A STUDY OF THE DACRYMYCES DELIQUESCENS COMPLEX

by

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ABSTRACT

The main objective of the present study was to determine whether the varieties of Dacrymyces deliquescens sensu Kennedy represent a single species or are three distinct species, and to study the life cycles of the fungi of this complex.

An unsuccessful attempt was made to grow these fungi through their life cycles in culture. Cultural characteristics were compared among the varieties as well as to those characteristics reported in the literature. To obtain single spore cultures for mating tests, eight methods and six media were tried without successful results. Of the 1560 spores isolated only three resulted in mycelial growth. Erroneous isolation and the presence of a number of spores were suspected in these cultures. The development of the basidium and of basidiospores were studied cytologically to determine the fate of the two nuclei remaining in the basidium. The two supernumerary nuclei remain in the hypobasidium and degenerate.

Conidia produced on the mycelium are globose, elliptical or oval for D. deliquescens (Merat) Duby var. deliquescens Kennedy, D. deliquescens var. ellisii (Coker) Kennedy and D. deliquescens var. minor (Peck) Kennedy respectively. It is suggested that these conidia are carried away by water rather than ejected by some mechanism, as has been previously proposed.

Color differences among cultures and different shape of conidia produced indicate a definite degree of separation while intermediate forms among the three organisms indicate close relationship. Whether there are three species or one species with three varieties involved cannot be determined without mating tests.

The suggested life cycle for D. deliquescens var. deliquescens follows: Basidiospores are uninucleate at the time of discharge. The nucleus soon divides and 3-septate, four-celled spores are formed. Germination is induced by a volatile substance which is produced in minute quantities. Cells of basidiospores act independently after germination of the spore. Spores germinate by germ tubes, by conidia or by a combination of the two. Production of conidia terminates the germination of individual cells. The maximum number of germ tubes produced by any one cell is two. Monokaryotic mycelium is produced upon germination of basidiospores and conidia. Dikaryotization takes place in an unknown way. Arthrospores may be produced on dikaryotic mycelium, and these germinate to produce dikaryotic mycelium. Basidia are formed on dikaryotic mycelium. The young basidium is cylindric, binucleate, and separated from the hyphae by a basal septum. A fusion nucleus is produced which gives rise upon division to four stichobasidially arranged daughter nuclei. One nucleus passes into each epibasidium and this later becomes the nucleus of the spore. Two nuclei remain in the hypobasidium and degenerate.

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A. INTRODUCTION

The family Dacrymycetaceae is treated either as one of the families in the Tremellales, or as a single family in the Dacrymycetales. The group is easily distinguished from all others by possession of bifurcate, "tuning fork" basidia and typically allantoid, transversely septate basidiospores. While specimens may be readily recognized as members of this family, identification to genus or to species often presents considerable difficulty. Until recently, generic separation was based mainly upon basidiocarp form. Since this is an extremely variable characteristic, as it is in other heterobasidiomycetous groups, genera were not clearly defined. In the most recent treatment of the family (Kennedy, 1958a), an attempt has been made to separate genera on the basis of microscopic characteristics. Since the latter have been found to be more reliable than macroscopic traits used in distinguishing other basidiomycetous groups, perhaps a more natural classification will result.

As with genera, species in this group have been distinguished mainly through use of macroscopic characteristics, of size, form, color, etc., all of which are extremely variable. Although more than forty species of Dacrymyces have been described, Kennedy (1958b) recognized only eight in her monograph of the genus. In her work, as in that of Kobayasi (1939), greater emphasis has been placed upon microscopic characteristics. As a result of this, many species have been reduced to synonymy and others have been treated as varieties. Not all taxonomists agree with this treatment (Klett, 1962), particularly with the designation of certain species as varieties. However, the group as a whole is so poorly known, that argument for either viewpoint has little factual basis.

Studies in this family, other than taxonomic, have not been numerous. Several species, including Dacrymyces deliquescens (Merat) Duby (Dangeard, 1895; Juel, 1898; Maire, 1902), Dacrymyces chrysocomus (Bull.) Tul. (Istvanffi, 1895), and Guepinia spathularia Schw. (Bodman, 1938) have been studied cytologically. Bulat (1953) grew Dacrymyces ellisii Coker in culture and described several types of conidia produced. Two later studies (Hanna and Bulat, 1953, Bulat, 1954) considered production of pigment in cultures of this species. Yen (1947 and 1949) reported on compatibility studies of several dacrymycetaceous fungi. Studies of the latter type could prove helpful in delimiting species within various genera. Information on cultural characteristics too, might be very useful in this respect. Furthermore, life cycles generally depicted for this group (as in Alexopoulos, 1952) are based largely on conjecture. Culture studies also could aid in determining relationships of the Dacrymycetaceae to other basidiomycetes.

Dacrymyces ellisii Coker and Dacrymyces minor Peck are species which in Kennedy's (1958b) monographic treatment have been reduced to varieties of Dacrymyces deliquescens. Kennedy states (1958a) "It is likely that this classification will be modified in the future, for developmental studies are very rare as yet". Although her statement refers to the family, it could be valid for this group of species.

The main objective of the present study has been to determine whether the varieties of Dacrymyces deliquescens, as treated by Kennedy, represent a single species or are three distinct species, and to study the life cycles of the fungi of this complex.

B. LITERATURE SURVEY

Dacrymyces deliquescens (Merat) Duby

In 1791 Bulliard described Dacrymyces deliquescens under the name of Tremella deliquescens. Nees established the genus Dacrymyces stillatus (=D. deliquescens). He spelled the generic name Dacryomyces. Merat (1821) gave a short description of the perfect stage of Tremella deliquescens Bull. Fries (1822) recognized the genus Dacrymyces and cited Tremella deliquescens as type species. His spelling of the generic name was Dacrymyces, and since he used this spelling in later works the change may be considered as intentional. Duby (1829) gave a description of Dacrymyces deliquescens and listed Tremella deliquescens Bull, as a synonym. The nature of the hymenial elements was first emphasized by Tulasne in 1853, who thought that these fungi might be related to the Tremellales. He also described the internal structure and the oidial (arthrospore) stage of Dacrymyces deliquescens. Kennedy (1958b) kept the name Dacrymyces deliquescens, but broadened the scope of the species considerably. In her monograph, the species includes the original Dacrymyces deliquescens (Merat) Duby as var. deliquescens Kennedy, Dacrymyces ellisii Coker as var. ellisii (Coker) Kennedy, and Dacrymyces minor Peck as var. minor (Peck) Kennedy. She based this treatment on the existence of collections that are intermediate in character between the three species.

The first cytological study of the genus Dacrymyces was made by Dangeard (1895) working with D. deliquescens. Dangeard noted the fusion of the primary nuclei and the organization of the nucleus. He mentioned only a single division of the fusion nucleus in the basidium, and stated

that one nucleus passed into each of the two basidiospores.

Istvanffi (1895) working with D. chrysocomus (Bull.) Tul. (=Guepiniopsis chrysocomus (Tul.) Brasf.) observed two divisions of the fusion nucleus. He also noted that only one nucleus passed into each basidiospore and thought that the remaining nuclei functioned in the production of a second crop of basidiospores.

Juel (1898) described the meiotic process in D. deliquescens. His account of nuclear division and basidiospore formation agrees with that of Istvanffi, but he did not postulate a second crop of basidiospores.

Maire (1902) confirmed Istvanffi's observations and postulation of a second generation of basidiospores. He went one step further and gave two as the chromosome number for Dacrymyces deliquescens.

Wager (1914) studied an unspecified species of Dacrymyces. He agreed with earlier workers in essentials concerning nuclear changes, but dismissed the probability of the production of a second crop of basidiospores. Instead he suggested the possibility of two nuclei passing into each basidiospore. Disagreeing with Maire he gave four as the chromosome number.

Gilbert (1921) worked with three species of Dacrymyces but did not give the specific names of these. He found centrosomes present in the nuclei and gave four as the chromosome number, confirming Wager's (1914) count. As for the two nuclei in the basidium after spore discharge, he stated that they remain in the probasidium (hypobasidium) and later degenerate.

