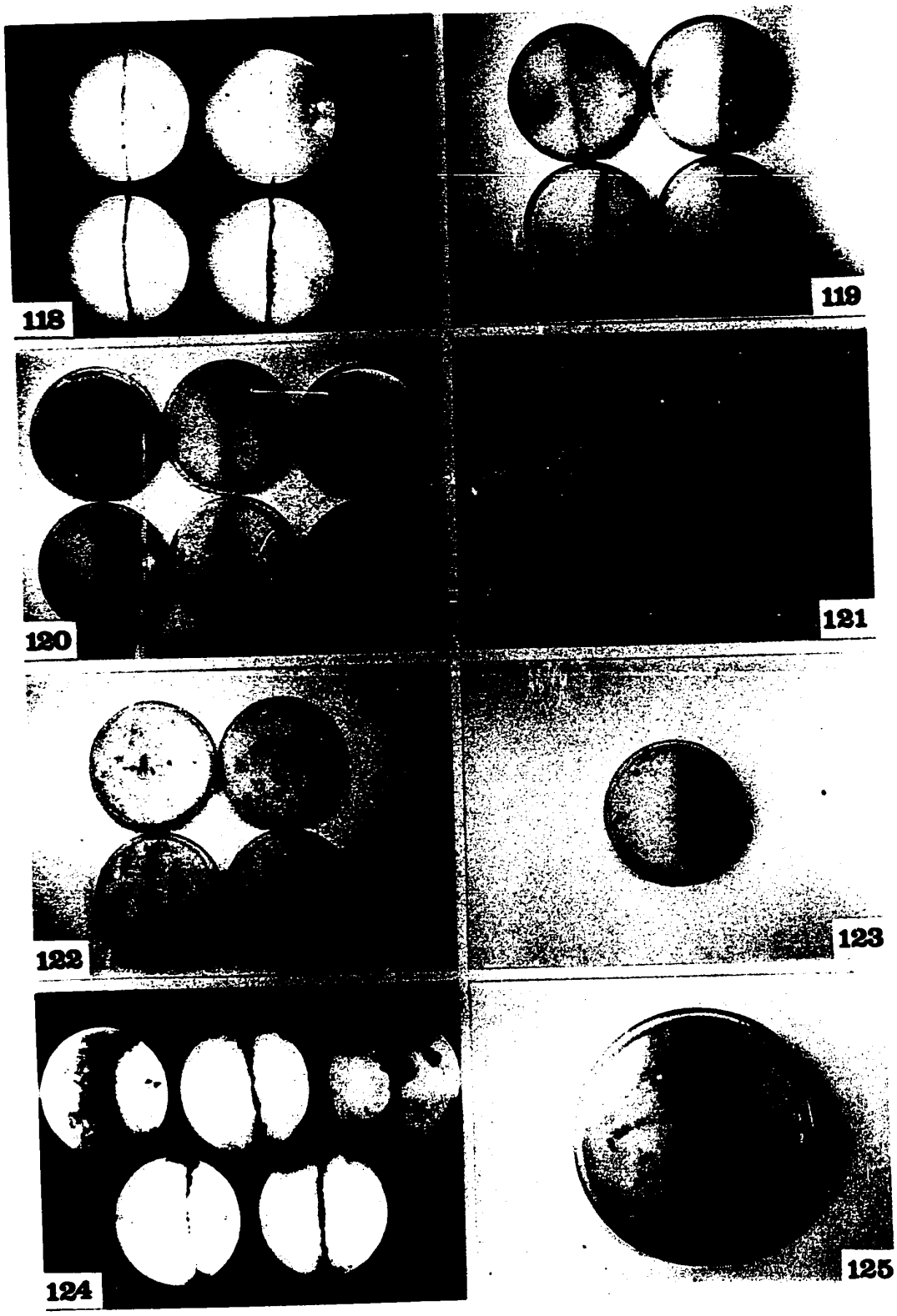
#271-2/#240-13

#272-2/#240-2

#271-2/#240-4

FIGURE 125. Double line formation between paired monospore isolates of *C. hirsutus*.

#306-4 X #240-2.


118
119
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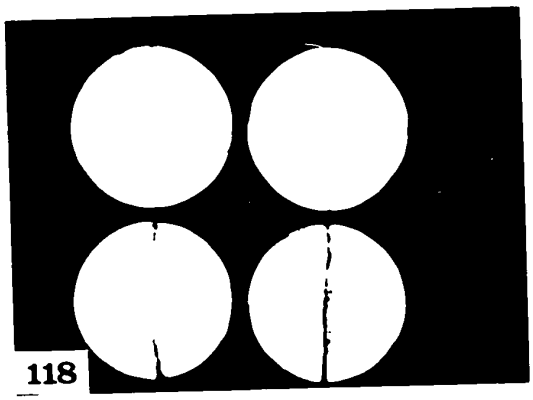
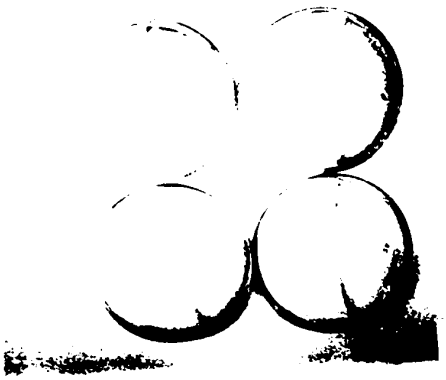


118



119

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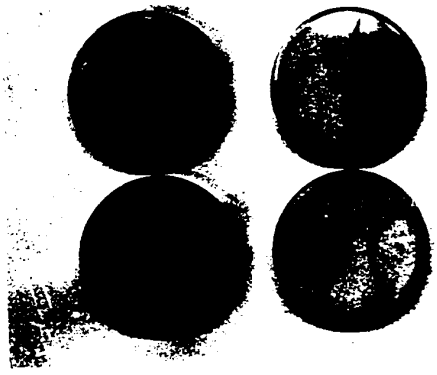

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125

FIGURES 126-129. Pairings between monospore isolates of #414 *C. versicolor* and #301 *C. hirsutus*.

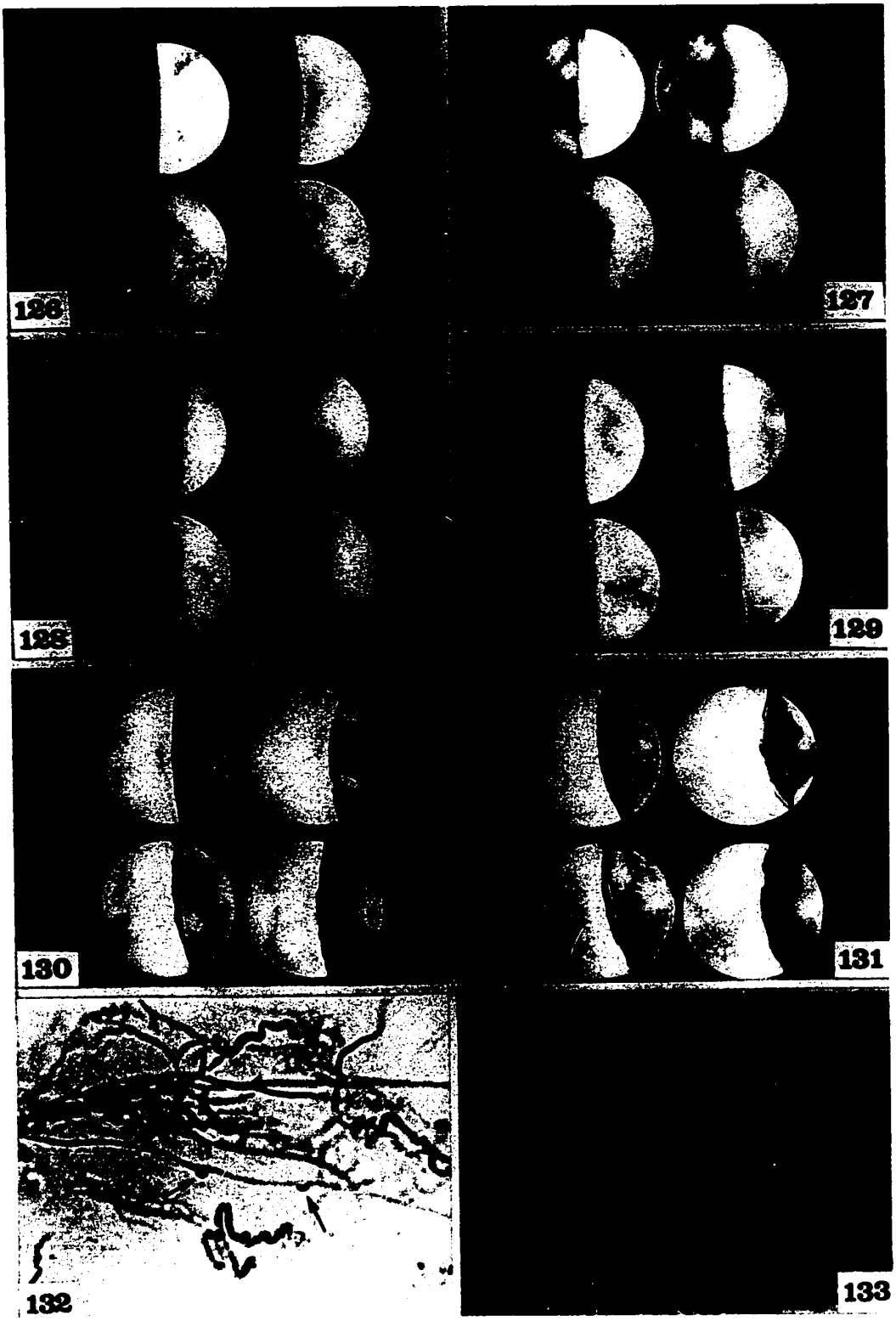
FIGURE 126.	#414-1 X #301-1	#414-1 X #301-2
	#414-1 X #301-3	#414-1 X #301-4
FIGURE 127.	#414-2 X #301-1	#414-2 X #301-2
	#414-2 X #301-3	#414-2 X #301-4
FIGURE 128.	#414-3 X #301-1	#414-3 X #301-2
	#414-3 X #301-3	#414-3 X #301-4
FIGURE 129.	#414-4 X #301-1	#414-4 X #301-2
	#414-4 X #301-3	#414-4 X #301-4

FIGURES 130-131. Pairings between monospore isolates of #301 *C. hirsutus* and #421 *C. hirsutus* from Sweden.

FIGURE 130.	#301-2 X #421-1	#301-2 X #421-2
	#301-2 X #421-3	#301-2 X #421-4
FIGURE 131.	#301-3 X #421-1	#301-3 X #421-2
	#301-3 X #421-3	#301-3 X #421-4

FIGURE 132. Incomplete clamp formation (arrows) in 'hybrid' cultures produced by pairing #415-1 *C. versicolor* with #301-3 *C. hirsutus*.

FIGURE 133. Normal clamp formation (arrows) in 'hybrid' cultures produced by pairing #415-2 *C. versicolor* with #301-3 *C. hirsutus*.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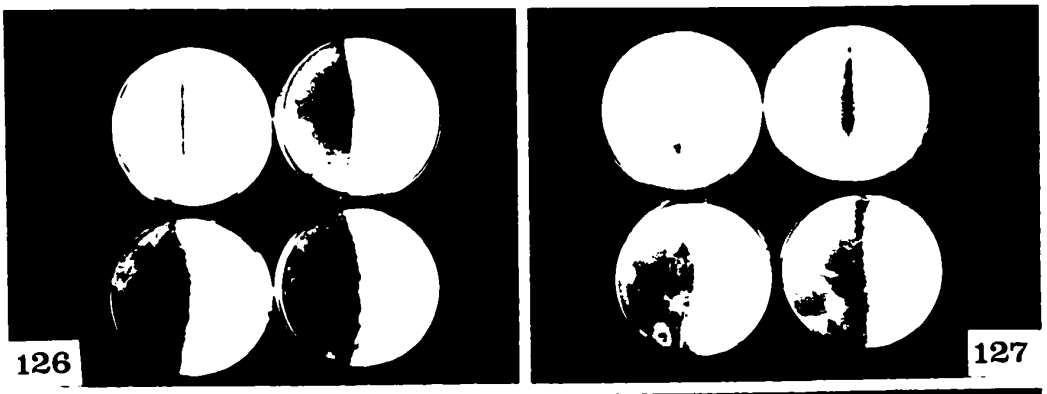
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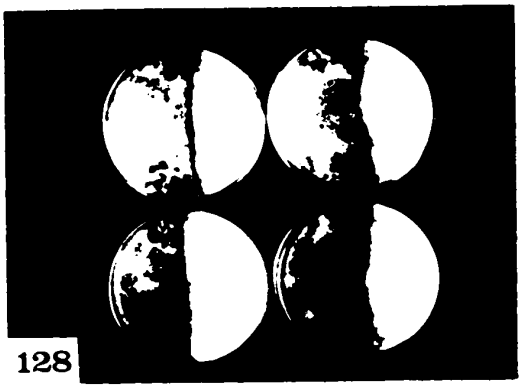
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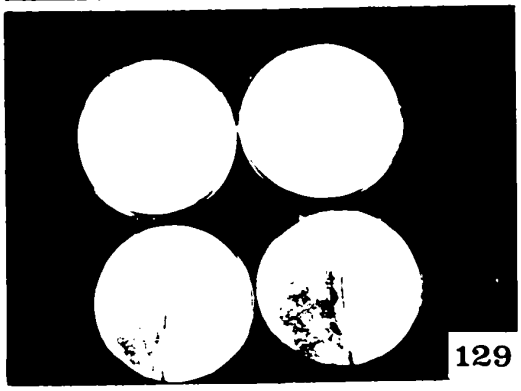
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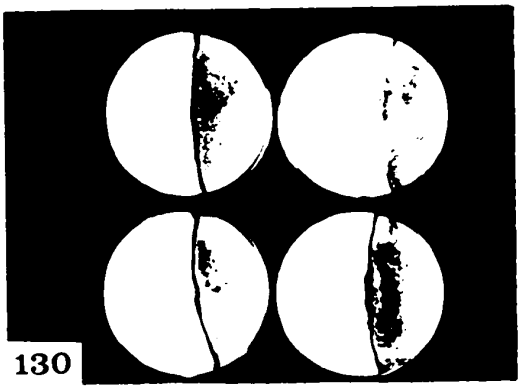
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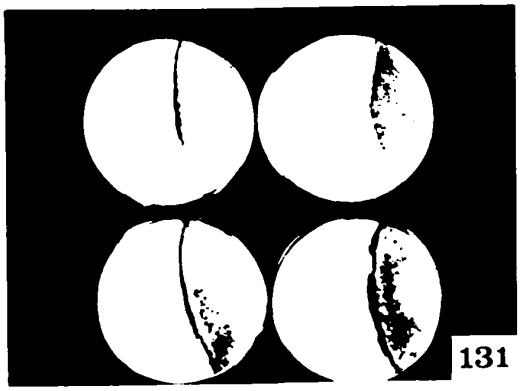
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CHAPTER V

CULTURAL STUDIES

INTRODUCTION

The vegetative mycelium of a polypore is, in most instances, the perennial overwintering stage and is present throughout most of the life cycle of these fungi. It is responsible for the decay and degradation of the woody substrate and, in time, basidiocarps will develop as direct outgrowths from this vegetative mycelium. Since this stage is so important, a biosystematic study would be incomplete without covering this part of the life cycle. Although it is possible to study the mycelium in its natural substrate, it is more convenient to grow it under uniform conditions on a standard artificial medium so that more precise comparisons between isolates of the same and different species may be made.