Bodman (1938) studied Guepinia spathularia Schw. (Dacryopinax spathularia (Schw.) Martin at present) and confirmed earlier descriptions

of the meiotic process. She disagreed with Wager's (1914) account of two nuclei passing into each spore, but stated that two nuclei pass into each epibasidium. She also noted that, in some cases, two nuclei remained in the hypobasidium, and that the supernumerary nuclei degenerated. Thus, her observations confirm those of Gilbert (1921).

There have been only two reported culture studies of D. deliquescens (Brefeld, 1888, Yen, 1949). Brefeld observed the germination of basidiospores in water and in liquid nutrient media, and first observed formation of conidia on thin walled hyphae. Conidia were borne singly or in groups of two or more upon short conidiophores, varying in shape from ellipsoid to ovoid. He gave the size of conidia as 3-7 x 2-5 u.

Yen studied the germination of basidiospores and arthrospores on nutrient agar. He reported that basidiospores are discharged as 1-celled spores and the formation of septa takes place after discharge. He stated that the four cells of basidiospores act independently from one another. He noted differences in the method of germination and in the color of cultures that were related to the kind of substratum the collection was made from. He obtained no fructification in cultures. Yen obtained 10 single spore cultures after extreme difficulties but unfortunately failed to mention the method used in obtaining them. He also observed the mycelial conidia and gave the size as 2.2 x 2.2 u or 2.0 - 2.5 x 4.0-4.5 u. Yen stated that D. deliquescens is a heterothallic species.

Dacrymyces ellisii Coker

Dacrymyces ellisii was described by Coker in 1920. Bresadola (1920) later described the same species as Dacrymyces harperi Bres. Lloyd (1923) and Neuhoff (1936) treated this species as Dacrymyces cerebriformis

Bref. However, Brefeld's (1888) D. cerebriformis is not definitely known to be identical to Dacrymyces ellisii Coker.

There are no known cytological studies of D. ellisii, but there have been three reports of work with this species in culture. Two of these (Hanna and Bulat, 1953; Bulat, 1954) were concerned with pigmentation and the effect of light on pigmentation respectively. The third (Bulat, 1953) concerns cultural characteristics of this species. Bulat reported the occurrence of three different types of conidial spores of Dacrymyces ellisii in cultures. One of these types measuring 4-5 x 1.8-2.5 μ occurred singly or in groups on hyphae. He stated that these were forcibly abstricted from the conidiophores, although he found no mechanism for this type of spore discharge. Thick walled, intercalary or terminal chlamydospores were also observed in his cultures. The third spore type observed was elongated ballistospores, averaging 17 x 2.6 μ in size, which germinated by budding and by repetition. On the basis of his observation of ballistospores, Bulat linked the family Sporobolomycetaceae to the Tremellales.

Dacrymyces minor Peck

Fries (1822) described D. deliquescens f. lutescens and this was later treated by Peck (1877) as a distinct species, D. minor. Most succeeding authors (Coker, 1920; Bourdot and Galzin, 1927; Neuhoff, 1936; Kobayasi, 1939; Martin, 1944, 1952) have followed Peck's treatment. However, Kennedy (1958b) considers it to be only a variety of D. deliquescens (Merat) Duby.

No cytological or cultural studies of D. minor have been reported in the literature.

Names of authors and of fungi concerning the early taxonomic treatment of the group were taken from Kennedy (1958b).

C. SEPARATION OF THE THREE VARIETIES OF DACRYMYCES DELIQUESCENS "KENNEDY"

The three species, or varieties, are usually distinguished from one another in the following way (based on Kennedy's (1958b) key for the genus Dacrymyces):

a. Basidiocarp greenish-yellow or amber, then dingy-yellow or orange; drying inconspicuous; scarcely coalescing; spores yellow in mass, finally 3-septate, usually on angiosperm wood.....D. deliquescens var. minor

a. Basidiocarp orange-yellow or reddish-orange; drying conspicuous; frequently coalescing to form irregular masses, erumpent, or raised clusters; spores orange in mass, early 3-septate.....b

b. Spores distinctly 3-septate, walls and septa usually becoming wide and gelatinous; arthrospores in the basidiocarp or in separate sporocarps (rarely absent); lacking radicating bases; hyphae smooth; on gymnosperm or angiosperm wood....D. deliquescens var. deliquescens

b. Spores distinctly or indistinctly septate, walls and septa narrow, arthrospores absent; attached

by a radicating base or short stalk; some hyphae rough (rarely all smooth); often in raised clusters; commonly on angiosperm wood.....D. deliquescens var. ellisii

D. MATERIALS

1. Organisms: All specimens used in this study were collected in British Columbia, the majority from the vicinity of Vancouver. These were collected by either Dr. R.J. Bandoni or by the writer. Dr. Bandoni's collection numbers bear the prefix BC-, and my specimens are indicated IM-. Of the 87 specimens studied, 39 were collected from angiosperm wood, and 46 specimens from gymnosperm wood. In two cases the substratum has been given only as decaying wood. The specimens were collected in the years of 1959-1962 inclusive. Collections were made during every month of the year, with the exception of August and December.

A list of the specimens used in the study is given in the Appendix. Collection data, substrata, date of collections, etc., are included. All the specimens listed there have been placed in the Mycological Herbarium of the University of British Columbia.

2. Culture media: A brief study was undertaken to determine the effects of some nutritional and environmental factors on the growth of D. deliquescens var. deliquescens. The basic synthetic medium described by Lilly and Barnett (1951), was modified (BMA). Freshly prepared potato dextrose agar (FPDA) was used extensively in this study because of its good support of mycelial growth of the fungus. PDA (difco) and malt ex-

tract agar (MEA) were also used. Difco malt agar (MA) was chosen for the study of spore germination. The contrast of color between the basidiospores and the medium, the homogeneous consistency, and the ease in preparation all made it preferable to other media. Water agar (WA) was tried for inducing basidiocarp formation. The formulae of the various media are given in the Appendix.

Branches of Acer macrophyllum Pursh and Tsuga heterophylla (Raf.) Sarg. were also used as substrate in culture studies. Only those branches on which D. deliquescens was growing were selected for this purpose. This was taken as insurance that the branches collected were suitable material for the growth of the fungus. The branches were decorticate and approximately 4 cm in diameter.

All water used in this study was distilled. Some was also treated with decolorizing carbon to purify it further.

3. Liquid cultures: 125 ml Erlenmyer flasks were used for liquid cultures. Basic medium (20 ml) and other appropriate substances were measured into the flasks. These were covered by aluminum foil and sterilized for 15 minutes at 15 psi. The flasks were inoculated after they cooled to room temperature. The use of a shaker was first tried, but was soon abandoned because of several drawbacks of the method. The number of cultures at any one time on the shaker was limited, the temperature in the vicinity of the shaker rose to 32° C after the third day of shaking, and no growth was found in the flasks. The raised temperature was suspected to be the growth inhibiting factor. Subsequently, inoculated flasks were stored on a table and shaken by hand from time to time.

E. METHODS

1. Care of collections: Freshly collected specimens were air dried at room temperature. Slow drying helped to preserve the material and did not greatly decrease the viability of basidiospores. When material was needed for study, the basidiocarps were soaked in distilled water long enough to swell and soften without causing loss of color. When used for obtaining basidiospores, basidiocarps were soaked for five minutes in distilled water.

2. Obtaining basidiospores: The method for obtaining basidiospores was that described by Mounce (1929), and has previously been used in the study of heterobasidiomycetous fungi by Barnett (1937) and others. Several basidiocarps on a piece of substratum were soaked. The piece was fastened to the cover of a Petri plate so that the basidiocarps faced downward when the lid was in place. The cover was rotated at intervals to help distribute spores on the agar surface. Individual spores were then picked out from the edges of spore deposits by the use of a fine needle. The same method was used for the study of spore germination but the basidiocarps were placed over microscope slides covered with an agar film.

3. Single spore cultures were required for use in mating tests. Single spores were transferred to plates from spore deposits. Media used in this series of transfers were PDA, both commercial and freshly prepared, MA, MEA, BMA, and WA.

The following methods were tried:

1. Spores were transferred to separate plates.

2. Plates were divided into quarters by pencil marks on the under sides of the plates, and one spore transferred to each quarter.

3. Two spores were transferred into each plate with approximately 1 mm of distance between the spores.

4. As a combination of No. 2 and No. 3, two spores were transferred to each quarter.

5. Distilled water was poured into a sterile Petri plate and spores shed from basidiocarps into the water. This water was used to flood the surface of agar plates. When the water evaporated the spores settled down on the agar surface.

6. A block of agar was cut from the immediate vicinity of germinating spore deposit, and transferred to a sterile plate. A single spore was transferred onto the block.

7. The colony of germinating spores was removed from the plate and single spores were transferred to the remaining agar.

8. Single spores already germinating were separated from the colony and transferred to new agar plates.

4. Multispore cultures: After obtaining spore deposits on the agar plates, portions of the deposits were transferred to new plates. In order to obtain pure cultures, two successive transfers were made from margins of the resulting mycelium. These third transfers were then used in this study.

The branches of Acer macrophyllum Pursh and of Tsuga heterophylla (Raf.) Sarg. were used as a substratum for multispore cultures. The branches were cut into pieces approximately 7 cm long. Some were then split longitudinally, exposing the wood. The sections were put singly on wet filter paper in small moist chambers and sterilized by autoclaving. Inoculation was made either by masses of germinating spores or by mycelium.

The cultures were kept under alternating light and dark periods, and at temperature ranging between 17° C and 21° C, for six weeks. When mycelium covered the surface of the substratum, some of these cultures were exposed to continuous light in an attempt to induce basidiocarp formation.

5. Glassware: The following techniques were utilized to insure glassware free of contaminating materials: Petri plates were washed with detergent (Labtone) in hot water. This was followed by extensive rinses in hot water, then in cold tap water. The plates were allowed to dry, inside turned downward, and then sterilized. In most cases, plates were placed in airtight containers and sterilized in a gas oven (3-4 hours above 180 degrees C). Less frequently autoclaving at 15 psi. for 20 minutes was used for this purpose. Applying the latter method the plates were used as soon as they were cool enough to be handled.

When liquid cultures were used, the washing procedure was essentially the same. However, these dishes received one additional rinse with carbon treated (demineralized) distilled water. This method probably did not result in glassware completely free of all foreign materials, but since all the experiments were of a comparative nature the results were useful.

Decolorizing carbon was added to distilled water at the approximate rate of 10 g/1000 ml. The two were boiled together for 5-10 minutes, and the carbon then removed by filtering.

6. Slides: Thin free hand sections or small fragments of the basidiocarps, picked by wedge shaped needle, were studied. The mycelium from cultures was removed by use of a sterile needle. Care was taken to limit the amount of agar as much as possible. This material was treated in one

of three ways: smeared on the slide, macerated on the slide, or left intact as put on the slide.

a. Staining of slides: The material for morphological study was stained using the KOH-phloxine technique of Martin (1934). A drop of 3% KOH and two drops of phloxine stain were added, the material was covered by a coverslip, the stain removed by absorbent paper and replaced by KOH.

For the observation of nuclei a modified version of Belling's iron-acetocarmine method was used. This modification was developed by Dr. R.J. Bandoni (personal communication). The material was smeared on the slide and treated with 3% KOH for about 4 minutes. This was removed by absorbent paper. Drops of acetocarmine dye were added and the slide heated. An iron needle was dipped into the dye three or four times, a coverslip was added, pressed, the excess dye removed and the slide allowed to cool down. The slides were warmed gently before observation in order to intensify the stain.

b. Preservation of slides: Repeated study, photography, and the use of a camera lucida made it necessary to have semipermanent slides. In such instances the edges of coverslip were simply sealed with paraffin or nail polish. Paraffin was always used for acetocarmine preparations and nail polish for slides stained with KOH-phloxine. Slides sealed with nail polish could be kept for a longer period than those sealed with paraffin.

c. Preparation of slides for the observation of spore germination: The germination of basidiospores was studied on slides prepared by a method similar to that described by Rawlins (1933). The medium was prepared, poured into tubes, sterilized, and stored. When needed it was

liquefied by heating the tubes in a hot water bath. A few drops of the medium were placed on warm, sterile slides and spread to form a thin film. Care was taken that the film did not reach the edges of the slide. Inoculation of the slides was done by placing basidiocarps over the agar film as has been described earlier. Inoculated slides were incubated in sterile moist chambers. Two media, FPDA and MA, were tested for the agar film on slides. FPDA was found to be too opaque; MA was therefore used.

Slide cultures were stained with phloxine, with or without KOH, for observation of conidia, hyphae and similar structures. Acetocarmine, preceded by KOH, was used for a nuclear stain. For observation of nuclei, slides were heated to melt the agar and the coverslip was then pressed to flatten the material. Paraffin was used to seal these slides.

7. Glass tubes for the study of spore germination: Another method was also used in the study of germination. A drop of distilled water was placed on a spore deposit. The capillarity of glass tubes, of about 0.2 mm inner diameter, was used to pick up water and spores. These tubes were placed on microscope slides, usually parallel with the length of the slide. A drop of water and a coverslip were added. Between observations the slides were kept in moist chambers. This method was used with relative ease and good results. The fact that material in the tubes could not be stained was a disadvantage of this method.

F. OBSERVATIONS

1. Distribution of specimens with respect to substrate. The types of wood on which the specimens were collected is given in Table 1.

2. Spore deposits. In the majority of cases the first visible spore deposit was obtained within 3-4 hours after the basidiocarp was placed over agar. However, this period varied from thirty minutes up to thirteen hours.

Basidiospores of D. deliquescens var. deliquescens obtained in these first deposits showed great variation. Freshly collected basidiocarps shed spores either 3-, or 1-septate, or with no septum at all. The proportion of these varied but all three kinds of spores were always found in the first deposits. Dried basidiocarps which had been rewet shed mostly 3-septate basidiospores, although 0-1 septate spores occurred frequently too.

When the basidiocarp of a freshly collected specimen was placed over agar overnight (9-10 hours), the highest proportion of spores was found to be 3-septate.

Successive transfers of a freshly collected basidiocarp were made and a series of spore deposits was obtained. The first visible deposit (I) was obtained in about an hour. Deposit II., with approximately the same macroscopic appearance, was obtained in about half an hour. Deposits III-VIII were obtained by transferring the basidiocarp with twenty minute intervals between transfers. No visible spore deposit resulted during the twenty minutes following deposit VIII. Of the six deposits, each obtained during a twenty minute period, the third (V) and fourth (VI) appeared to contain the maximum number of spores. Deposit VII already showed a definite decrease in number of spores.

Microscopic observation of the spore deposits revealed marked differences. Deposits I and II contained mostly nonseptate or 1-septate spores. There was always a small number of 3-septate mature basidiospores

Host	Dacrymyces deliquescens var.		
	deliquescens	ellisii	minor
Gymnosperms			
Pinus	-	-	2
Thuja	3	-	1
Tsuga	9	-	-
unidentified	29	-	2
Angiosperms			
Alnus	2	5	-
Acer	8	-	1
Betula	-	-	1
Cytisus	-	1	-
Populus	-	1	-
Rubus	6	1	-
Salix	-	1	-
Sambucus	11	1	-
Unidentified			
decaying wood	1	-	1
Gymnosperms	41	-	5
Angiosperms	27	10	2
Unidentified	1	-	1
Total	69	10	8

Table 1. Distribution of the three varieties of D. deliquescens with respect to substrate.

also present. The number of 3-septate spores nearly equalled that of the other kinds of spores in deposit III. In deposit IV the number of 3-septate spores definitely exceeded the others. From deposit V on the 0-1-septate spores occurred only occasionally. Table 2 shows the results in terms of percentages.

Deposit	percentage of	
	0-1-	3-
	septate basidiospres	
I	over 90%	less than 10%
II	over 90%	less than 10%
III	60 - 40%	40 - 60%
IV	40 - 25%	60 - 75%
V	25 - 10%	75 - 90%
VI	less than 10%	over 90%
VII	less than 10%	over 90%
VIII	less than 10%	over 90%

Table 2. The proportions of 0-1-septate basidiospores vs. 3-septate basidiospores in spore deposits, given in percentage ranges.

3. Basidiospores. Mature basidiospores of D. deliquescens (Merat) Duby var. deliquescens Kennedy (Figs. 1-6, 13, 53, 55) are deep orange, or occasionally ochraceous in mass. Most of the mature basidiospores appeared to contain four nuclei (Figs. 1-3, 53, 55). When the number of nuclei was less than four, those cells without a nucleus always seemed to be the ones at the ends of the spore (Fig. 4-6).