Among the early workers in this field, Long and Harsch (1918) pioneered the study of fungi in pure, artificial culture and they used 10 different media to establish criteria for separation of wood decay species. Fritz (1923) was another early worker in this field who deserves mention for her work on distinguishing fungi by use of cultural criteria. Cultural characters have attained considerable importance in recent years, owing to the work of Nobles (1958b, 1965) who has devised and described a key for the

identification of cultures of 149 species of wood-decay Basidiomycetes. Nobles (1958a) is also responsible for developing a rapid test to determine the presence or absence of an extracellular oxidase in cultures of wood-decay fungi. This enzyme, which is detected by applying an alcoholic solution of gum guaiac directly to the mycelium, has been shown by Nobles to occur in cultures of fungi which cause white rots of wood, and not to occur in cultures of fungi which cause brown rots of wood. On the basis of this test and other correlating characters of sexuality, Nobles (1958b) suggested that the Polyporaceae can be divided into two groups, a primitive group consisting of species which produce no extracellular oxidase and a more advanced group consisting of species which do produce extracellular oxidase. The four taxa *C. hirsutus*, *C. versicolor*, *C. pubescens* and *C. zonatus* all produce extracellular oxidase and are included in a group of 16 species described by Nobles (1965) as being, "so similar in macroscopic appearance and in microscopic characters that no precise criteria for separation are available". Nobles (1965) futher states that, "isolates of *P. hirsutus*, *P. pubescens*, *P. zonatus* and *P. versicolor* that remain white can be identified with certainty only when fruit bodies are formed, from which single spore cultures can be isolated and paired with single spore isolates from fruit bodies collected in nature". Since basidiocarps are rarely produced in culture they are at present impossible to separate. Van der Westhuizen

(1958) has published the cultural characteristics complete with key for 16 species of South African wood-decay fungi, including *C. hirsutus*. His treatment is somewhat superficial, being concerned with the presence or absence of such features as chlamydospores, oidia etc. and gives little information on the microstructure of the mycelium. Mycelial microstructure has, in fact, been poorly described and illustrated for most of the polypores. Nobles does include line drawings and plates of parts of the vegetative mycelium of a number of species, but the mycelium of the four species of *Coriolus* is not illustrated. One feature of the vegetative mycelium which is of particular interest is the 'fiber hyphae', described by Nobles (1965) as "hyphae with thick, refractive walls, hyaline or brown and lumina narrow or apparently lacking, ...which usually arise as the elongated terminal cells of a hyphae and are thus aseptate". These elements are reported to occur in cultures of the four taxa previously mentioned. Nobles and Frew (1962) have illustrated 'fiber hyphae' of *Pycnoporus cinnabarinus* as terminal cells, but most of their illustrations are of parts of 'fiber hyphae'. Van der Westhuizen (1963) has described and illustrated branched and unbranched, thick-walled 'fiber hyphae' with clamp connections from cultures of *Cerrena unicolor*.

The term 'hypha' has already been shown to be meaningless in its application to the microstructure of the basidiocarp and the same criticism can be levelled against its

application to the vegetative mycelium. It has been used by various authors to describe a group of cells, a single cell or part of a cell. Since the vegetative mycelium, like the basidiocarp is composed of discrete cells, it is more meaningful to describe the mycelium in terms which reflect its cellular construction. The term 'hypha' will therefore be restricted to mean a group of two or more elongate cells joined end to end.

In addition to describing the microstructure, there are several other methods of characterizing the vegetative mycelium. Kaarik (1965) has carried out an extensive study of the ability of cultures of wood-decay fungi to oxidize phenolic compounds. From her results she was able to divide the wood-decay fungi into four groups, I - those which produce neither laccase or tyrosinase; II - those which produce tyrosinase but no laccase; III - those which produce laccase but no tyrosinase and; IV - those producing both laccase and tyrosinase. Since *C. hirsutus*, *C. pubescens*, *C. versicolor* and *C. zonatus* were all included in group III, this report is further evidence of the close relationship of these taxa.

Growth rates in culture have been investigated by a number of workers. The usual method is to grow cultures in agar plates and measure their daily increase in diameter. Humphrey and Siggers (1933) studied the effects of temperature on the rate of growth in culture of 56 species of wood-decay fungi. Their results show that *C. hirsutus* had an

optimum temperature for growth of 34°C and was inhibited at 44°C, while *C. versicolor* had an optimum of 28°C and was inhibited at 36-38°C. It is important to note that in the study by Humphrey and Siggers, only one isolate was used for most species and consequently no account was taken of differences which might be present between isolates of the same species. Although Cowling and Kelman (1964) found no significant differences between cardinal temperatures of 94 isolates of *Fomes annosus*, including both dikaryotic and monokaryotic ones, Cartwright and Findlay (1958) had earlier stated that, "...it has been found that there may be considerable differences in rates of growth of different isolations (strains) of the same fungus". The effects of temperature on growth rates in culture of a number of wood-decay fungi have been studied by Cartwright and Findlay (1934). These workers found that the depth of media in a petri dish does not affect growth rate and they conclude that temperature curves may be used to characterize certain species in culture, enabling them to be recognized and distinguished from related species. They only used one isolate of most species investigated, but used 5 replicates and published their results in the form of a graph showing average increase in diameter of colony per day plotted against temperature. They gave 28-30°C as the optimum temperature for *C. versicolor*, with inhibition occurring at 38°C.

The work of Henningsson (1967) must also be mentioned, since he has studied the temperature relations of wood-decay

fungi. He reported his results in graphical form and from them the following cardinal temperatures have been taken.

C. hirsutus optimum 32-33°C, inhibited at 45°C.

C. versicolor optimum 30°C, inhibited at 35°C.

C. zonatus optimum 30°C, inhibited at 37°C.

It can be said that the optimum and inhibitive temperatures proposed for the same species of wood-decay fungi by various workers agree reasonably well. It seems, therefore, that cardinal temperature is another criterion which may be used by taxonomists to characterize and identify fungi.

MATERIALS AND METHODS

Monokaryotic isolates were grown from single spores and all were positively identified by compatible mating tests with monokaryotic cultures derived from typical basidiocarps of each species. Some of the monokaryons were paired to provide dikaryons of known parentage. This was done by placing a small piece of mycelium from each of two compatible monokaryons at opposite sides of a malt agar plate. After the two cultures came into contact reciprocal dikaryotization took place. Subcultures were then taken from each side of the plate and grown separately. For example, isolate #237-52 and isolate #237-45 were placed at opposite sides of a malt agar plate. After dikaryotization had taken place a piece of mycelium was taken from the #237-52 side of the plate, established as a dikaryotic culture and numbered #237-52/45. Similarly a piece of mycelium was taken from the #237-45

side of the plate, established as a dikaryotic culture and numbered #237-45/52. Although the resulting two dikaryotic cultures have exactly the same nuclear composition, it is possible that cytoplasmic factors might have an effect on growth rate.

Wild type, dikaryotic cultures were isolated from locally collected basidiocarp tissue or from the wood substrate immediately below a basidiocarp. These cultures were positively identified by use of the Buller phenomenon by pairing with known monospore isolates which became dikaryotic if conspecific with the dikaryon under test.

Cultures for microstructural investigation were grown in the dark at 20°C on Nobles malt agar in 9 cm plastic petri plates. Cultures of various ages were examined to determine changes in microstructure over a period of time. Microscopic observations of cultures were made by removing small pieces of mycelium, staining with KOH-phloxine-Congo red, as previously described, and examining under the microscope. A camera lucida was used to make the line drawings. Attempts were made to stain the nuclei in the vegetative cultures of these species using the methods described by Lu (1962) and Furtado (1970), but these proved unsuccessful. It was possible, however, to visualize the nuclei by placing a piece of agar and mycelium on a slide, adding a drop of 5% KOH and a touch of 1% aqueous phloxine, and squashing under a coverslip. In places where the phloxine was very dilute, it concentrated around the nuclei leaving the rest

of the cytoplasm unstained. The nuclei appeared hyaline with a halo of stained material around them. The terms used to describe the macroscopic characters of the mycelium are taken from Nobles (1965).

The growth rate experiments were set up as follows. 9 cm plastic plates containing approximately 20 ml of Nobles agar were prepared and inoculated at the edge with 5 mm discs cut from the growing margin of stock cultures maintained on the same medium. Three replicates of each isolate were used. The plates were incubated in the dark at the specified temperatures for four days, and the radial growth measured by holding a ruler to the underside of a plate held up to the light. In most cases the margin of the mycelium was even, but if it were irregular, several measurements were taken and averaged. The plates were returned immediately to the growth chamber for a further period of four days after which they were again measured. The difference between the four day and eight day readings was calculated and averaged for each set of replicates. The average daily growth rate in millimeters per day was calculated for each isolate. When cultures were grown at higher temperatures, it was sometimes necessary to take readings at three and seven days, since at these temperatures, the mycelium completely covered the plates in eight days.

OBSERVATIONS AND RESULTS

Cultural characters of *C. hirsutus*

a. Macroscopic cultural characters.

The advancing edge of the mycelium was even and flat on the agar. At about 5 mm from the advancing edge the aerial hyphae began to develop and the mycelial mat became raised and downy. With advancing age patches of mycelium became interwoven and felty. After about four weeks an occasional small patch of mycelium developed a pore surface, while other areas became tufted and irregular. The reverse of monokaryotic cultures became bleached after four weeks while that of the dikaryotic cultures often developed patches of brown discoloration. Most of the cultures remained translucent and both monokaryotic and dikaryotic cultures gave a strong, blue positive reaction when tested with gum guaiac solution, indicating the presence of extracellular oxidase.

b. The microstructure of the mycelium.

The advancing zone consists of thin-walled, hyaline hyphal ends, 2.5-4 μ in diameter, with end cells 200-500 μ long. New end cells develop as branches from subterminal cells and grow into the marginal zone. The terminal cells at the advancing margin do not branch. Clamp connections occur at each septum of dikaryotic cultures and each cell has two nuclei. At distances of 1 cm or more from the margin, differentiation of the thin-walled cells has occurred.

Two types of thick-walled elements differentiate from the thin-walled cells, depending on the location in a hypha. One type developed from end cells while the other type developed by differentiation of intercalary cells. In young cultures end cells elongated, branched and became thick-walled (Figures 134, 136). The branches were 3-4 μ in diameter, straight and long and these elements comprised most of the aerial mycelium of young cultures. They are responsible for the downy condition. In older cultures end cells also developed into thick-walled elements, but these were characterized by a larger number of shorter branches (Figure 137). All of these short-branched, thick-walled, end cells were readily stained with Congo red, as were the long-branched end cells. The second type of thick-walled element which developed from intercalary cells appeared less frequently than the first type, although different isolates vary in the proportions of each. Thin-walled, intercalary cells produced a number of thin branches, 1.5-2.5 μ in diameter, which extended by apical growth until they were 200 μ or more long. Cell wall thickening began before the branches were completely developed and eventually the whole cell became thick-walled (Figure 142). The cells on either side of these thick-walled elements remained thin-walled. Partial wall thickening of cells in older cultures sometimes occurred and occasionally irregular outgrowths developed on them (Figure 135). Both chlamydospores and oidia were frequently found in older cultures, the oidia being mostly

uninucleate (Figures 139, 140). The brown discoloration which was noticed in the reverse of some dikaryotic isolates was found to be localized in pigment vesicles on older parts of the mycelium (Figure 138). Pigment from these vesicles diffused into the agar and was responsible for the brown discolouration.

The mycelium which developed within the agar was very variable. Some cells were less than 1 μ in diameter while others were as large as 6 μ in diameter (Figure 141).

No differences have been detected in microstructure between monokaryotic and dikaryotic isolates of *C. hirsutus* with the exception of clamp connections which, of course, do not occur in the monokaryon and the brown discolouration of the reverse which has not been observed in monokaryotic isolates. In all other respects they are identical.

Cultural characters of *C. pubescens*

a. Macroscopic cultural characters.

The advancing edge of the mycelium was even and flat on the agar. Aerial hyphae began to develop at about 5 mm from the advancing edge and the mycelial mat became raised, white and downy. With advancing age the mycelium became interwoven and formed a thick, tough, white skin over most of the surface of the plate. Mycelial development was more profuse than that of *C. hirsutus*, and frequently grew up the sides of the petri plate and onto the underside of the lid. Pore surfaces were not developed by any isolate and

the mycelium remained fairly uniform with increasing age. The reverse of all isolates became bleached. Both monokaryotic and dikaryotic isolates gave a strong, blue positive reaction when tested with gum guaiac solution, indicating the presence of extracellular oxidases.

b. The microstructure of the mycelium.

The advancing zone of the mycelium consisting of end cells 2.5-4.5 μ in diameter, was indistinguishable from that of cultures of *C. hirsutus*. Similarly, new end cells developed as branches from subterminal cells and grew into the advancing zone. Clamp connections occurred at each septum of dikaryotic cultures and each cell contained two nuclei. The two types of thick-walled elements, previously described for cultures of *C. hirsutus*, also occurred in cultures of *C. pubescens*. End cells located at least 1 cm from the margin usually became elongated and branched and developed into thick-walled elements with capillary lumina (Figure 146). Occasionally, shorter, more branched thick-walled elements were formed, but these were quite rare. The most abundant thick-walled elements, which constituted the bulk of the aerial mycelium of older cultures, developed from intercalary cells by profuse production of long branches which extended, in some cases, up to 250 μ from the original cell (Figure 144). These branches became interwoven as they developed and formed the thick, tough pellicle, characteristic of this species. Both types of thick-walled end cells were stained by Congo red, but the intercalary

thick-walled elements appeared not to take up this stain. Wall thickening of most cells occurred to some extent with increasing age of the cultures, particularly in the surface zone. Chlamydospores and oidia were not found in any isolates of this species.

The hyphae which developed submerged in the agar were extremely variable, ranging in diameter from 1-7 μ.

No differences were detected between monokaryotic and dikaryotic cultures of this species, except for the clamp connections which do not occur in monokaryotic cultures.