4. Single spore cultures. Mature basidiospores of D. deliquescens var. deliquescens were used for spore isolations. The results are shown in Table 3. The inoculated plates were kept either until the agar started

to dry out or contamination occurred. No plate was discarded until two weeks after inoculation.

Of the 1560 spores transferred only three resulted in mycelial growth. Statistically this means 0.19% successful germinations. The three cultures obtained by this method were lost through overgrowth by contaminants.

5. Germination of basidiospores of D. deliquescens var. deliquescens. Viable basidiospores, when in groups and under moist conditions, germinated readily. Three different methods of spore germination on agar were observed. Spores germinated by germ tubes, by globose, or oval conidia, or by germ tubes and conidia simultaneously. These kinds of germination occurred at the same time and in the same spore deposit.

a. Germination of basidiospores by germ tubes. Some of the basidiospores germinated by production of stout germ tubes. (Figs. 14-20, 56-59). There seems to be no specified place where germination starts. It was noted, however, that most spores produced the first germ tube at either end of the spore (Figs. 16-17, 56-59). The number of germ tubes produced by a spore varied from one to eight. When eight germ tubes were produced, two germ tubes always originated from each of the four cells of the basidiospore. It was not unusual to find spores having two germ tubes, both originating from the same cell, while the other three cells of the spore were apparently unchanged.

In cells on which the formation of a germ tube took place, the nucleus remained unchanged or divided to produce two daughter nuclei. The presence of two nuclei in a cell with one germ tube indicated the future formation of a second germ tube. One nucleus passed into each germ

Method	Medium	Number of plates	Number of spores per plate	Number of spores total	Germination
1	PDA	60	1	60	-
	FPDA	100	1	100	2
	MA	120	1	120	1
	MEA	40	1	40	-
	BMA	120	1	120	-
	WA	30	1	30	-
2	MA	40	4	160	-
	FPDA	30	4	120	-
3	MA	40	2	80	-
4	MA	40	8	320	-
	FPDA	40	8	320	-
5	FPDA	20	1	20	-
6	MA	20	1	20	-
7	MA	20	1	20	-
8	MA	20	1	20	-x
	FPDA	10	1	10	-x
Total		750	-	1560	3

x - no further germination after separating and transferring the spores.

Table 3. Isolation of mature basidiospores of D. deliquescens (Merat) Duby var. deliquescens Kennedy.

tube. When the elongating germ tube reached a certain length, which showed a great variation, the nucleus divided. Development of a septum took place either soon after the nucleus divided, or septation was delayed. When spores germinated in water septation occurred soon after nuclear division. On FPDA septation was often delayed. In one case there were four nuclei present in a long germ tube and no septum was observed.

b. Germination of basidiospores by conidia. Some of the basidiospores germinated by the production of conidia (Fig. 12). One, two, three (Fig. 8) or all four (Figs. 7, 9-10) cells of the spore produced conidia simultaneously. These conidia were borne on spicules on the cells. The observation of nuclear conditions was difficult because conidia separated from the spores during the staining process. Nuclei were observed moving into the outgrowths of the cells on which conidia were borne. The conidia were uninucleate in most cases. Some with two nuclei were also observed but these were always oval in shape, and probably in the first stage of germination.

c. Germination of basidiospores by germ tube and conidia. Some of the spores germinated by a combination of the two methods discussed earlier. These produced germ tubes as well as conidia. Spores occurred with germ tubes and conidia in equal number (Fig. 29), others possessed more germ tubes than conidia (Figs. 21-22, 24), still others had more conidia than germ tubes (Fig. 11). Some cells of the spores had one germ tube and one conidium (Fig. 24), some two germ tubes and no conidia (Figs. 21-22), and some just one conidium (Fig. 21).

Germ tubes usually bore conidia. These were on spicules (conidiophores) on the germ tubes (Figs. 22-23, 25-29). Conidia occurred singly

or in clusters of two to six spores. Nuclear conditions were similar to those discussed earlier for germ tubes and for conidia respectively.

6. Multispore cultures were produced by the germination of spore masses. Disks of mycelium from these were transferred to sterile plates. Most of the cultures that developed from these inoculations resulted in circular colonies, as was expected. In some cases, however, irregular growth formations appeared. These were noted in plates which were observed at frequent intervals, and in which condensation had occurred. Small colonies developed along lines, originating from the central colony. When drops of sterile water were placed on circular colonies and made to flow on the agar surface, the development of small colonies occurred along the path of the water drops after 8-12 days. Conidia formed on the mycelium were transferred by the water and produced the small colonies upon germination. These conidia are pale yellow by transmitted light, globose, 1-celled, uninucleate 2.4 x 2.4 μ. Attempts to observe the formation of these conidia failed. As soon as any kind of liquid was added the conidia separated from the mycelium and were carried away. Conidiophores, similar to those mentioned in connection with germ tubes were always present on the hyphae (Figs. 36-37).

7. Germination of conidia of D. deliquescens var. deliquescens. Conidia, whether borne on basidiospores or upon conidiophores on the mycelium, germinated in the same way. They increased in volume and produced one or two germ tubes. Different stages of nuclear division occurred in expanding conidia. The most advanced stage of nuclear division observed was the separation of the two daughter nuclei (late anaphase). In those cases where two nuclei were present in conidia the formation

of germ tubes was already apparent. One nucleus passed into each of the two germ tubes and monokaryotic mycelium developed.

8. Arthrospores (often called oidia) were observed in all D. deliquescens var. deliquescens specimens studied. They frequently occurred in separate sporocarps but were also present, even if only a few in number, in the basidiocarps. They were usually oblong in shape but irregular forms were also frequent. Arthrospores were one or two celled and the cells each contained two nuclei. The nuclei often lay close to one another and at times it was difficult to distinguish both. Arthrospores occurred in chains (Figs. 30-35, 63), in pairs (Figs. 31-34, 64) or singly (Fig. 62), depending on the extent to which the chains had broken.

a. Germination of arthrospores of D. deliquescens var. deliquescens. Arthrospores for the study of germination were taken from conidial sporocarps. These sporocarps were soaked in sterile water until they became very soft. Sporocarps were then treated as the basidiocarps as discussed earlier under "Obtaining basidiospores". The only exception was the addition of a drop of sterile water on the sporocarp before that was placed over the agar. The excess water usually dropped on the agar surface taking a great number of arthrospores with it. This drop was spread on the agar for a better distribution of arthrospores.

Arthrospores germinated readily, and almost exclusively by germ tubes (Figs. 31, 33-34). No more than two germ tubes were produced by any cell. Germination by conidia was observed only on two occasions. One of the conidia was produced similar to those produced on basidiospores. The second one, a globose conidium, appeared on a short (15 μ), slender conidiospore. A dikaryotic mycelium developed upon germination of the arthrospores.

9. Development of basidiocarp on wood-cultures. Twenty-eight inoculations were made on wood; 50% of the inoculations were unsuccessful, 25% were contaminated by Trichoderma sp., and 25% produced mycelium. Of the seven successful inoculations five were made on Tsuga heterophylla (Raf.) Sarg., and two on Acer macrophyllum Pursh. Development started with the formation of a gelatinous area of approximately 2 cm in diameter around the inoculum. Mycelium grew out from the edges of these areas and covered the surface of the substratum in two months. The production of surface mycelium as well as of white aerial mycelium was abundant. The cultures changed color, the originally white mycelium became orange-yellow in four or five days. Apart from this no other changes were observed. Only one developing basidiocarp was noted after nine weeks. The substratum for this was Acer macrophyllum Pursh. The beginning of epibasidial development was the most advanced stage observed (Fig. 44).

10. Development of the basidium. There was only one basidiocarp of D. deliquescens var. deliquescens available from culture, therefore observations on the development of basidia were made on material obtained from basidiocarps collected in their natural habitat.

Young basidia appeared on binucleate hyphae (Fig. 38). They were cylindrical, 25-50 μ long, separated from the hyphae by basal septa, and filled with nearly homogenous, granular cytoplasm which readily took up stains (Figs. 39-41). Two longitudinally arranged nuclei were usually present in the middle of the basidium (Fig. 39). When the top of the basidium became flat it indicated the beginning of epibasidial development (Fig. 42). Two epibasidia developed and the basidium appeared as a bifurcate "Tuning fork" structure (Figs. 42-47).

Nuclear conditions during this period of development varied but the end results were always the same. The two nuclei in the probasidium fused resulting in the fusion nucleus. This divided twice and gave rise to four daughter nuclei, arranged along the longitudinal axis of the hypobasidium (stichobasidial arrangement) (Fig. 46). In some cases the fournucleate stage appeared in the cylindrical probasidium (Figs. 41-43), in others the fusion nucleus was still present in basidia with developing epibasidia (Fig. 45). With more advanced stages of epibasidial development the cytoplasm appeared to become thinner, and it did not take up stains as readily as before.

One nucleus passed into each epibasidium while the two others remained in the hypobasidium, and usually moved up close to the top of the hypobasidium (Figs. 47-49). The nucleus migrated toward the top of the epibasidium. By the time the nucleus reached the top of the epibasidium a short sterigma was always present there (Fig. 61). The development of the two epibasidia was not always simultaneous, but the fully developed epibasidia of the same basidium always appeared nearly equal in length.

11. Development of basidiospores. While the nucleus was moving toward the tip of the epibasidium small vesicles appeared on the tip of the sterigma (Figs. 48-49, 61). These vesicles increased rapidly in size. They were filled with cytoplasm. A correlation was noted between the size of the developing basidiospores and the ability of the basidium to take up stains. The larger the basidiospores were the fainter the basidium appeared after staining.

The nucleus passed from the epibasidium through the sterigma into the spore (Fig. 51). The great majority of the basidiospores were aseptate at the time of spore discharge. In some cases the basidiospores became 1-septate while still attached to the sterigmata. These spores contained two nuclei. In all these cases, however, two nuclei were present in the hypobasidium.

12. Deterioration of the basidium. After the spores were discharged from the basidium, it appeared to contain two nuclei and only threads of cytoplasm or none remained. The two nuclei as a rule were found at the top of the hypobasidium. In a very few cases, however, one of the nuclei was observed near the centre of the hypobasidium (Fig. 50). The basidium became hyaline, appeared to be completely empty, and collapsed (Fig. 52). The actual deterioration of the nuclei was not observed.

13. Cultural characteristics of the three varieties of Dacrymyces deliquescens. A series of studies (Magasi, 1962) made with D. deliquescens var. deliquescens revealed that the color of cultures of this variety varies under different conditions. A definite correlation was found to exist between the sugar (sucrose) concentration of the medium and the intensity of the orange color of the cultures. With increase in the sugar content of the medium an increasingly darker orange color was obtained in cultures. D. deliquescens var deliquescens was found to be deficient for thiamine. By changing the thiamine concentration of the medium color of developing cultures varied. Varying light intensity and the time of exposure also effected the color of cultures. Under the same nutritional and environmental conditions, however, the color of different D. deliquescens var. deliquescens cultures did not show such variation.

Multispore cultures of the three varieties were made on the same medium, at the same time, and were kept under the same conditions. Cultures of the three varieties when grown under identical conditions always showed consistent differences in coloration. Cultures of D. deliquescens var. deliquescens were the darkest orange-yellow of the three varieties, D. deliquescens var. ellisii was definitely paler orange-yellow or yellow, and D. deliquescens var. minor was yellow with a typical olivaceous tinge on MA.

The exact cardinal temperatures have not been determined but the ranges obtained for the three varieties were the same as follows:

Minimum	between	5° C and 10° C
Optimum	between	10° C and 20° C, most likely very close to 15° C
Maximum	between	25° C and 30° C

There were no significant differences in the rate of growth of the three varieties.

Microscopic study of cultures revealed some differences among the three varieties of the species. Table 4 shows the kinds of spores that were observed during the examination of cultures.

Spore forms	Dacrymyces deliquescens var.		
	deliquescens	ellisii	minor
germination of basidiospores	germ tube and conidia conidia germ tube	germ tube and conidia	not observed
conidia on germination	globose (oval very rare)	elliptical	not observed
conidia on hyphae	globose	elliptical	oval
arthrospores	present in older dikaryotic cultures	none	none
chlamydospores	present in liquid cultures	present	none
basidiospores in cultures	none	none	none

Table 4. Spore forms of the three varieties of Dacrymyces deliquescens sensu "Kennedy".

G. DISCUSSION

1. Distribution of specimens with respect to substrate. Kennedy (1958b) gave the habitat for D. deliquescens var. deliquescens as "gymnosperm or less frequently angiosperm wood". Of the sixty-nine specimens studied forty-one have been collected on gymnosperm wood, twenty-seven

on angiosperm wood, and one on unidentified decaying wood, agreeing with Kennedy's statement. According to Kennedy D. deliquescens var. ellisii occurs on "angiosperm and very rarely on gymnosperm wood", and the statement is well supported by the ten collections on angiosperm wood while none has been collected on gymnosperm wood. Five collections of D. deliquescens var. minor have been collected on gymnosperm wood, two on angiosperm wood, and one on unidentified wood. This distribution neither proves nor disproves Kennedy's statement for this variety, "angiosperm or rarely gymnosperm wood", because of the small number of collections studied.

2. Spore deposits. Upon drying the basidiocarp the development of reproductive structures is interrupted for a length of time. This inactive period is avoided in those cases where freshly collected material is used at once in order to obtain spores. In this way the normal life functions of the fungus are not disturbed to any greater extent.

The basidiospores of D. deliquescens are usually one-celled at the time of spore discharge. In a few instances spores become one-septate while still on the basidium. Soon after discharge the one-celled spores become one-septate, four-celled mature basidiospores are formed. These observations agree with those of Bessey (1950), Gaumann (1928) and of Yen (1947). Based on this information high percentages of one-and two-celled spores were expected to be present in spore deposits. This assumption, however, proved to be right only in the first few deposits (Table 2).

Moisture conditions of the basidiocarp seem to influence the release of spores from the basidiocarp. When the moisture content of the basidiocarp is high the spores are released from the basidiocarp as

soon as they are discharged from the basidium. Decrease in moisture content has a retarding effect on the speed of spore release. Since the spores become 3-septate soon after discharge this can happen while they are still in the basidiocarp, and they are already 3-septate by the time of their release. This explains the increasing number of 3-septate spores in later spore deposits when the decrease of water content of the basidiocarp decreases the speed of spore release.

The presence of mostly 3-septate spores from air dried and rewet basidiocarps seems to justify this explanation. Spores are still being produced and discharged by the basidia at the beginning of the drying process but they are not released partly because of the unnatural position of the basidiocarps on drying and partly because of the decreased moisture content of the basidiocarps. The spores mature on the basidiocarp and remain there until the basidiocarp becomes wet again. Then these are the first ones to be released.

Buller (1922) reported that the necessary time for the full development of basidiospores of D. deliquescens is twenty-three minutes and an additional twenty-seven minute period is required before the spores are discharged. It is obvious that a period of time is necessary for the fungus to reach the normal functioning condition after the basidiocarp has been rewetted. A further period is necessary for basidial development. Fifty minutes (Buller, 1922) is required between the completion of basidial development and the discharge of the first spores produced by these basidia. By the time the mass production of fresh spores takes place the slow drying out of the basidiocarp is already in progress, due to the artificial conditions under which the spore deposits are obtained, and the retarding

effect on spore release creates the situation under which the spores obtained in the deposits are mostly 3-septate.

3. Basidiospores. Basidiospores are considered to be mature when they become 3-septate, not when they are discharged from the basidium as one-celled spores. A mature basidiospore contains four nuclei, one in each of its cells.

Basidiospores obtain their original single nucleus from the basidium. This was observed and reported previously by Dangeard (1895), Istvanffi (1895), Juel (1898), Maire (1902), and Gilbert (1921), for Dacrymyces, and also by Bodman (1938) for another genus of the family. This seems to be proved by the count of five nuclei whenever binucleate undischarged spores were observed. Of the five nuclei two were seen in the hypobasidium, one in the epibasidium or already in the spore, and two in the second spore. The meiotic division of the fusion nucleus resulted in the production of four daughter nuclei. It seems to be justified to suggest that the fifth nucleus is the result of a mitotic division of a daughter nucleus already in the spore. Wager (1914) was the only one who suggested the possibility of two nuclei passing into each spore. However, he did not actually observe this.