Growth rates in culture

Growth rates of monokaryotic isolates of *C. hirsutus* and *C. pubescens* are shown in Tables 4-6. The individual growth rates of isolates of #237 (Table 4) show some variation, isolate #237-52 was particularly slow-growing. Isolates of #207 (Table 5) also showed some variation in growth rates, with #207-8 and #207-9 being particularly slow. Similar variation is shown in individual growth rates of isolates of #300 (Table 6).

The dikaryotic isolates, whose growth rates are shown in Tables 7 and 8, were produced by pairing the two constituent monokaryons and subculturing from one or other side of the pairing as previously described. Reciprocal pairs of dikaryons are grouped together. Although in most cases the members of a reciprocal pair grew at approximately the

same rate, it should be noticed that #237-45/52 grew approximately twice as fast as #237-52/45 at all three temperatures (Table 7). The average growth rates for dikaryotic and their constituent monokaryotic cultures of #300 and #237 are illustrated in Figure 147. At 17°C the dikaryotic cultures appeared to have a slightly slower growth rate than their separate constituent monokaryotic isolates, but at 23°C the dikaryotic cultures had much the faster growth rates.

The growth rates of freshly collected wild-type dikaryotic isolates of *C. hirsutus* and *C. pubescens* are shown in Tables 9 and 10, respectively, and their average growth rates with respect to temperature are plotted in Figure 148. There appears to be more variation between growth rates of individual dikaryotic cultures than was observed between monokaryotic isolates. Five of the six dikaryotic isolates of *C. hirsutus* had an optimum temperature of 26°C, the remaining isolate had an almost constant growth rate from 26-30°C (Table 9). Of the six dikaryotic isolates of *C. pubescens*, five had an optimum temperature of 33°C, the remaining one having an optimum of 30°C (Table 10). Two of the six dikaryotic isolates of *C. hirsutus* failed to grow at 36°C, the remaining four made little growth. Similarly, three of the six dikaryotic isolates of *C. pubescens* failed to grow at 36°C, but one isolate, #333, was able to make considerable growth at this temperature.

DISCUSSION

The vegetative mycelium of both species studied has been shown to have a basic cellular construction. Individual terminal or intercalary cells may become differentiated into thick-walled elements. Three types of thick-walled elements have been observed in cultures of both species. These are terminal elements with few long branches, terminal elements with many short branches and intercalary elements with long branches. It is probable that the first two types represent opposite ends of a range of elements which is continuously variable from one type to the other. The long-branched type are produced during early growth of the culture while the short-branched types appear to develop at a later stage. The intercalary types appear to develop last in the aging cultures. All three types would, in the terminology of Nobles (1965) be described as 'fiber hyphae', which were defined as "hyphae with thick refractive walls, hyaline or brown, and lumina narrow or apparently lacking". Nobles (1965) also states that branching of fibers may be absent, rare or frequent, depending on species. With regard to the origin of 'fiber hyphae', Nobles considered them to arise usually, as the elongated terminal cell of a hypha and therefore to be aseptate. Van der Westhuizen (1963) described 'fiber hyphae' of *Cerrena unicolor* to be nodose-septate and terminal in origin. Fiber hyphae of *C. hirsutus* were also described by Van der Westhuizen (1958) to be "very

numerous, with walls thick and refractive, lumina narrow or lacking, aseptate, branched 1.5-3 μ". Zycha and Knopf (1966) have described 'fiber hyphae' of *C. versicolor* as "...much branched, curving and interwoven,... with walls thickened, lumina visible at bases of branches only", but they gave no information on terminal or intercalary position. References to the intercalary development of thick-walled elements have not been discovered and it is concluded that this type of element has not been previously described.

The proportion of each type of thick-walled element differed between cultures of the two species. In most cultures of *C. hirsutus*, the short-branched, terminal elements were particularly common; these elements were rarely found in cultures of *C. pubescens*. The usefulness of this observation as a taxonomic criterion is reduced because some cultures of *C. hirsutus* have been found which have few elements of this type. The intercalary, thick-walled elements were particularly abundant in cultures of *C. pubescens* where they became interwoven to form a thick tough pellicle. Most cultures of *C. hirsutus* lacked this feature, but again, some cultures were found which approached this condition. More important differences between the intercalary elements formed by each species were detected. Those of *C. hirsutus* commonly had 3-4 branches and those of *C. pubescens* had 8-15 branches or more. The number of branches on these intercalary elements appears to be a useful criterion which may be employed to separate these

two species. It is difficult to make comparisons between the thick-walled elements formed in cultures of *C. hirsutus* and *C. pubescens* with those formed by cultures of other species, since these elements have not been completely described and illustrated in their entirety for any other species. All thick-walled elements in cultures of *C. hirsutus* and *C. pubescens* differentiate from single cells, and therefore must be aseptate. They consequently differ from those of *Cerrena unicolor*, which were described as septate by Van der Westhuizen (1963).

In general, observations made in this study agree with the cultural descriptions provided by Nobles (1965) for *C. hirsutus* and *C. pubescens*. The 'bovista' type of binding hypha to which Nobles refers appears to be the same as the intercalary type of thick-walled element. The absence of chlamydospores and oidia in cultures of *C. pubescens* and their presence in cultures of *C. hirsutus* is also in agreement with Nobles' observations, although the usefulness of this criterion for distinguishing these two species is limited, since not all isolates of *C. hirsutus* form these asexual spores.

The short-branched, terminal, thick-walled elements found in cultures have a number of characteristics in common with the binding elements found in basidiocarps of these species. The number of branches and the short length of the branches, together with the development from an end cell, are characteristics of both types of elements. The

cultural elements are not contorted as the basidiocarp elements are, because they do not develop within a dense tissue and their branches are consequently less restricted in their direction of growth.

It must be remembered that the cultures are growing mainly on and to a lesser extent in an artificial substrate. Under natural conditions most growth takes place within the woody substrate and little superficial growth is made, except in places where the humidity is high, for example beneath logs and possibly in cracks in the wood. The submerged mycelium in artificial culture probably more nearly approaches the natural mycelium permeating the wood substrate, but, unfortunately for taxonomic purposes, both submerged mycelium in artificial culture and naturally occurring mycelium in wood are totally devoid of useful criteria and are consequently of little value in delimiting species. Thick-walled elements have not been observed in the submerged mycelium of artificial culture, nor have they been observed in preparations of naturally occurring mycelium in wood.

Although the cultural elements appear at first sight to have a binding function in that they become interwoven and form a tough pellicle, their true function may be to form an insulating layer on the surface of the substrate to prevent its drying out. One other point worth noting is that the intercalary thick-walled elements act as a block to the translocation processes which take place in a hypha

and effectively sever the hypha at the point where they develop. The distal end of the hypha, to remain viable, must therefore regain contact with the substrate or by anastomosis link up with another hypha.

With regard to growth rates, the optimum temperature for growth of *C. hirsutus* in artificial culture has been determined to be 26°C which is some 7°C lower than that reported by other workers. In this study, six isolates were used for each species and it has been found that each isolate had an almost constant optimum growth rate over a narrow temperature range. For example, isolate #324 had almost the same growth rate at 23°C, 26°C and 30°C, while #388 had the same growth rate at 26°C, 30°C and 33°C. It probably gives a truer picture to say that *C. hirsutus* has an optimum temperature range of 23-33°C, and that, within this range, temperature has little effect on growth.

The results of the growth rate studies of *C. pubescens* show a more clearly defined average optimum temperature of 33°C, although again, isolates varied, several of them showing a double peak. For example, #350 showed growth rates at 26°C of 9.8 mm per day, at 30°C of 9.0 mm per day and at 33°C of 10.4 mm per day. *C. pubescens* appears more tolerant of higher temperatures than *C. hirsutus*.

Humphrey and Siggers (1933) have suggested that there is a downward shifting of the optimum temperature over a period of time, and since, in this study, the cultures were grown at the indicated temperatures for several days before

initial measurements were taken, this may partly explain the lowered optimum temperatures for *C. hirsutus*, compared with that reported by other workers. Cultures were grown at indicated temperatures for several days before taking initial readings to remove possible sources of error which could be caused by differences in inoculum potential. Although all attempts were made to use inocula of the same age, different pieces of inoculum do vary in their ability to grow when placed on fresh medium.

Although the primary purpose of the growth rate experiments was to determine the optimum temperature for each species and to gain information on variations within each species, some of the other results obtained have proved to be unusual.

There is an indication that dikaryotic cultures of *C. pubescens* had a faster growth rate at 20°C and 23°C than did their component monokaryotic isolates when grown separately (Figure 147). Since this type of experiment has not been done before, more evidence is needed to establish that the dikaryon has the faster growth rate at higher temperatures.

Of particular interest were the results of the growth rates of reciprocal pairs of dikaryons (Tables 7, 8). Although in most cases, the reciprocal pairs had approximately the same growth rates, several instances occurred where the two cultures had different growth rates. This was especially noticeable at 23°C, where, for example, the

growth rate for #237-52/45 was 4.4 mm per day, while that for its reciprocal culture #237-45/52 was 9.2 mm per day. Similarly the growth rate for #237-36/13 was 6.2 mm per day, while that for its reciprocal culture, #237-13/36 was 8.9 mm per day. As each pair of cultures had the same nuclear composition, there must be some cytoplasmic factors which are causing an inhibition or acceleration of growth rate. Interactions between nuclei and cytoplasm which affect growth rate have not been reported before and consequently there is a need for further investigation of this phenomenon.

To conclude this chapter, the following cultural descriptions are provided.

C. hirsutus

Macroscopic - Advancing edge of mycelium even and appressed, aerial mycelium arising 5 mm from edge and forming raised, downy mat. Occasional patches becoming interwoven, felty and white, the remainder being slightly translucent. Reverse usually becoming discoloured brown with age. Growth rapid, plates covered in 10 days at 23°C.

Microscopic - Advancing zone of thin-walled, hyaline, hyphal ends, 2.5-4 μ in diameter, the end cells being 200-500 μ long. Branches arise from subterminal cells and clamp connections develop at each septum. Aerial mycelium composed of thick-walled elements of two types. Terminal thick-walled elements initially with 2-6 long branches, 3-4 μ in

diameter, but later terminal elements formed with 10-20(40) short, stubby branches. Intercalary thick-walled elements with 2-4 long branches 1.5-2.5 μ in diameter formed sparingly or in patches of interwoven mycelium. Chlamydospores and oidia formed by some isolates.

C. pubescens

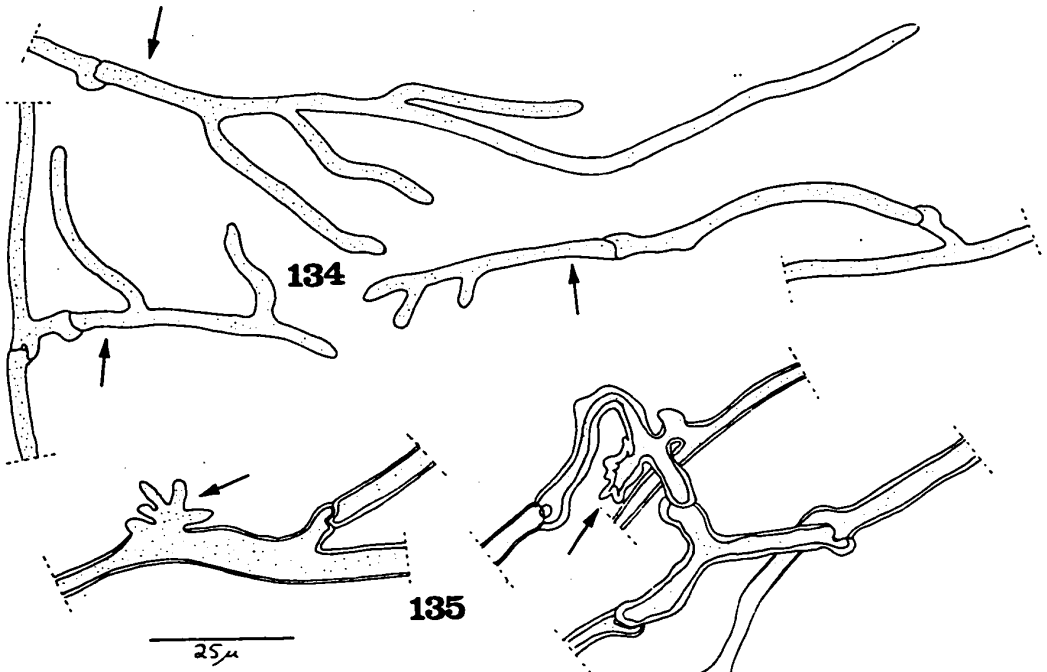
Macroscopic - Advancing edge of mycelium even and appressed, aerial mycelium arising 5 mm from edge and initially forming raised, white, downy mat, later becoming interwoven to form thick, tough, white pellicle over most of plate surface, mycelium frequently growing onto sides and top of plate. Reverse becoming bleached. Growth rate rapid, plates covered in 10 days at 23°C.

Microscopic - Advancing zone of thin-walled, hyaline, hyphal ends, 2.5-4.5 μ in diameter, end cells being 200-600 μ long. Branches arise from subterminal cells and clamp connections develop at each septum. Aerial mycelium composed of thick-walled, elements of two types. Terminal thick-walled elements initially with 1-6 long branches, 3-4.5 μ diameter, later terminal elements formed with 4-8 short branches. Intercalary thick-walled elements with 8-15 long branches 1.5-2.5 μ in diameter, abundant and interwoven to form the thick pellicle.

FIGURE 134. Branched, terminal cells (arrows) arising from clamped, thin-walled cells, taken 4-5 mm from advancing edge of culture of *C. hirsutus*.

FIGURE 135. Partial cell wall thickening in older cells in culture and development of outgrowths (bulbils) (arrows). *C. hirsutus* culture.

FIGURE 136. A long-branched, thick-walled element arising by differentiation of a single, terminal cell and connected to a thin-walled cell by a clamped septum (arrow). *C. hirsutus* culture.


134
135
25μ

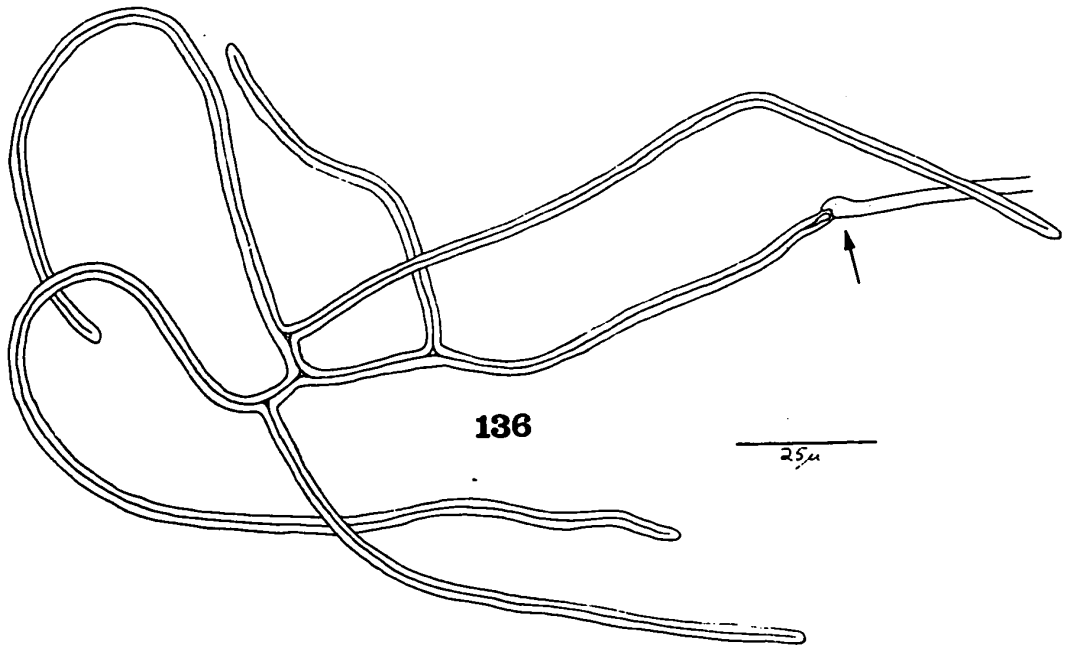

136
25μ

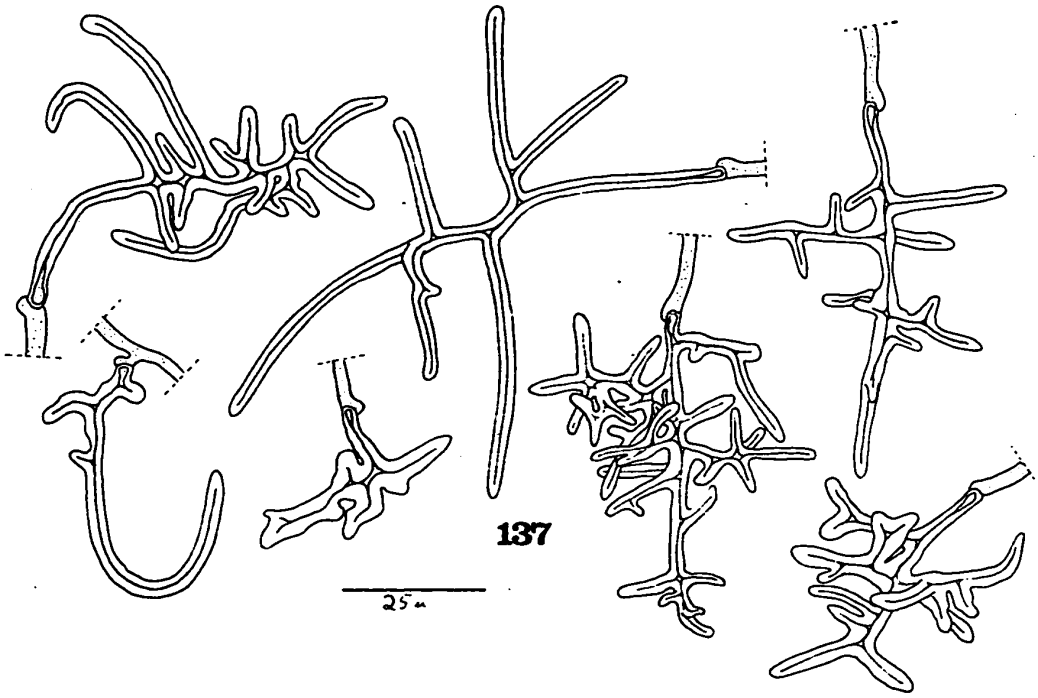
FIGURE 137. Various short-branched, thick-walled elements each arising by differentiation of a single terminal cell. *C. hirsutus* culture.

FIGURE 138. A pigment vesicle (arrow) developing as an outgrowth from a thin-walled, submerged cell in culture of *C. hirsutus*.

FIGURE 139. Chlamydospores from cultures of *C. hirsutus*.

FIGURE 140. Uninucleate oidia (arrows) developing by fragmentation of a binucleate hypha of *C. hirsutus*.

FIGURE 141. Large, thin-walled, submerged cells occasionally found in cultures of *C. hirsutus*.


137
25 u

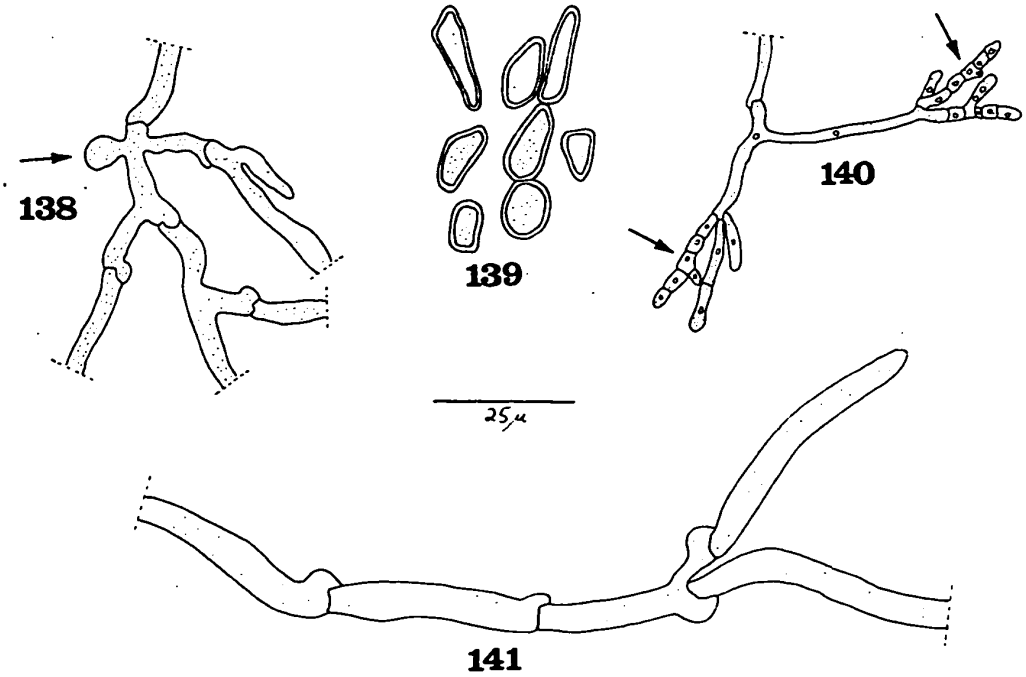
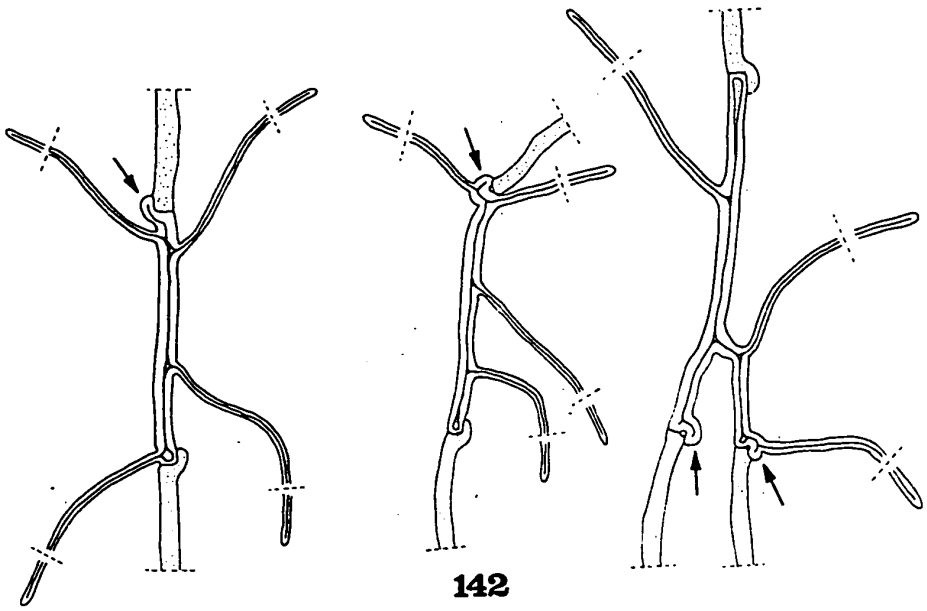

138
139
140
25 u
141

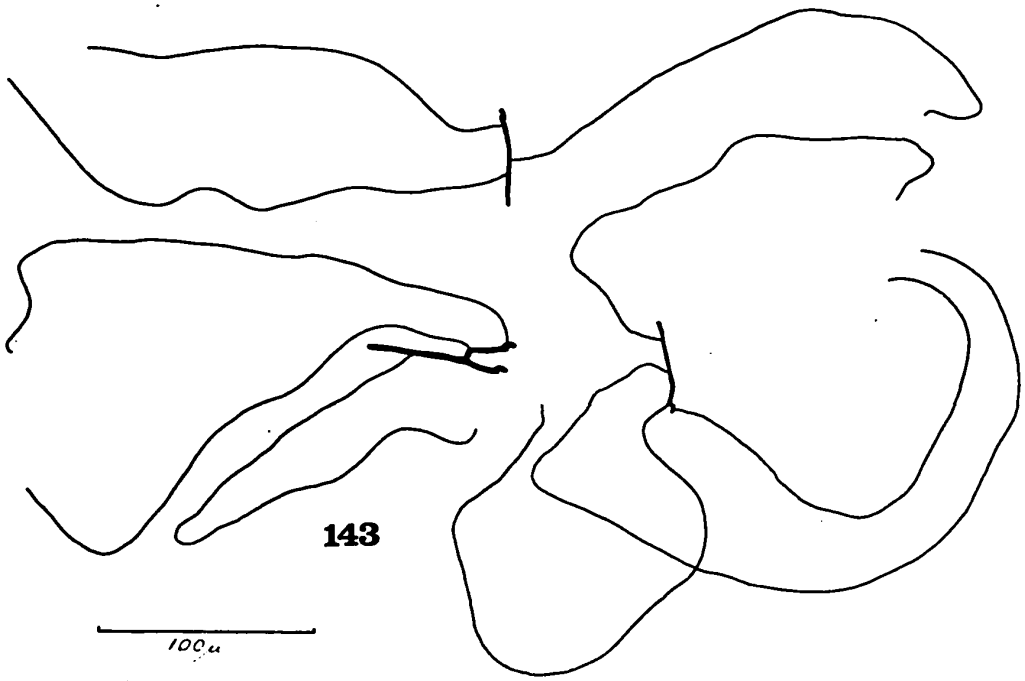
FIGURE 142. Central portion of intercalary, thick-walled elements with thickened clamps (arrows) each arising by differentiation of a single intercalary cell. *C. hirsutus* culture.

FIGURE 143. Intercalary, thick-walled cells as in Figure 142, but showing full extent of branches. *C. hirsutus* culture. (Thickness of branches is not to scale).



142

25μ

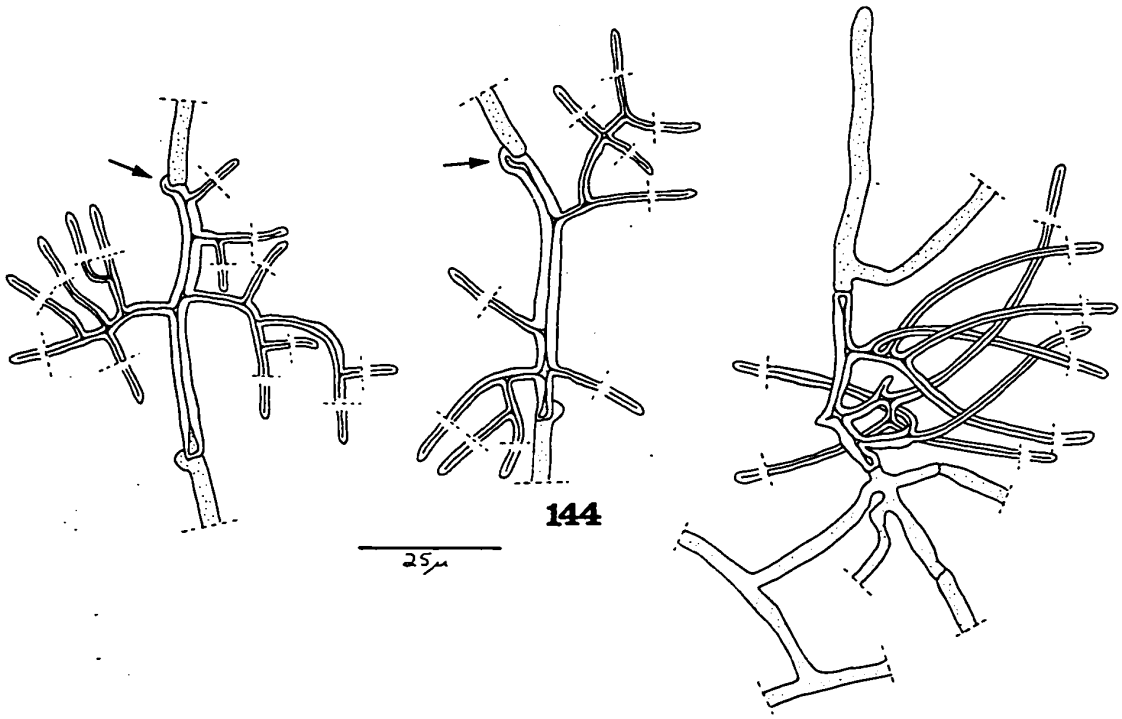


143

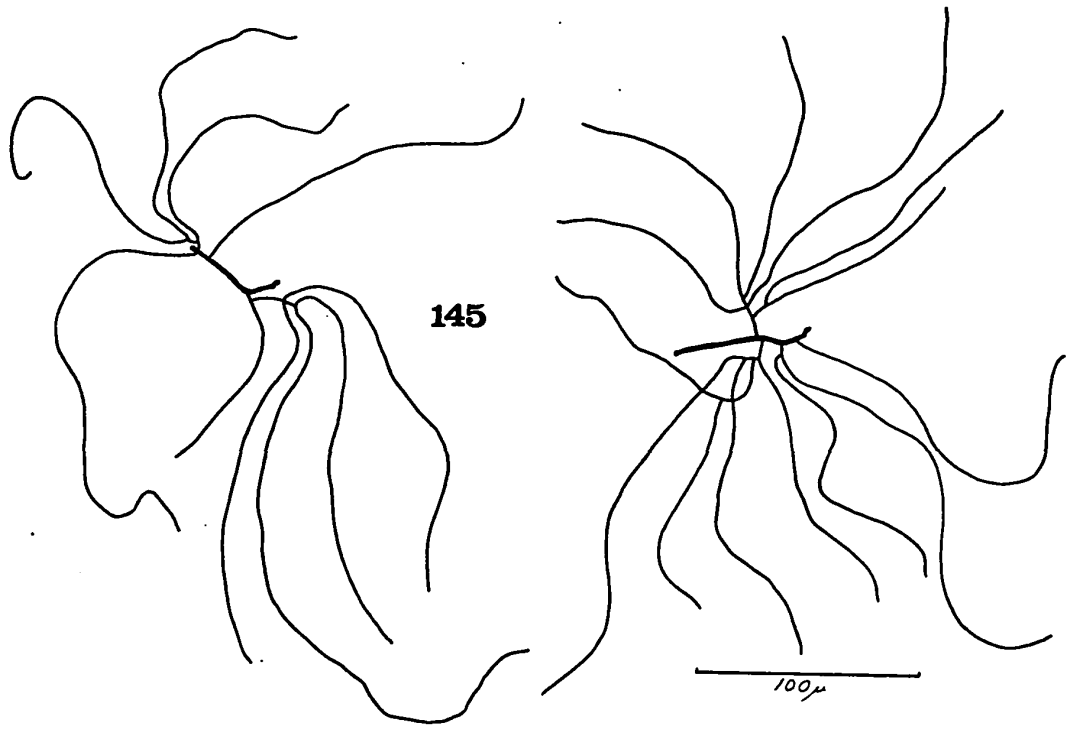
100μ

FIGURE 144. Central portion of intercalary, thick-walled elements. Two at the left have thickened clamps (arrows) and are from dikaryotic cultures, one at the right has simple septa and is from a monokaryotic culture. *C. pubescens* cultures.