The discharged, mostly unicellular, uninucleate spores undergo a series of changes already discussed above. In those cases where the number of nuclei was found to be less than four it seems to be probable that the unseen nuclei were present but did not stain satisfactorily. The fact that cells without nuclei were always the ones at the ends of the spore, suggested that the formation of septa may have taken place without completed normal nuclear division. This suggestion, however, has

not been supported by actual observation. Another explanation is that the cell of the spore from which the nucleus is missing has been damaged to some extent and the nucleus already disappeared but other contents of the cell are still present.

4. Single spore cultures. One of the main objectives of the present study was to get information about the relationship among the three varieties of D. deliquescens ("Kennedy"). Mating tests were hoped to provide much of the necessary information. For mating tests single spore cultures were required. There is only one study reported in the literature in which single spore cultures of D. deliquescens (Merat) Duby are mentioned. Yen (1949) mentioned the extreme difficulties he encountered in obtaining ten single spore cultures. Unfortunately he made no mention of the method he used in obtaining them.

Spores of D. deliquescens germinate readily on almost any kind of culture medium, and even in distilled water but only when they are in masses. Six different media and eight different methods were tried without success for obtaining single spore cultures.

The first method was the one most frequently used in studies of this kind. Spores were isolated and placed individually in Petri plates. Of the 470 spores isolated on six different media (Table 3), there were only three cultures obtained, two on FPDA and one on MA. All three cultures were lost through contamination.

The fact that all three cultures in which germination occurred were contaminated raised the question whether the contaminant (Trichoderma sp.) could produce some kind of substance which induces germination of Dacrymyces spores. The fact that there were other cultures also contamin-

ated by Trichoderma sp. without any visible effect on the germination of single spores made this possibility doubtful. However, a series of double inoculations were made to determine this. It does not seem likely that in those three cultures Trichoderma had any effect on the germination. Rather there is the possibility of erroneous isolation and the presence of a number of spores in these cultures.

The ready germination of spores in masses, and the failure of germination of single spores suggested that the substance produced by the spores, and necessary for germination could be produced in small quantities by individual spores. This quantity could not be enough, at least not under cultural conditions, to induce germination in the spore which produced it. This suggestion is also supported by the fact that spores, which are already germinating, when removed from the spore mass and transferred to a new plate ceased growing. In this separation there is a possibility that some of the tender germ tubes were damaged during the transfer.

When four spores were transferred one to each quarter of a plate the distance between the spores was too great for any produced substance to diffuse from one spore to another. Therefore this isolating method might also be considered a true isolation.

Yen (1947) studied Calocera cornea Batsch. in culture and reported it to be a heterothallic tetrapolarous species. He suggested that the same might be true for Dacrymyces deliquescens. Considering this suggestion and assuming that the amount of substance which induces germination is small, other methods of isolation were tried. Two spores, both from the same basidiocarp, or from different basidiocarps, were placed close to one another. No germination occurred.

Germinating basidiospore masses may very likely produce the inducing substance in excess and this could penetrate the culture medium around the spore mass. Small blocks of agar were cut from the immediate vicinity of germinating spore colonies, transferred on fresh medium and inoculated. The failure of germination in these cases could be explained by assuming that substance could diffuse from the spore mass to the agar before it was cut out, then the substance might have diffused from the agar block into the agar below it, reducing the concentration below the effective level.

Therefore, in the next step the spore mass was removed and the intact agar, supposedly containing the necessary substance for germination, was inoculated. The negative result can be explained in but one way, i.e. the substance must be volatile and so disappears rapidly.

There are many species of fungi the spores of which germinate only after having been exposed to a cold treatment. Denyer (1961) and Kneebone (1951) reported basidiospore germination only after the spores were kept in cold storage, at -7° C, for up to twelve months, while without this treatment they failed to germinate. However, in those cases the treatment was necessary for germination of spore masses as well as of single spores. Dacrymyces spores in masses do not need any kind of treatment for germination; all that is necessary for them is moisture.

It would seem that a certain substance is produced by the basidiospores of D. deliquescens that induces germination. This substance is produced in small quantities by individual spores, and does not induce germination in the spore that produced it. Furthermore this substance is suspected to be volatile. The failure of inducing germination in

single spores is probably due to the small quantity produced or possibly to the quality of the substance. Spore masses produce sufficient quantity of the substance to support germination.

After the failure of obtaining single spore cultures of D. deliquescens for mating tests this part of the study had to be dropped.

Similar results were also obtained with the isolation of single spores of D. ellisii and of D. minor.

5. Germination of basidiospores of D. deliquescens var. deliquescens in mass. Three different kinds of basidiospore germination were observed and there seems to be no rule as to which one of the three may be expected to take place.

Yen (1949) reported differences on germination dependent on the kind of substratum on which the collection was made. He observed germination exclusively by germ tubes in collections from gymnosperm wood. Collections from angiosperm wood germinated either by the formation of conidia and a very few germ tubes or by the formation of germ tubes and conidia.

In the present observations D. deliquescens var. deliquescens spores germinated by any of the three methods regardless of the original substratum, of the kind of medium on which germination was observed, and of the conditions under which germination took place. Furthermore, the three methods of germination often occurred intermixed in the same spore deposit.

D. deliquescens var. ellisii spores were never observed to germinate exclusively by germ tubes in this study. There were always at least some elliptical conidia also present.

Based on the present observations, on the distribution of the three varieties given by Kennedy (1958b) it is suggested that in Yen's study there could have been more than one variety of D. deliquescens involved. This suggestion is also supported by Yen who raised the question that there might have been more than one species studied by him under the same name, the collections from different substrata belonging to different species.

It is suggested that Yen's collections from gymnosperm wood could be D. deliquescens var. deliquescens while those from angiosperm wood could be either the same or D. deliquescens var. ellisii.

a. Germination of basidiospores by germ tubes. The four cells of basidiospores acted independently from one another on germination. As has been mentioned in the observations, there seems to be no rule determining where germination begins or how it will take place. All four cells of a spore may produce one or two germ tubes, or any of the cells may produce germ tubes less or more in number than others, or may be unchanged while others germinate.

Nuclear conditions also showed variation during germination. In those cases where the nucleus remained unchanged and passed into the germ tube the possibility of the formation of a second germ tube on the same cell was eliminated. In those cases where a nuclear division, definitely a mitotic one, took place there was the possibility for the formation of a second germ tube, or in the case of mixed germination that of a conidium.

The nucleus of the elongating germ tube divided producing two daughter nuclei. Usually a septum was formed right after the nuclear division. The length of the cells of the germ tube showed a great variation, but remained between the limits of 7-20 μ. These figures agree with those of Yen (1949).

It was noted that on FPDA, the richest medium used in the study, septation was often delayed, and two or even four nuclei were observed in one elongated cell. Bessey (1950) suggested that on media supporting vegetative growth of fungi well, elongation is often very rapid and septation cannot keep up with that rate of growth. The missing septa are formed later either in succession or simultaneously.

b. Germination of basidiospores by conidia. Unlike the germination by germ tubes, when germination took place by conidia only, the number of conidia produced directly on the short spicules of the spore was always one per cell. In this case no nuclear division was observed. The nucleus passed from the cell into the conidium hence eliminating the possibility of the formation of another conidium on the same cell.

Another kind of conidia-producing spore germination was also observed, and this is the type previously reported by Brefeld (1888) and Yen (1949). A short conidiophore is produced by the cell and conidia are formed on it. Conidia are borne singly or in groups. The number of conidia in a cluster varies from two to six. Brefeld gave the size of these conidia as 3-7 x 2-5 μ. This disagreed with Yen's measurements of 2-3.5 μ in diameter. The present measurements agree with those of Yen. Brefeld is known to have given inaccurate measurements in all of his work.

Difficulties in observations of nuclear conditions have been mentioned above, and were also reported by Yen (1949). However, it was obvious from observations that conidia, produced singly or in clusters, were uninucleate.

c. Germination of basidiospores by germ tubes and conidia. The combination of germination by germ tubes and by conidia was the most frequent means of basidiospore germination.

As was stated earlier, the cells of basidiospores act independently from one another. Germ tubes and conidia might be produced on the same spore. Furthermore, they can be produced on the same cell. Here, however, the sequence of germination creates some limitations.

When a germ tube is the first product of germination, and when there is a nuclear division before a nucleus passes into the germ tube, there are two possible further sequences. There may be either a second germ tube produced or a conidium.