FIGURE 145. Intercalary, thick-walled cells as in Figure 144, but showing full extent of branches. *C. pubescens* culture. (Thickness of branches is not to scale).

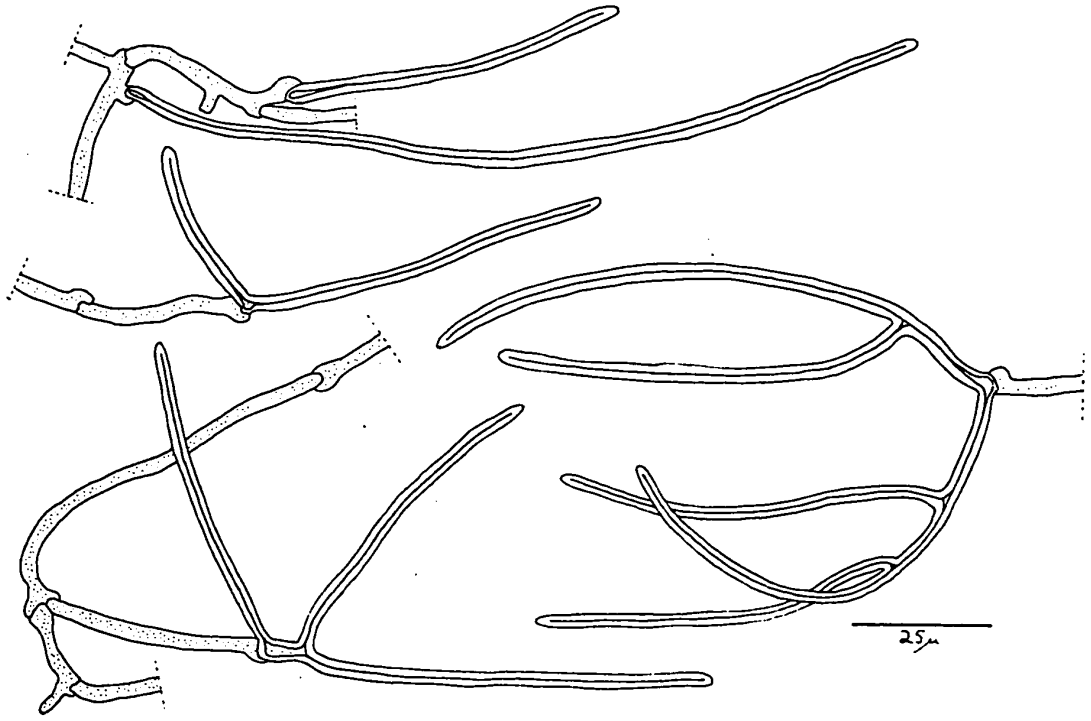


144



145

FIGURE 146. Long-branched, thick-walled, terminal elements arising by differentiation of single cells. An intercalary cell (arrow) is shown developing into a thick-walled element of the type shown in Figure 145. *C. pubescens* culture.


25µ

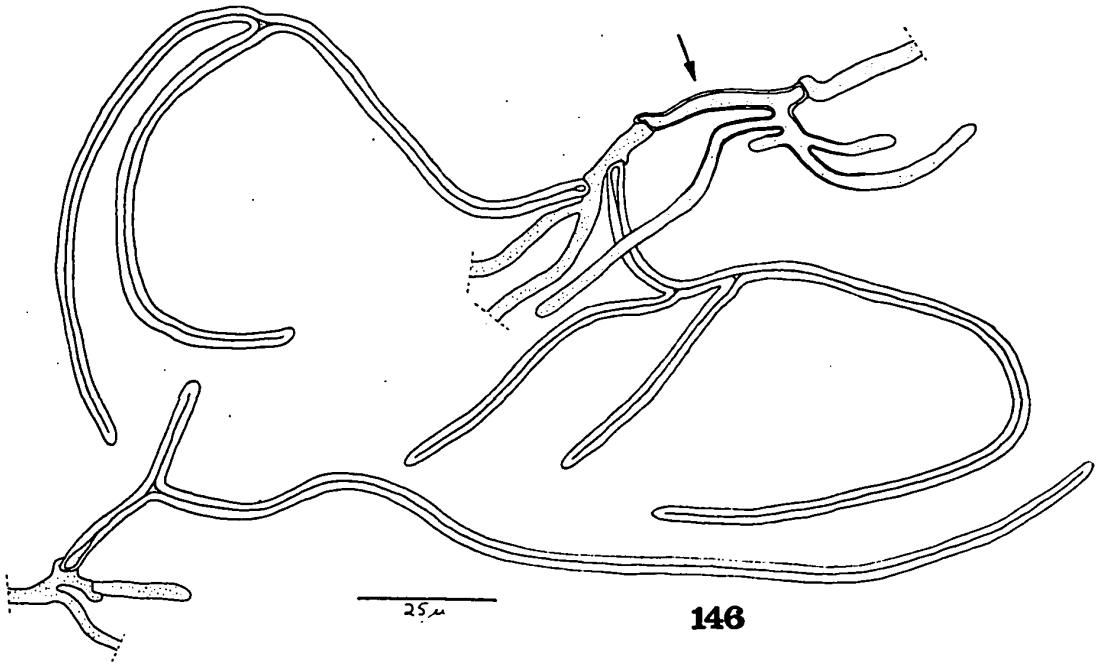
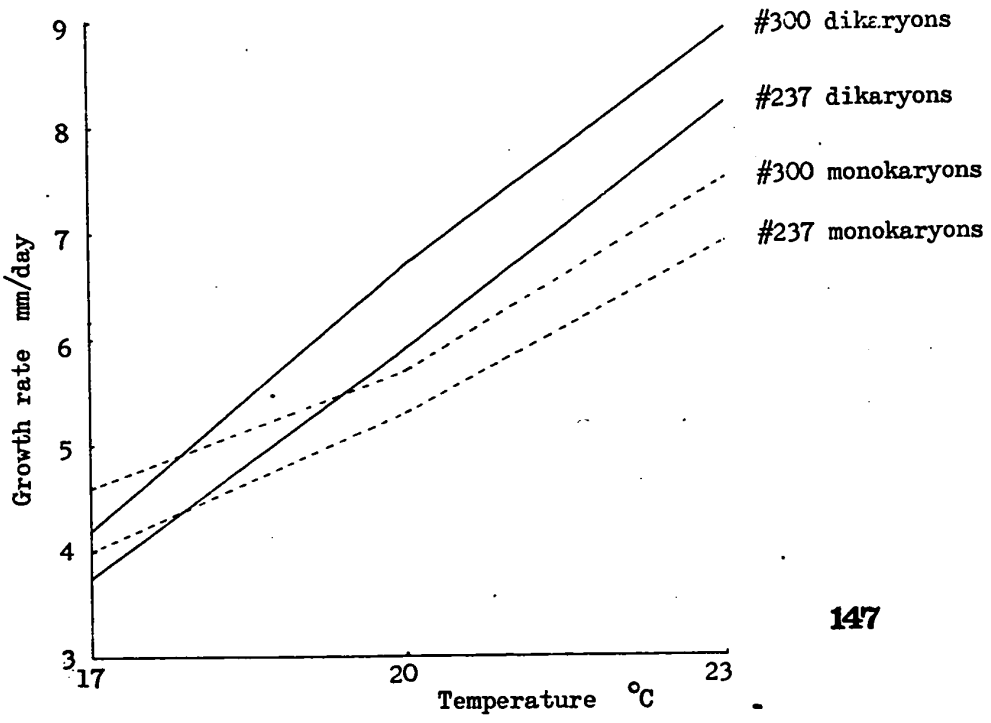

25µ

FIGURE 147. Effect of temperature on radial growth rates of monokaryotic and dikaryotic cultures of *C. pubescens*.

FIGURE 148. Effect of temperature on radial growth rates of dikaryotic cultures of *C. hirsutus* and *C. pubescens*.


#300 dikaryons
#237 dikaryons
#300 monokaryons
#237 monokaryons
Growth rate mm/day
9
8
7
6
5
4
3
17
20
23
Temperature °C
147

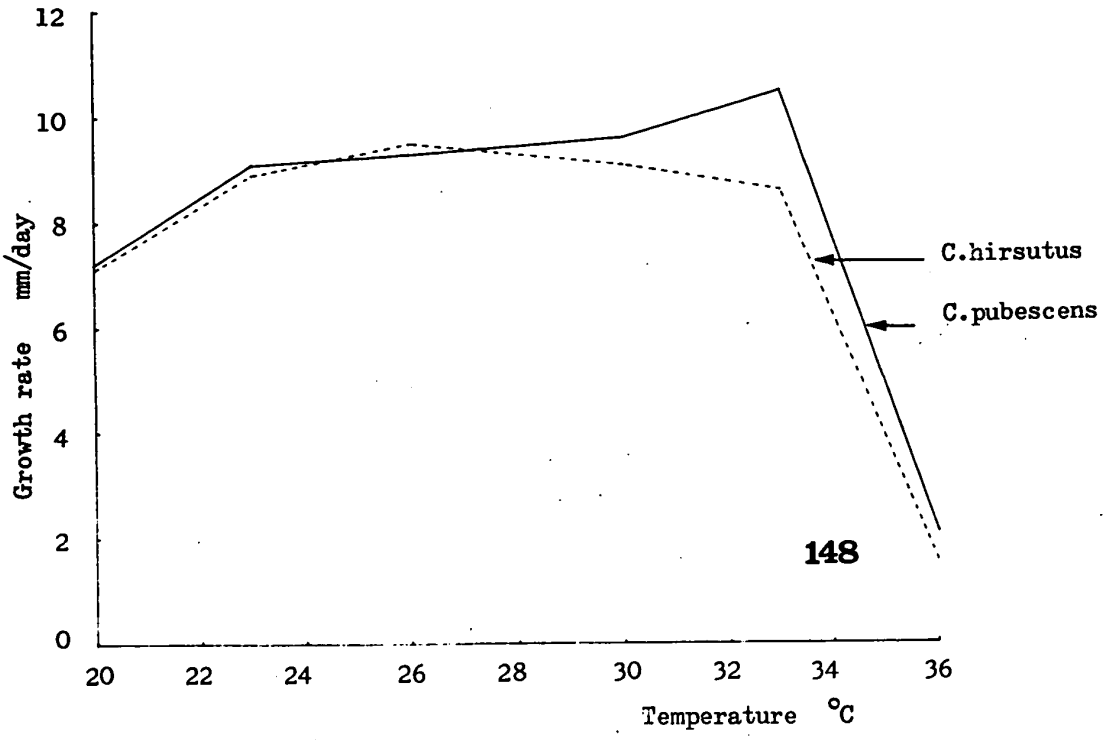

Growth rate mm/day
12
10
8
6
4
2
0
20
22
24
26
28
30
32
34
36
Temperature °C
C.hirsutus
C.pubescens
148

TABLE 4. Effect of temperature on radial growth rates (mm/day) of 8 monokaryotic isolates of *C. pubescens* #237.

TABLE 5. Effect of temperature on radial growth rates (mm/day) of 7 monokaryotic isolates of *C. hirsutus* #207.

TABLE 6. Effect of temperature on radial growth rates (mm/day) of 8 monokaryotic isolates of *C. pubescens* #300.

TABLE 4

Isolate	Temperature			
	17°C	20°C	23°C	26°C
#237-13	4.0	5.25	7.25	6.9
#237-36	4.25	5.5	6.75	7.4
#237-45	4.0	5.5	7.5	7.0
#237-48	4.25	6.0	7.5	7.9
#237-52	3.0	4.0	5.25	-
#237-63	4.0	5.25	6.5	7.5
#237-67	4.25	5.5	7.0	7.25
#237-68	4.0	5.25	7.5	7.25
Average	4.0	5.3	6.9	7.3

TABLE 5

Isolate	Temperature			
	17°C	20°C	23°C	26°C
#207-6	4.25	5.5	7.0	8.5
#207-7	4.0	4.5	5.5	5.5
#207-8	2.5	3.25	4.25	-
#207-9	2.75	4.0	4.75	6.25
#207-10	3.75	4.25	5.25	5.75
#207-12	3.75	4.5	5.75	5.9
#207-13	3.75	4.75	6.0	6.75
Average	3.5	4.4	5.5	6.4

TABLE 6

Isolate	Temperature		
	17°C	20°C	23°C
#300-1	3.5	5.5	6.75
#300-2	4.25	5.5	7.5
#300-3	4.75	5.75	7.5
#300-4	5.5	6.5	8.25
#300-5	5.0	5.5	7.5
#300-6	4.25	5.5	7.75
#300-7	5.0	6.0	7.5
#300-9	4.25	5.5	7.25
Average	4.6	5.7	7.5

TABLE 7. Effect of temperature on radial growth rates (mm/day) of reciprocal pairs of dikaryotic cultures of *C. pubescens* #237.

TABLE 7

Isolate	Temperature 17°C	20°C	23°C
#237-52/45	2.2	3.7	4.4
#237-45/52	4.2	6.3	9.2
#237-52/13	3.0	4.7	5.7
#237-13/52	2.1	3.9	5.0
#237-63/13	3.8	5.9	8.9
#237-13/63	3.7	5.9	7.6
#237-68/36	4.1	6.3	9.1
#237-36/68	4.0	6.0	8.6
#237-67/48	3.9	6.7	9.3
#237-48/67	4.2	6.3	9.6
#237-63/45	4.1	6.2	9.2
#237-45/63	4.5	6.3	9.3
#237-36/13	3.5	5.7	6.2
#237-13/36	3.7	6.0	8.9
#237-45/36	4.3	6.9	9.7
#237-36/45	4.7	6.9	11.0
Average	3.75	5.9	8.2

TABLE 8. Effect of temperature on radial growth rates (mm/day) of reciprocal pairs of dikaryotic cultures of *C. pubescens* #300.

TABLE 8

Isolate	Temperature 17°C	20°C	23°C
#300-5/3	4.5	6.8	8.9
#300-3/5	4.5	6.7	9.1
#300-4/9	3.7	6.9	8.2
#300-9/4	4.0	6.6	8.2
#300-2/10	4.5	6.7	9.3
#300-10/2	4.3	6.6	9.0
#300-1/7	4.3	6.4	8.7
#300-7/1	4.5	6.9	8.7
#300-4/1	4.5	6.9	9.7
#300-1/4	4.3	6.9	9.2
#300-7/9	3.9	6.3	8.1
#300-9/7	3.9	6.1	7.3
#300-6/5	4.2	6.4	9.8
#300-5/6	4.3	6.9	9.4
#300-5/2	4.1	6.8	9.1
#300-2/5	4.4	6.9	8.9
Average	4.2	6.7	8.9

TABLE 9. Effect of temperature on radial growth rates (mm/day) of unrelated dikaryotic cultures of *C. hirsutus*.

TABLE 10. Effect of temperature on radial growth rates (mm/day) of unrelated dikaryotic cultures of *C. pubescens*.

TABLE 9

Isolate	Temperature 20°C	23°C	26°C	30°C	33°C	36°C
#324	7.2	9.0	9.3	9.2	8.1	0.0
#362	6.7	8.6	10.0	9.8	9.3	1.3
#366	7.5	9.3	9.4	8.8	9.3	0.0
#367	6.8	8.1	8.4	7.3	7.3	2.7
#372	7.4	9.0	9.7	9.4	7.3	2.8
#388	7.2	9.3	10.2	10.2	10.3	2.9
Average	7.1	8.9	9.5	9.1	8.6	1.6

TABLE 10

Isolate	Temperature 20°C	23°C	26°C	30°C	33°C	36°C
#330	6.8	8.1	7.8	8.8	10.4	2.7
#331	7.7	9.5	10.0	10.3	11.0	3.8
#333	7.9	9.5	10.2	10.4	11.3	6.2
#350	6.8	8.9	9.8	9.0	10.4	0.0
#390	7.6	9.6	8.2	9.3	8.9	0.0
#393	6.7	9.0	9.6	9.6	10.8	0.0
Average	7.2	9.1	9.3	9.6	10.5	2.1

CHAPTER VI

SUMMARY

Much of the difficulty in the identification of fungi of the Polyporaceae results from the fact that the basidiocarps, which are the structures providing the taxonomic characters, are polymorphic in many species. Basidiocarps are extremely variable in appearance and have a plasticity of form which is frequently moulded by their environment. The conditions found in a particular ecological niche where a basidiocarp is developing has a profound effect on the final size, shape and colouration of the basidiocarp. This kind of variation is consequently not due to variation in the genotype. Polymorphism is so great that species descriptions often overlap, which makes it almost impossible to draw a dividing line between two closely related species and to identify individuals belonging to them with any degree of confidence. In such cases the experience of the collector is instrumental in pushing a particular type of basidiocarp one way or another into which ever species he thinks best. This has resulted in the erection of artificial species where basidiocarps, which on macroscopic appearance fall within a certain range, are said to be conspecific. Unfortunately, no two collectors draw the dividing line between two species in the same place, consequently, a basidiocarp may be assigned to species A

by one collector, while another collector may categorically state that it is obviously species B. Such is the case with the species complex containing the five taxa *Coriolus hirsutus*, *C. pubescens*, *C. versicolor*, *C. velutinus* and *C. zonatus*. These five taxa have been given species rank by earlier workers primarily on the basis of the macroscopic appearance of their basidiocarps. All five taxa have been reported to occur in Alberta. Various authors have accepted some of these taxa as good species and have rejected others. Bondartsev (1953) considered *C. velutinus* to be synonymous with *C. pubescens* which he recognized as a good species together with *C. hirsutus*, *C. versicolor* and *C. zonatus*. Overholts (1953), on the other hand, accepted the taxa *C. hirsutus*, *C. pubescens*, *C. versicolor* and *C. velutinus* as good species, but claimed that *C. zonatus* was a form of *C. versicolor*. This kind of inconsistency in the taxonomy of this group results from the fact that each worker has his own ideas of the acceptable range of variation of each species. Before it is possible to circumscribe and delimit a species on the grounds of its macroscopic characteristics, it is necessary to make a collection of typical representatives of the species and in addition to collect other specimens which are thought to belong to the species. The resultant species description will then be broad enough to include those specimens which were considered sufficiently similar to the typical representatives of the species to be this species. This can be regarded as a circular

argument, since the species description is based on the examined collections whose identification is based on the species description.

These inherent disadvantages of the artificial or form species concept can be overcome in certain instances by resorting to the natural or biological species concept. The advantage of the latter concept is that the conspecificity of two individuals can be proven by simply test-mating cultures of the two individuals. The results of this test are, in almost all cases, unequivocal and different workers carrying out the same test will get exactly the same results. A species description can then be based on positively identified individuals.

INTERFERTILITY STUDIES

The principle of the biological species concept has been applied to the five taxa *C. hirsutus*, *C. pubescens*, *C. versicolor*, *C. velutinus* and *C. zonatus* reported to occur in Alberta, the study area. The results of interfertility tests have shown that there are only two good species extant in this area. These species are *C. hirsutus* and *C. pubescens*. Individual basidiocarps which on macroscopic appearance would have been classified by other workers as *C. versicolor*, *C. velutinus* or *C. zonatus* have been proven by interfertility tests to be compatible with basidiocarps of either *C. hirsutus* or *C. pubescens*, but not with both. The limits of the species *C. hirsutus* and *C. pubescens*, as formerly

described, were too narrow to accomodate these variants and consequently the variants have been given separate species status by other workers. The use of interfertility tests has clearly demonstrated that there are two, intersterile species present in Alberta.

It has been possible to test-mate cultures derived from basidiocarps of *C. versicolor* which were collected in Ontario with local isolates of *C. hirsutus* and *C. pubescens*. It was expected that *C. versicolor* would prove to be intersterile with the Albertan species, but this was not the case. The isolates of *C. versicolor* were partially fertile with those of *C. hirsutus* and to a lesser extent with those of *C. pubescens*. When monospore isolates of *C. versicolor* were paired with those of *C. hirsutus* the cultures of *C. versicolor* became dikaryotic in many instances and developed clamp connections. When these 'hybrid' cultures were subcultured, some appeared to revert to simple septate monokaryons. It was noticed that in some cultures cell division was impaired in that the hook cell of the clamp connection failed to fuse with the subterminal cell, trapping a nucleus in the hook cell. The result of this incomplete cell division was that the terminal cell remained dikaryotic while the subterminal cell became monokaryotic. Branches which arose from the subterminal cells developed into simple septate hyphae. It must be emphasized that the formation of a 'hybrid' dikaryon is only the first step in the life cycle of these fungi and must be followed by

development of a basidiocarp, nuclear fusion, meiosis and the production of viable spores before a true hybrid can be claimed. Further research in this area of hybrid formation would give a new insight into the sexuality and speciation of these fungi.

A second surprising result in the interfertility studies was that Swedish isolates of *C. hirsutus* were inter-sterile with Canadian isolates of this species. Strict adherence to the biological species concept dictates that the Swedish taxon named *C. hirsutus* can not be the same species as the Canadian taxon named *C. hirsutus*. Obviously, a more widespread study is needed to confirm this initial observation, and, should it be confirmed, a number of nomenclatural problems would need to be resolved. One possible solution would be to allow the Swedish taxon to retain the species name *C. hirsutus*, since it is in the type locality, while the North American taxon could perhaps become *C. nigromarginatus* as this specific epithet was originally applied to the North American taxon by Murrill (1906).

BASIDIOCARP MACROSTRUCTURE

By using interfertility tests it was possible to accumulate positively identified collections of basidiocarps of *C. hirsutus* and *C. pubescens* from the study area. These collections were then used to assess the range of variation of basidiocarps within each species and to determine stable

taxonomic characters for each species. This approach removes all of the uncertainty of whether a particular specimen does or does not belong to a certain species and the consequent species description which results from the study of a positively identified collection will be based on a solid foundation.

Specimens of *C. pubescens* have frequently been found which are much larger and thicker than the usual type. These large, thick basidiocarps are outside the previously described size range for the species. It has been necessary to expand the species description so that they are included. These large, thick basidiocarps have been observed to develop in a dry microhabitat where there is high light intensity which points to an interaction between a developing basidiocarp and its microenvironment. Basidiocarps which develop under shaded, moist conditions are invariably thin, applanate and paler in colour.

There are few macroscopic characters of the basidiocarp which can be used to distinguish between *C. hirsutus* and *C. pubescens*. The most reliable characteristics are the colouration of the pore surface and the dissepiment thickness. The age of a basidiocarp is of great importance and must be taken into account when making comparisons. Young basidiocarps of both species have thick dissepiments, but as the dissepiments grow downwards, they taper so that the pore becomes larger in diameter while the wall becomes narrower in thickness. Older basidiocarps of *C. pubescens* have

longer and narrower dissepiments than those of *C. hirsutus*. The smoky coloured pore surface of basidiocarps of *C. hirsutus* is a constant character of this species. Basidiocarps of *C. pubescens* always lack this pigmentation.

BASIDIOCARP MICROSTRUCTURE

A study of the microstructure of basidiocarps of *C. hirsutus* and *C. pubescens* has indicated that, while basidiocarps of both species have the same basic type of construction, there are specific differences which are useful taxonomic criteria. The complex mode of development of basidiocarps of both species has been elucidated and has thrown a new light on basidiocarp construction. It is more meaningful to regard a basidiocarp as being constructed of individual, elongate cells rather than to refer to its construction in terms of hyphae. The meaning of the term hypha depends on the way a particular author uses the term. Hypha has been used to mean a group of cells joined end to end, a single elongate cell or a part of a single elongate cell. A basidiocarp is constructed of individual elongate cells joined end to end. A cell can occupy one of two possible positions, intercalary or terminal. Little differentiation of intercalary cells takes place. They form a continuous system and are the generative hyphae of Corner (1932a, 1932b). Terminal cells are the cells which become differentiated into specialized structures, the type of structure depending on their location in the basidiocarp.

They may develop into thick-walled, branched, structural cells, thick-walled, unbranched cells of the pileus surface, thin-walled basidia or they may constitute the growing margin of the basidiocarp. End cells which are at the growing margin of the basidiocarp invariably develop into elongate, thick-walled, branched, skeletal elements. Those that differentiate at some distance from the margin develop into much-branched contorted binding elements because, in this position, the developing branches of a cell are hindered in their growth and they must weave around and between other thick-walled elements. There is no clear distinction between skeletal and binding elements since cell differentiation takes place at all distances from the growing margin. End cells which are formed near the upper surface of the basidiocarp develop into thick-walled, unbranched elements and become a part of the trichoderm. End cells which develop in the hymenium become basidia. A skeletal or binding element is a discrete structure differentiated from a single cell, and is consequently not a hypha. Intercalary cells are all generative and, in the early stages of basidiocarp development, are thin-walled. New growing points arise from generative cells and, after elongation by apical growth, become delimited as new end cells. In this manner new end cells are continually being formed to replace those which have differentiated into binding or skeletal elements.

There are fundamental differences between specialized elements of the basidiocarps of *C. hirsutus* and *C. pubescens*.

Skeletal elements of *C. hirsutus* have two or three short branches while those of *C. pubescens* have a larger number of long tapering branches. It is interesting to note that, from the few basidiocarps of *C. versicolor* which have been examined, skeletal elements of this species are unbranched and aciculiform. Binding elements of *C. hirsutus* have few branches and the branches have swollen ends, while binding elements of *C. pubescens* have many branches which remain isodiametric.

The microstructure of basidiocarps of *C. hirsutus* and *C. pubescens* appears to be basically different from that described for other species of polypore. Corner (1953) has described the microstructure of basidiocarps of *Polyporus sulphureus* and *P. squamosus* and found that intercalary cells developed long whip-like processes and became binding elements. A distinction must be made between an intercalary or terminal position for specialized structural components. Merely stating that both are binding elements ignores their different mode of development and suggests relationships which may be false. Van der Westhuizen (1963) claimed that interseptal elongation of thick-walled hyphae took place in basidiocarps of *Cerrena unicolor*. In the present study, apical growth was the only type of elongation noted. There may be many types of basidiocarp development which must be understood before relationships within this group will be detected. The microstructure of so few basidiocarps has been described in detail that it is as yet impossible to

comprehend the phylogenetic implications of this type of study. The microstructure of many more species needs to be elucidated before relationships become apparent. There is no doubt that the microstructure is more stable than the macrostructure and will therefore play a role of increasing importance in the taxonomy of this group.

CULTURAL STUDIES

The microstructure of the mycelium of *C. hirsutus* and *C. pubescens* and its differentiation in artificial culture have been studied. Cultures of both species are initially composed of elongate cells which individually become differentiated into discrete structures. Single, terminal or intercalary cells may develop into thick-walled elements. Terminal cells either developed a few long branches or numerous shorter branches. Intercalary cells differentiate later in aging cultures by developing a number of whip-like processes from a single cell. These thick-walled elements, both terminal and intercalary, have been collectively referred to as 'fiber' hyphae by Nobles (1965), but it had not been demonstrated that a fiber hypha developed from a single cell. Short-branched, thick-walled, terminal elements were particularly common in cultures of *C. hirsutus*, and rare in cultures of *C. pubescens*. There are consistent differences in structure of intercalary elements between the two species. The intercalary elements of *C. hirsutus* commonly had three to four branches, while those of

C. pubescens had eight to fifteen or more branches. The number of branches which develop from intercalary elements appears to be a useful taxonomic criterion. As thick-walled cultural elements of *C. hirsutus* and *C. pubescens* always develop from single cells and therefore are aseptate, they must be considered different from those of *Cerrena unicolor* which have been reported by Van der Westhuizen (1963) to be septate. Cultural elements of other species of polypore have not been described or illustrated in their entirety, therefore the significance in terms of relationships of this type of comparison can not be assessed until the detailed cultural microstructure of many more species is elucidated.

The results of studies of growth rates of isolates of *C. hirsutus* and *C. pubescens* in culture has indicated that considerable variation exists between isolates of the same species. There does not appear to be a clearly defined optimum temperature for cultures of *C. hirsutus*, since within the range 23-33°C temperature had little effect on growth rate. Cultures of *C. pubescens* showed a more clearly defined optimum temperature of 33°C and this species appeared more tolerant of higher temperatures than did *C. hirsutus*.