When a conidium is the first product of germination this is in no case followed by the production of either an additional conidium or a germ tube. The same appears to be true for those cases where conidiophore and a cluster of conidia was formed. No explanation is offered as to why the formation of conidia terminates the sequence of germination while the formation of either conidia or germ tubes is possible after the formation of the first germ tube.

This combination of the different methods of germination also seems to support Yen's (1949) statement concerning the independent behavior of the cells of basidiospores.

In certain, and not unusual, cases all four cells of a given basidiospore may exhibit a different kind of germination, and even then there is another possibility.

Cells of basidiospores of D. deliquescens var. deliquescens may germinate in any of the following five ways:

1. One germ tube is produced without nuclear division
2. Two germ tubes are produced after one nuclear division
3. One germ tube is produced directly after nuclear division, and this is followed by the production of a conidium
4. One conidium is produced without nuclear division
5. A cluster of conidia is produced on a conidiophore, under unobserved nuclear conditions.
6. Multispore cultures. Conidia formed on the mycelium have been reported for many basidiomycetous fungi. Nobles (1948) included the presence or absence of conidia as a permanent cultural characteristic for identification of some fungi. Fomes annosus (Fr.) Cke., F. officinalis (Vill. ex Fr.) Faull, Polyporus berkeleyi Fr., P. guttalatus Peck, P. sulphureus Bull. ex Fr., Poria carbonica Overh. of the Polyporaceae, and Pholiota adiposa Fr. of the Agaricaceae possess mycelial conidia of the species she studied. Barnett (1937) reported mycelial conidia on hetero-basidiamycetous fungi (Auricularia, Exidia).

For D. deliquescens Yen (1949) was the first, and only one, to report the presence of mycelial conidia. The results of the present observations revealed some differences from those of Yen. He reported that conidia formed only in cultures from angiosperm wood. In the present case conidia were observed in cultures from gymnosperm as well as from angiosperm wood. Yen reported globose and elliptical conidia in cultures of D. deliquescens (Merat) Duby. In the present study no elliptical conidia were noted in D. deliquescens var. deliquescens cultures still on conidiophores. As was mentioned before, some elongated conidia were seen but those were free, contained two nuclei and were presumed to be

germinating. D. deliquescens var. ellisii cultures, however, produced elliptical conidia on conidiophores which developed on the mycelium. These were uninucleate and measured 4-5.5 x 2-3 u. These figures are very close to Yen's measurements for elliptical conidia of D. deliquescens.

The suggestion that Yen's collections from angiosperm wood might have been different varieties of D. deliquescens is further supported here. It seems possible that all collections from gymnosperm wood, and collections from angiosperm wood with globose conidia belong to D. deliquescens var. deliquescens, and those from angiosperm wood with elliptical conidia to D. deliquescens var. ellisii.

7. Germination of conidia of D. deliquescens var. deliquescens. The fact that there was only one kind of conidial germination observed, and that was by germ tubes, does not prove Yen's (1949) observations to be erroneous. He reported that conidia sometimes give rise to other conidia instead of producing germ tubes on germination. Our observations in connection with conidial germination are similar in every other respect.

8. Arthrospores are formed on dikaryotic hyphae and so the arthrospores are also dikaryotic. Since they are formed by the breaking up of hyphae arthrospores exhibit a great variation in shape, depending on the part of hyphae from which they originate. Most of them are oblong, originating from straight simple hyphae but those from around the branching of hyphae are very irregular in shape.

Arthrospores are almost always present in basidiocarps of D. deliquescens var. deliquescens. The presence of arthrospores is an exclusive feature of this variety and readily distinguishes it from the other two varieties.

Some arthrospore formation was observed in old multispore cultures on agar. Yen (1949) found these only in cultures from gymnosperm wood. The absence of arthrospores in cultures from angiosperm wood alone does not prove that at least some of these collections belong to the variety ellisii. However, along with other phenomena already discussed strengthens the suspicion that it could be so.

a. Germination of arthrospores of D. deliquescens var. deliquescens takes place shortly after they are under favorable conditions. Arthrospores appeared to germinate almost exclusively by binucleate germ tubes which gave rise to dikaryotic mycelium. This observation is in agreement with that of Yen (1949). It is not possible to agree with the following statement of Alexopoulos (1952): "Before germination each oidium (arthrospore) splits into two uninucleate cells. Each of these then germinates producing primary mycelium". It is true that the two nuclei lie very close to one another at times in the arthrospore and therefore it is difficult to see them both. The germ tubes and developing mycelium, however, are readily distinguishable as dikaryotic structures.

Similar to the germination of basidiospores, the maximum number of germ tubes produced by any one cell of arthrospores was two. The two occasions on which germination by conidia was observed do not permit the stipulation that arthrospores germinate exclusively by germ tubes.

9. Development of basidiocarp on wood-cultures. Using different media and incubating the cultures under different conditions effected the development of cultures. No basidiocarp was obtained in agar cultures, however, during the twenty-seven months (June 1960 - Aug. 1962) of culture work with D. deliquescens. There was one occasion when a yellowish

structure macroscopically resembling a basidiocarp and two basidiospore-like spores were found on 1.5% water agar after nine weeks of inoculation. The yellowish structure was found to be a mycelial accumulation. No basidium in any stage of development was observed in the structure. The spores were arthrospores resembling basidiospores. Yen (1949) worked with D. deliquescens in cultures for two and a half years, but was unable to obtain basidiocarps.

Basidiocarps of higher basidiomycetous fungi (Polyporales, Agaricales) are obtained with relative ease in cultures (Badcock 1941, 1943; Cartwright and Findlay 1958; Etter 1928; Papazian 1950; etc.). Barnett (1937) obtained basidiocarps of Auricularia in culture but these were not typically formed basidiocarps.

It was hoped that wood on which the fungus develops under natural conditions would be a more suitable substratum than agar to obtain basidiocarps on, even after autoclaving and under laboratory conditions. The gelatinous slimy substance which developed around the inoculum and contained great numbers of conidia appeared to be similar to that very often found around basidiocarps in nature. Mycelium developed by the germination of conidia.

The only basidiocarp that was obtained from twenty-eight wood cultures was pustulate, bright orange-yellow and contained arthrospores as well as basidia in the early stages of development. This basidiocarp was compared to young basidiocarps of the collection from which the culture was made and appeared to be identical. Even though the basidiocarp was harvested before its full development it seems to be safe to state that with the modification of this method basidiocarps of D. deliquescens can

be cultivated under laboratory conditions.

10. Development of the basidium. The work of students of this group (Dangeard 1895; Istvanffi 1895; Juel 1898; Maire 1902; Wager 1914; Gilbert 1921; Bodman 1938) proved that there are two primary nuclei present in the young dacrymycetaceous basidium. This is general among Basidiomycetes and D. deliquescens is no exception. The binucleate probasidium is separated from the hyphae by a basal septum, and is filled with dense homogeneous, finely granular cytoplasm. The fusion of the primary nuclei produces the fusion nucleus which is always considerably larger than haploid nuclei. The fusion nucleus is the only stage in the life-cycle of the fungus which is diploid in the true sense of the word. Although the dikaryotic phase may take up a very large portion of the life-cycle, two haploid nuclei giving the cells a double chromosome content, the only true 2N condition is satisfied only between the time of the fusion of the primary nuclei and the meiotic division of the fusion nucleus. The time of nuclear fusion cannot be defined in terms of morphological development of the basidium. Fusion nuclei can still be present in basidia on which epibasidia have already appeared while, usually, the meiotic division occurs in the cylindrical probasidium. Bodman (1938) thought it possible "that fusion initiates in the basidium the series of morphological changes that are attendant upon the maturation of the nuclei, and that the two proceed simultaneously; meiosis at some times running slightly ahead, and at others the morphological development gaining the ascendency".

Juel (1898) described the details of the meiotic process. The stages of this process were not observed since a complete cytological observation is beyond the scope of this study. It was noted, however,

that the fusion nucleus divided into two daughter nuclei and the strands of the spindle appeared nearly parallel with the longitudinal axis of the basidium. The two nuclei were usually found in the upper one third of the basidium. This might be due to cytoplasmic flow in the enlarging basidium. The second, meiotic, division follows and the four daughter nuclei are lined up in the basidium in stichobasidial arrangement.