It is interesting that dikaryotic cultures of *C. pubescens* showed a higher growth rate at 20°C and 23°C than did their component monokaryotic isolates when grown separately. More work is needed to substantiate this claim. A second interesting observation was that cytoplasmic factors may cause an inhibition or acceleration of growth

rate. This type of reciprocal cultural analysis has not been reported before and consequently more work needs to be done to confirm this supposition.

Hansen (1958) has said that "one of the more complicated problems in mycology is to find criteria for a natural classification of the Polyporales". Wherever possible, taxonomic studies of selected species should begin with individuals positively identified by means of test-mating. Then, close attention to the detailed microstructure of both the basidiocarp and the mycelium in artificial culture will provide more stable criteria on which to base a natural classification.

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APPENDIX I

COLLECTIONS USED IN THIS STUDY

Collections of *C. pubescens*.

Acc. #.	Substrate	Date	Location
101	*Betula*	13.9.68	Winterburn, Alta.
112	*Betula*	13.9.68	Winterburn, Alta.
122	*Betula*	5.10.68	Winterburn, Alta.
130	*Betula*	19.10.68	Winterburn, Alta.
131	*Betula*	19.10.68	Winterburn, Alta.
132	*Betula*	19.10.68	Winterburn, Alta.
136	*Betula*	19.10.68	Winterburn, Alta.
137	*Betula*	19.10.68	Winterburn, Alta.
138	*Betula*	19.10.68	Winterburn, Alta.
139	*Betula*	19.10.68	Winterburn, Alta.
149	*Betula*	30.11.68	Winterburn, Alta.
151	*Betula*	30.11.68	Winterburn, Alta.
152	*Betula*	30.11.68	Winterburn, Alta.
155	*Betula*	30.11.68	Winterburn, Alta.
156	*Betula*	30.11.68	Winterburn, Alta.
170	*Betula*	30.11.68	Winterburn, Alta.
172	*Betula*	30.11.68	Winterburn, Alta.
173	*Betula*	30.11.68	Winterburn, Alta.
185	*Betula*	1.5.69	Winterburn, Alta.
186	*Betula*	1.5.69	Winterburn, Alta.
188	*Betula*	1.5.69	Winterburn, Alta.

Acc. #.	Substrate	Date	Location
189	*Betula*	1.5.69	Winterburn, Alta.
196	*Betula*	20.5.69	Devon, Alta.
198	*Betula*	21.5.69	Wabamun, Alta.
199	*Betula*	21.5.69	Wabamun, Alta.
200	*Betula*	21.5.69	Wabamun, Alta.
214	*Betula*	3.9.69	Wabamun, Alta.
215	*Betula*	3.9.69	Wabamun, Alta.
216	*Betula*	3.9.69	Wabamun, Alta.
219	*Betula*	3.9.69	Wabamun, Alta.
221	*Betula*	3.9.69	Wabamun, Alta.
230	*Betula*	10.9.69	Pigeon Lake, Alta.
233	*Betula*	10.9.69	Breton, Alta.
234	*Betula*	20.9.69	Wabamun, Alta.
236	*Betula*	7.10.69	Winterburn, Alta.
237	*Betula*	7.10.69	Winterburn, Alta.
238	*Betula*	7.10.69	Winterburn, Alta.
239	*Betula*	7.10.69	Winterburn, Alta.
241	*Betula*	7.10.69	Winterburn, Alta.
242	*Betula*	7.10.69	Winterburn, Alta.
243	*Betula*	31.8.69	Winfield, Alta.
253	*Betula*	9.8.70	Devon, Alta.
254	*Betula*	9.8.70	Devon, Alta.
255	*Betula*	9.8.70	Devon, Alta.
256	*Betula*	9.8.70	Devon, Alta.
257	*Betula*	9.8.70	Devon, Alta.
261	*Betula*	21.8.70	Boyne Lake, Alta.

Acc. #.	Substrate	Date	Location
262	*Betula*	21.8.70	Boyne Lake, Alta.
263	*Betula*	21.8.70	Boyne Lake, Alta.
264	*Betula*	21.8.70	Boyne Lake, Alta.
266	*Betula*	21.8.70	Boyne Lake, Alta.
267	*Betula*	21.8.70	Boyne Lake, Alta.
269	*Betula*	21.8.70	Boyne Lake, Alta.
270	*Betula*	21.8.70	Boyne Lake, Alta.
272	*Betula*	21.8.70	Boyne Lake, Alta.
274	*Betula*	21.8.70	Boyne Lake, Alta.
276	*Betula*	21.8.70	Boyne Lake, Alta.
279	*Betula*	31.8.70	Winterburn, Alta.
280	*Betula*	31.8.70	Winterburn, Alta.
282	*Betula*	31.8.70	Winterburn, Alta.
283	*Betula*	31.8.70	Winterburn, Alta.
284	*Betula*	31.8.70	Winterburn, Alta.
285	*Betula*	31.8.70	Winterburn, Alta.
286	*Betula*	31.8.70	Winterburn, Alta.
287	*Betula*	31.8.70	Winterburn, Alta.
288	*Betula*	31.8.70	Winterburn, Alta.
289	*Betula*	31.8.70	Winterburn, Alta.
293	*Betula*	15.9.70	Edmonton, Alta.
294	*Betula*	15.9.70	Wabamun, Alta.
300	*Betula*	11.10.70	Edmonton, Alta.
309	*Betula*	24.9.70	Winterburn, Alta.
310	*Betula*	24.9.70	Winterburn, Alta.
330	*Betula*	24.8.71	Winterburn, Alta.

Acc. #.	Substrate	Date	Location
331	*Betula*	24.8.71	Winterburn, Alta.
332	*Betula*	24.8.71	Winterburn, Alta.
333	*Betula*	24.8.71	Winterburn, Alta.
336	*Betula*	24.8.71	Winterburn, Alta.
339	*Betula*	27.8.71	Edmonton, Alta.
346	*Betula*	30.8.71	Chisholm, Alta.
347	*Betula*	30.8.71	Chisholm, Alta.
348	*Betula*	30.8.71	Chisholm, Alta.
350	*Betula*	30.8.71	Chisholm, Alta.
352	*Betula*	30.8.71	Chisholm, Alta.
353	*Betula*	30.8.71	Chisholm, Alta.
390	*Betula*	31.8.71	m.40, Wabasca Rd., Alta.
392	*Alnus*	7.9.71	Smoky Fire Tower, Alta.
393	*Alnus*	7.9.71	Smoky Fire Tower, Alta.
398	*Betula*	16.5.70	Prince Albert, Sask.
399	*Betula*	16.5.70	Prince Albert, Sask.
400	*Betula*	16.5.70	Prince Albert, Sask.
401	*Betula*	16.5.70	Prince Albert, Sask.
404	*Betula*	26.9.71	Entwistle, Alta.
405	*Betula*	26.9.71	Entwistle, Alta.
407	*Alnus*	2.10.71	Lake Eden, Alta.
417	*Betula*	24.9.70	Winterburn, Alta.
418	*Betula*	24.9.70	Winterburn, Alta.
419	*Betula*	24.9.70	Winterburn, Alta.

Collections of *C. hirsutus*.

Acc. #.	Substrate	Date	Location
119	*Betula*	27.9.68	Winterburn, Alta.
123	*Betula*	12.10.68	Edmonton, Alta.
157	*Betula*	5.10.68	Elk Island, Alta.
159	*Populus*	20.10.68	Seba Beach, Alta.
161	*Malus*	12.2.69	Edmonton, Alta.
168	*Betula*	30.11.68	Winterburn, Alta.
175	*Populus*	6.4.69	Cooking Lake, Alta.
184	*Populus*	29.4.69	Elk Island, Alta.
192	*Betula*	3.5.69	Lacombe, Alta.
193	*Betula*	3.6.69	Lacombe, Alta.
194	*Betula*	8.5.69	Edmonton, Alta.
207	*Populus*	19.8.69	Gorge Creek, Alta.
208	*Populus*	19.8.69	Gorge Creek, Alta.
209	*Populus*	19.8.69	Gorge Creek, Alta.
210	*Populus*	20.8.69	Gorge Creek, Alta.
211	*Populus*	20.8.69	Gorge Creek, Alta.
213	*Populus*	3.9.69	Wabamun Lake, Alta.
220	*Betula*	3.9.69	Wabamun Lake, Alta.
222	*Populus*	3.9.69	Wabamun Lake, Alta.
223	*Betula*	7.9.69	Lacombe, Alta.
226	*Populus*	10.9.69	Pigeon Lake, Alta.
228	*Populus*	10.9.69	Pigeon Lake, Alta.
229	*Populus*	10.9.69	Pigeon Lake, Alta.
231	*Populus*	10.9.69	Pigeon Lake, Alta.

Acc. #.	Substrate	Date	Location
240	*Betula*	7.10.69	Winterburn, Alta.
244	*Populus*	20.8.69	Gorge Creek, Alta.
245	Hardwood	25.6.69	Shuswap Lake, B.C.
246	*Betula*	16.9.68	Edmonton, Alta.
249	*Populus*	28.5.70	Amisk Lake, Alta.
250	*Populus*	31.5.70	Brazeau Dam, Alta.
251	*Prunus*	12.7.70	Kaleden, B.C.
259	*Sorbus*	17.8.70	Edmonton, Alta.
268	*Betula*	21.8.70	Boyne Lake, Alta.
271	*Betula*	21.8.70	Boyne Lake, Alta.
273	*Betula*	21.8.70	Boyne Lake, Alta.
277	*Populus*	21.8.70	Boyne Lake, Alta.
278	*Populus*	23.8.70	Edmonton, Alta.
290	*Populus*	31.8.70	Thunder Lake, Alta.
291	*Populus*	7.9.70	Sylvan Lake, Alta.
292	*Populus*	7.9.70	Sylvan Lake, Alta.
296	*Populus*	11.10.70	Edmonton, Alta.
297	*Populus*	11.10.70	Edmonton, Alta.
298	*Populus*	11.10.70	Edmonton, Alta.
299	*Populus*	11.10.70	Edmonton, Alta.
301	*Populus*	11.10.70	Edmonton, Alta.
302	*Betula*	11.10.70	Edmonton, Alta.
303	*Populus*	11.10.70	Edmonton, Alta.
304	*Populus*	18.10.70	Bowden, Alta.
305	*Populus*	18.10.70	Bowden, Alta.
307	*Populus*	18.10.70	Bowden, Alta.

Acc. #.	Substrate	Date	Location
317	*Populus*	27.7.71	Cypress Hills, Sask.
320	*Populus*	10.8.71	Swan Hills, Alta.
323	Hardwood	29.7.71	Mount Robson, B.C.
324	*Populus*	18.8.71	Clearwater Bridge, F.T.R. Alta.
325	*Populus*	18.8.71	Clearwater Bridge, F.T.R. Alta.
326	*Populus*	10.8.71	25m. N. Whitecourt, Alta.
362	*Populus*	30.8.71	m.24 Wabasca Rd., Alta.
366	*Populus*	30.8.71	m.24 Wabasca Rd., Alta.
367	*Populus*	30.8.71	m.24 Wabasca Rd., Alta.
370	*Populus*	30.8.71	m.24 Wabasca Rd., Alta.
372	*Populus*	30.8.71	m.24 Wabasca Rd., Alta.
375	*Populus*	31.8.71	m.32 Wabasca Rd., Alta.
379	*Populus*	31.8.71	m.32 Wabasca Rd., Alta.
381	*Populus*	31.8.71	m.32 Wabasca Rd., Alta.
388	*Populus*	31.8.71	m.40 Wabasca Rd., Alta.
389	*Populus*	31.8.71	m.40 Wabasca Rd., Alta.
391	*Populus*	31.8.71	m.40 Wabasca Rd., Alta.
408	*Populus*	2.10.71	Lake Eden, Alta.
409	*Populus*	9.10.71	Magnolia, Alta.
420	*Betula*	24.9.70	Winterburn, Alta.
421	*Fagus*	31.10.71	Lk. Lygnern, Sweden.
422	*Fagus*	31.10.71	Lk. Lygnern, Sweden.
423	Hardwood	31.10.71	Lk. Lygnern, Sweden.
424	Hardwood	31.10.71	Lk. Lygnern, Sweden.

Collections of *C. versicolor*.

Acc. #.	Substrate	Date	Location
414	*Fagus*	14.10.71	Hensall, Ontario
415	*Fagus*	14.10.71	Hensall, Ontario
416	*Ulmus*	14.10.71	Hensall, Ontario

APPENDIX II

HERBARIUM MATERIAL

(Cryptogamic Herbarium, Department of Botany, University of Alberta, Edmonton)

Collections listed as *C. pubescens*.

Acc. #.	Date	Location	Substrate	Observations
4373	5.5.68	Alta.	--	Weathered
2862	8.9.65	Alta.	*Betula*	Confirmed
2863	8.9.65	Alta.	*Betula*	Confirmed
2864	27.8.61	Alta.	*Betula*	Confirmed
2865	24.8.67	Alta.	*Populus*	*C. hirsutus*
2866	24.8.67	Alta.	*Populus*	*C. hirsutus*
2867	20.8.47	Alta.	*Populus*	*C. hirsutus*
2868	18.10.38	Alta.	*Betula*	Confirmed
2869	3.10.48	Alta.	*Populus*	Doubtful
2870	27.8.47	Alta.	*Betula*	Confirmed
2871	27.8.47	Alta.	*Betula*	Confirmed
2872	19.8.52	Alta.	*Populus*	*C. hirsutus*
2859	1.10.67	Alta.	*Populus*	*C. hirsutus*
2860	13.9.68	Alta.	*Betula*	Confirmed
2861	13.9.68	Alta.	*Betula*	Confirmed
4005	15.9.31	Alta.	--	Confirmed
2873	3.10.56	Alta.	*Betula*	Confirmed
2874	17.9.56	Alta.	Hardwood	*C. hirsutus*
2875	24.8.67	Alta.	*Populus*	*C. hirsutus*

Acc. #.	Date	Location	Substrate	Observations
2876	21.5.63	Alta.	Wood	Confirmed
2877	16.8.62	Alta.	*Betula*	Confirmed

Collections listed as *C. hirsutus*.

4371	21.6.68	Alta.	*Populus*	Weathered
4459	3.10.70	Alta.	*Populus*	Confirmed
4579	26.9.71	Alta.	*Populus*	Confirmed
2772	5.6.63	Alta.	*Picea*	Confirmed
2773	20.8.67	Alta.	*Populus*	Confirmed
2774	7.10.64	Alta.	*Populus*	Confirmed
2775	11.10.24	Mass.	*Carya*	Confirmed
2756	17.4.55	Iowa	Hardwood	Confirmed
2757	21.9.54	Iowa	Hardwood	Confirmed
2758	21.9.54	Iowa	--	Confirmed
2759	24.9.59	Alta.	*Populus*	Confirmed
2760	6.9.67	Alta.	*Populus*	Confirmed
2764	10.9.47	Alta.	*Populus*	Confirmed
2765	22.9.46	Alta.	*Populus*	Confirmed
2766	18.10.35	Alta.	*Populus*	Confirmed
2767	10.9.47	Alta.	*Populus*	Confirmed
2768	6.8.48	Minn.	*Populus*	Confirmed
2769	8.7.29	Alta.	*Betula*	*C. pubescens*
2781	4.10.25	Mass.	*Acer*	Confirmed
2782	26.10.24	Mass.	Wood	Confirmed
2783	18.10.25	Mass.	*Betula*	Confirmed
2784	16.6.26	N.S.	*Betula*	Confirmed

Acc. #.	Date	Location	Substrate	Observations
2785	11.10.24	Mass.	*Carya*	Confirmed
2786	4.10.25	Mass.	*Acer*	Confirmed
2776	22.10.32	N.S.	*Fagus*	Confirmed
2777	22.10.32	N.S.	*Fagus*	Confirmed
2778	30.3.24	Mass.	--	Confirmed
2779	4.10.25	Mass.	*Acer*	Confirmed
2780	26.10.24	Mass.	Hardwood	Confirmed
4145	2.9.70	Alta.	*Populus*	Confirmed
4151	-.9.70	Alta.	*Betula*	Confirmed
4167	29.9.70	Alta.	*Betula*	Confirmed
2761	21.9.66	Alta.	*Populus*	Confirmed
2762	21.9.66	Alta.	*Populus*	Confirmed
2763	20.10.68	Alta.	*Populus*	Confirmed
4052	13.5.70	Alta.	*Betula*	Confirmed
2787	2.9.58	Ariz.	*Acer*	Confirmed
2788	14.10.61	N.Y.	*Acer*	Confirmed
2789	1.9.58	Ariz.	Hardwood	Confirmed
Collections listed as *C. versicolor*.				
2921	18.10.33	Colo.	*Betula*	Confirmed
2922	9.9.65	Alta.	*Populus*	*C. hirsutus*
2923	13.7.67	Alta.	*Populus*	*C. hirsutus*
2924	1.8.63	B.C.	Wood	Confirmed
2925	7.10.64	Alta.	*Populus*	*C. hirsutus*
4293	23.7.35	China	--	Confirmed
4118	--	--	--	*C. hirsutus*
2935	-.8.67	Alta.	*Populus*	*C. hirsutus*

Acc. #.	Date	Location	Substrate	Observations
2936	24.8.67	Alta.	*Populus*	*C. hirsutus*
2937	4.6.66	B.C.	--	Confirmed
3532	4.6.68	Texas	--	Confirmed
3960	12.6.69	Hawaii	--	Confirmed
3955	25.6.68	Hawaii	--	Confirmed
3968	13.6.69	Hawaii	--	Confirmed
2926	24.6.53	Iowa	Hardwood	Confirmed
2927	31.7.48	Minn.	*Populus*	Confirmed
2928	21.9.54	Iowa	Hardwood	Confirmed
2930	21.9.54	Iowa	Hardwood	Confirmed
2929	7.6.68	B.C.	--	Confirmed
2931	23.10.26	N.S.	*Picea*	Confirmed
2932	26.10.24	Mass.	Hardwood	Confirmed
2933	16.6.26	N.S.	Wood	Confirmed
2934	24.9.33	N.S.	*Fagus*	Confirmed
2938	22.10.32	N.S.	*Fagus*	Confirmed
2939	18.10.25	Mass.	*Acer*	Confirmed
2940	11.8.27	N.S.	Hardwood	Confirmed
2941	16.6.26	N.S.	Wood	Confirmed
2942	16.8.25	N.S.	*Fagus*	Confirmed
2943	11.8.27	N.S.	Hardwood	Confirmed
2944	18.10.25	Mass.	*Acer*	Confirmed
2945	20.7.25	N.S.	*Fagus*	Confirmed
2946	6.9.68	Alta.	--	*C. hirsutus*
2947	26.10.24	Mass.	Wood	Confirmed

Collections listed as *C. zonatus*.

Acc. #.	Date	Location	Substrate	Observations
2954	17.8.47	Alta.	*Populus*	*C. hirsutus*
2955	16.10.51	Alta.	*Betula*	*C. hirsutus*
2956	20.8.47	Alta.	*Populus*	*C. hirsutus*
2957	24.9.51	Alta.	*Populus*	*C. hirsutus*

(Herbarium of the Northern Forest Research Centre, Edmonton)

Collections listed as *C. pubescens*.

Acc. #.	Date	Location	Substrate	Observations
WINF(M)4557	24.6.66	Sask.	*Betula*	Confirmed
DASFP1448	25.10.54	--	*Betula*	Confirmed
DASFP873	7.9.49	Sask.	*Betula*	Confirmed
5502	25.8.66	Sask.	*Salix*	*C. hirsutus*
CFB8856	27.9.64	N.Y.	*Acer*	Confirmed
CFB4832	7.9.61	Alta.	*Betula*	Confirmed
DACFP174	21.5.71	Alta.	*Populus*	Doubtful
DACFP2817	4.9.48	Ont.	*Acer*	Confirmed
CFB5111	25.8.62	Alta.	*Alnus*	Doubtful
DACFP248	25.6.52	Alta.	*Populus*	Doubtful
CFB4412	29.6.60	Alta.	*Populus*	*C. hirsutus*
WINF(M)11130	6.8.68	N.Y.	*Acer*	Confirmed
WINF(M)176	5.9.23	Sask.	*Acer*	Confirmed
WINF(M)83	10.9.47	Man.	*Quercus*	Confirmed

Collections listed as *C. hirsutus*.

DACFP188	20.8.52	Alta.	*Populus*	Confirmed
DACFP2815	28.6.52	Alta.	*Betula*	*Cerrena unicolor*
DACFP765	7.8.53	Alta.	*Betula*	Confirmed
CFB5162	22.6.62	Alta.	*Populus*	Weathered
CFB4889	23.8.61	Alta.	*Populus*	Confirmed
WINF(M)8428	21.8.67	Sask.	*Betula*	Confirmed
WINF(M)8241	12.9.67	--	*Populus*	Confirmed

Acc. #.	Date	Location	Substrate	Observations
WINF(M)10872	24.7.68	Man.	*Populus*	Confirmed
WINF(M)87	23.9.47	Sask.	*Populus*	Confirmed
WINF(M)86	24.11.32	Ont.	*Pyrus*	Confirmed
WINF(M)2016	30.9.65	Man.	*Betula*	*C. pubescens*
WINF(M)12503	15.9.68	Sask.	*Prunus*	*C. pubescens*
WINF(M)3284	8.7.65	Man.	*Betula*	*Cerrena unicolor*
WINF(M)3313	17.8.65	Man.	*Betula*	*Cerrena unicolor*
WINF(M)3292	6.7.65	Man.	*Populus*	Weathered
3251	17.8.65	Man.	*Populus*	Doubtful
WINF(M)3297	19.8.65	Man.	*Populus*	*Cerrena unicolor*
17906	12.10.47	Alta.	*Populus*	Confirmed
WINF(M)10276	27.8.68	Man.	*Populus*	Confirmed
5499	15.9.66	Man.	*Populus*	Confirmed
WINF(M)7944	11.8.67	Man.	*Quercus*	Confirmed
WINF(M)2026	11.9.64	Sask.	*Populus*	Confirmed
Collections listed as *C. versicolor*.				
WINF(M)8748	-.10.67	Ont.	*Acer*	Confirmed
WINF(M)8813	25.10.67	Man.	*Betula*	*C. hirsutus*
WINF(M)-	6.10.65	Man.	*Populus*	*C. hirsutus*
DASFP679	15.8.52	Sask.	*Populus*	*C. hirsutus*
WINF(M)2430	6.10.65	Man.	*Populus*	*C. hirsutus*
36-1	9.10.36	P.Q.	*Fagus*	Confirmed
WINF(M)3328	1.9.65	--	*Populus*	*Bjerkandera adusta*
WINF(M)4204	6.6.66	--	*Acer*	*Stereum* spp.
WINF(M)6749	18.5.67	Man.	*Fraxinus*	Confirmed

Acc. #.	Date	Location	Substrate	Observations
17562	23.9.47	Sask.	*Populus*	*C. hirsutus*
S100	1.9.47	Man.	*Populus*	*C. hirsutus*
WINF(M)2018	--	--	*Populus*	*C. hirsutus*
WINF(M)2930	27.9.65	Man.	*Alnus*	Very young
WINF(M)4910	11.8.66	Sask.	*Betula*	*Stereum* spp.
WINF(M)-	17.8.67	Man.	*Betula*	Confirmed
WINF(M)6727	16.5.67	Man.	*Betula*	*C. hirsutus*
DACFP3515	7.10.57	N.Y.	*Acer*	Confirmed
Collections listed as *C. zonatus*.				
5505	13.9.66	Sask.	--	*C. hirsutus*
CFB6509	13.8.63	Alta.	*Betula*	*C. hirsutus*
CFB6108	22.9.63	Alta.	*Betula*	*C. hirsutus*
DACFP603	9.7.52	Alta.	*Populus*	*C. hirsutus*
DACFP66	11.9.52	Alta.	*Populus*	*C. hirsutus*
DACFP245	17.9.52	Alta.	*Populus*	*C. hirsutus*
DACFP176	31.7.52	Alta.	*Populus*	*C. hirsutus*
DACFP246	10.9.52	Alta.	*Populus*	*C. hirsutus*
DACFP249	8.8.52	Alta.	*Populus*	*C. hirsutus*
DACFP250	12.8.52	Alta.	*Populus*	*C. hirsutus*
CFB6100	23.6.63	Alta.	*Populus*	*C. hirsutus*
CFB6127	13.8.63	Alta.	*Populus*	*C. hirsutus*
CFB6130	20.8.63	Alta.	*Populus*	*C. hirsutus*
CFB6132	7.6.63	Alta.	*Populus*	*C. hirsutus*
Collections listed as *C. velutinus*.				
CFB6621	14.8.65	Alta.	*Populus*	*C. hirsutus*

Acc. #.	Date	Location	Substrate	Observations
WINF(M)98	18.9.47	Man.	*Populus*	*C. hirsutus*
CFB6614	15.7.65	Alta.	*Populus*	*C. hirsutus*
DASFP721	5.8.52	Man.	*Populus*	*C. hirsutus*
WINF(M)2019	--	--	*Malus*	*C. hirsutus*
DASFP923	-.5.36	Sask.	*Populus*	*C. hirsutus*
Collections listed as *Polyporus resinosus*.				
5408	30.8.66	Sask.	*Betula*	*C. hirsutus*

APPENDIX III

Collections with cultures received from the Plant Research Institute, Ottawa

Collections listed as *C. pubescens*.

Acc. #.	Date	Location	Substrate	Observations
DAOM53503	3.10.59	N.Y.	--	Confirmed
DAOM94017	8.9.62	Ont.	*Acer*	Confirmed
DAOM94039	8.9.62	Ont.	*Betula*	Confirmed
DAOM17540	18.9.47	Man.	*Populus*	*T. sauveolens*
DAOM73309	20.9.55	Ont.	*Alnus*	Confirmed
DAOM94026	8.9.62	Ont.	*Acer*	Doubtful

Collections listed as *C. hirsutus*.

DAOMF1304	20.11.29	N.Y.	*Ulmus*	Doubtful
DAOM72381	24.9.44	Ont.	*Betula*	Doubtful
DAOM94064	-.12.54	S.Africa	--	No basidiocarp
DAOMF9884	13.6.40	B.C.	*Alnus*	Doubtful
DAOM10240	21.10.41	B.C.	*Prunus*	Doubtful
DAOM21158	24.7.48	Ont.	*Thuja*	Doubtful

APPENDIX IV

Sample size for interfertility tests.

Since the four mating types of a tetrapolar species of a Basidiomycete result from a meiotic division, they occur in equal numbers. The probability of a spore sample of a certain size containing the four mating types can be calculated from the following formula where P = the probability of the four mating types being present in a sample of n size.

$$P = \frac{1}{4^{n-1}} (4^{n-1} - 3^n + 2^n - 1)$$

This information is useful in determining the size of sample to be used in interfertility tests.
The following values for P have been calculated for various sample sizes (n).

n = 8, P = 0.62

n = 10, P = 0.78

n = 12, P = 0.88

n = 13, P = 0.91

n = 14, P = 0.93

n = 15, P = 0.95

n = 16, P = 0.96

n = 17, P = 0.97

Derivation.

The general formula for determining the probability

that in a sample of n size, there will be at least one each of r objects is:-

$$P = \frac{1}{r^n} \left[r^n - \binom{r}{1}(r-1)^n + \binom{r}{2}(r-2)^n - \binom{r}{3}(r-3)^n \ldots \pm \binom{r}{r-1}(1)^n \right]$$

where $\binom{r}{1}$ is the number of combinations of r objects taken 1 at a time.

This formula is derived as follows:-

The total number of combinations of r objects in a sample n is given by r^n, but this includes in addition to the favourable cases, the cases with (r-1) objects.

Subtracting this we get $r^n - \binom{r}{1}(r-1)^n$, but this subtracts too much, namely, $\binom{r}{2}$ of the cases with (r-2). Therefore this must be added to get $r^n - \binom{r}{1}(r-1)^n + \binom{r}{2}(r-2)^n$.

Again, this adds too many cases with (r-3) objects, and by continuing in this manner, the following formula is arrived at.

$$P = \frac{1}{r^n} \left[r^n - \binom{r}{1}(r-1)^n + \binom{r}{2}(r-2)^n - \binom{r}{3}(r-3)^n \ldots \pm \binom{r}{r-1}(1)^n \right]$$

If, for example, a sample of 8 spores is used, and there are 4 possible mating types, then n = 8 and r = 4.

Substituting for n and r,

$$P = \frac{1}{4^8} \left[4^8 - \binom{4}{1}(3)^8 + \binom{4}{2}(2)^8 - \binom{4}{3}(1)^8 \right]$$

$$P = \frac{1}{4^8} \left[4^8 - 4(3)^8 + 4(2)^8 - 4(1)^8 \right]$$

$$P = \frac{4}{4^8}\left[4^7 - (3)^8 + (2)^8 - (1)^8\right]$$

$$P = \frac{1}{4^7}\left[4^7 - (3)^8 + (2)^8 - (1)^8\right]$$

$P = 0.62.$

i.e. if $r = 4$,

$$P = \frac{1}{4^{n-1}}\ (4^{n-1} - 3^n + 2^n - 1)$$

The formula for determining the probability of both mating types appearing in a sample of size n, for a bipolar species is given by:-

$P = \frac{2^n - 2}{2^n}$ substituting for various values of n,

if $n = 2$, $P = 0.5$

$n = 3$, $P = 0.75$

$n = 4$, $P = 0.87$

$n = 5$, $P = 0.94$

$n = 6$, $P = 0.97$