There has been more disagreement concerning the fate of the four daughter nuclei than in any other part of the life-cycle. Different authors worked with different species of the group but even then there should not be so much disagreement unless the different theories have been based on inadequate observations. Dangeard (1895) was the only one who observed only a single division of the fusion nucleus and stated that one of the daughter nuclei passed into each of the epibasidia. His observation concerning nuclear division can be considered inadequate. All other authors of the group are in agreement in the production of four daughter nuclei, through meiosis. Istvanffi (1895), Maire (1902), Wager (1914) and Bodman (1938) stated that two nuclei pass into each epibasidium. Istvanffi and Maire postulated that one nucleus passes into each basidiospore and that there are two crops of spores from the same basidium. Wager suggested that two nuclei pass into each basidiospore. Bodman dismissed all these possibilities and stated that one nucleus is received by each spore and the remaining two nuclei deteriorate in the epibasidium.

Gilbert (1921) was the only one who stated that one nucleus passes into each epibasidium, consequently into each spore. Two nuclei remain in the hypobasidium and later degenerate. Gilbert and Bodman

had the same opinion about the fate of the nuclei remaining in the basidium. The contradiction concerning the place of degeneration might be justified since they worked with two different genera of the family.

With the development of basidiospores the cytoplasm continues to flow out of the basidium, into the spores. By the time the spores reach mature size, cytoplasm is almost absent in the basidium. Occasional cytoplasmic threads are present in the basidium. The amount of this available cytoplasm and the two nuclei in the hypobasidium (in Dacrymyces) or in the epibasidia (in Guepinia) makes a second crop of basidiospores unlikely. Observations made during the present study seem to agree with those of Gilbert.

11. Development of basidiospores. Two sterigmata develop on the top of epibasidia after they pierce the gelatinous layer of the basidiocarp. Basidiospores first appear as small swellings on the sterigmata. They quickly enlarge, and are filled with cytoplasm from the basidium. The cytoplasmic flow carries the nucleus from the epibasidium into the spore. Buller (1922) reported that it takes only twenty-three minutes from the time the basidiospores first appear on the sterigmata until they reach their mature size.

Basidiospores are usually discharged as unicellular structures. This statement is in agreement with those of Bessey (1950), Buller (1922), Gauman (1928) and Yen (1949). In some cases, however, the nucleus of the spore divides and a septum is formed before the spore is discharged. This was also reported by Bessey, Gauman and Yen.

12. Deterioration of the basidium. After spore discharge the basidium contains two nuclei and very little cytoplasm. Basidia in this stage are hyaline and soon collapse. Actual deterioration of the basidium was not observed in the study but Gilbert (1921) noted that this is the fate of the empty basidium which, in this stage is hardly more than a cell wall.

13. Cultural characteristics of the three varieties of D. deliquescens. The color of different cultures from the same mycelium is an extremely variable feature depending upon environmental and nutritional conditions. For species of Dacrymyces this has been proved by Bulat (1954) and the present results supported this. However, under identical conditions even different cultures of the same species (or variety) appeared to have the same coloration. This has been reported previously by Yen (1949). Different varieties of D. deliquescens under the same conditions always exhibited differences in coloration. Yen (1949) noted that cultures originating from gymnosperm wood and some from angiosperm wood were orange-yellow in color while others from angiosperm wood had light orange-yellow color. Considering the reasons discussed above, and on the basis of observations made during this study it seems probable that the deeper orange-yellow cultures were those of D. deliquescens while the light orange-yellow were those of D. ellisii. Color differences observed among varieties of D. deliquescens were persistent and could, in some cases, be used for the separation of the varieties in culture work. Color, however, is thought to be an unreliable feature of fungi; therefore other characteristics of cultures should be taken into consideration as well.

Far more significant and more dependable differences are revealed by the microscopic examination of cultures of the three varieties. The shape of mycelial conidia is different in all three varieties. D. deliquescens var. deliquescens can be readily distinguished by its globose conidia. At times it may be difficult to separate the elliptical conidia of D. deliquescens var. ellisii from the oval conidia of D. deliquescens var. minor.

Arthrospore formation is the exclusive feature of D. deliquescens var. deliquescens. Whenever arthrospores are present in a D. deliquescens culture it can be said with certainty that the culture is an older dikaryotic culture of the variety deliquescens.

Bulat (1953) reported the formation of thick-walled chlamydospores for D. ellisii which are "spherical to ovoid, 6-12 x 5-8 μ, which may be intercalary or terminal". These were found only in cultures of D. deliquescens var. ellisii on solid media during the present study. Chlamydospore formation was also observed in some old liquid cultures of D. deliquescens var. deliquescens, but not on solid medium in cultures of either this variety or of var. minor.

Bulat (1953) reported three kinds of colonies for D. ellisii. His "small colonies" were observed in all three varieties of D. deliquescens during the present study. The origin of these colonies has been explained earlier and it was suggested that water carries the conidia from the "giant colony" to the other parts of agar surface. Bulat claims that conidia are projected in some way from the culture. However, he found no mechanism for this method. On the basis of his theory there seems to be no explanation why the "giant colonies" do not eject conidia,

why certain spots of the agar surface are completely free from the "small colonies", why there is always at least one line of "small colonies" originating from the "giant colony" (this is apparent in the photographs he presented), and why there is an aggregation of "small colonies" around the edge of the Petri plate while with the exception of the one line originating from the "giant colony" the agar surface is free from "small colonies". If it is true that water carries the conidia from the giant colony, as suggested here, all these questions can be readily answered. Bulat's method of changing covers of Petri plates from over cultures to over sterile agar was tried. No colony formation was observed.

Gelatinous pustules, similar to those reported by Bulat, were occasionally noted during the course of the present study. These were found to be bacterial contaminants.

The size of conidia for D. ellisii was given by Bulat as 4-5 x 1.8-2.5 μ the present measurements for var. ellisii were 4-5.5 x 2-3 μ. Yen (1949) reported the ellipsoid conidia in cultures from angiosperm wood as 4-4.5 x 2-2.5 μ. His measurements compared to the other two suggest D. ellisii, especially when other characteristics are also considered.

Bulat reported the presence of elongate ballistospores on four occasions in eight months of culture work with D. ellisii. On the basis of the presence of ballistospores, which germinated by budding or repetition he suggested the possible relationship between Tremellales and Sporobolomycetaceae. Since no structures remotely resembling ballistospores were found during the course of the present study it is not

possible to question the validity of this suggestion at the present time.

14. Conclusions of the study. Due to the complete failure of single spore germination one of the questions, to determine whether the three varieties of D. deliquescens, as treated by Kennedy, represent a single species or are three distinct species, remains unanswered. Differences, like those in distribution concerning substratum, color differences in cultures, or the different shape of conidia produced, indicate a definite degree of separation. On the other hand, intermediate forms (Kennedy, 1958b, Olive, 1947) suggest that the three-deliquescens, ellisii, and minor - are only varieties of the same species. Whether the differences separate species or only varieties, or the intermediate forms indicate varieties or closely related species cannot be determined without mating tests. To answer this question, further studies are needed, first to find a method of obtaining single spore cultures, then to make mating tests.

The life cycle of D. deliquescens var. deliquescens can be summarized in the following points.

1. Basidiospores are usually uninucleate at the time of discharge but the nucleus soon divides and after two divisions the spore is 3-septate, four celled.

2. It is suggested that germination is induced by a volatile substance which is produced in small quantities by the spores. The amount of this substance is insufficient to induce germination in the spore that produced it.

3. Cells of basidiospores act independently of one another upon germination.

4. Germination takes place by globose conidia, by germ tubes or by the combination of conidia and germ tubes.

5. Production of conidia terminates the germination of individual cells. The maximum number of germ tubes produced by any single cell is two.

6. Mycelium produced on germination of basidiospores or of conidia is monokaryotic.

7. Dikaryotization of mycelium takes place but the method is unknown.

8. Arthrospores may be produced by dikaryotic mycelium. Germination of arthrospores produces dikaryotic mycelium.

9. Basidia are formed on dikaryotic mycelium. The young basidium is cylindric, binucleate, and separated by a basal septum from the hyphae.

10. A fusion nucleus is produced. Two divisions of the fusion nucleus give four stichobasidially arranged daughter nuclei.

11. One nucleus passes into each epibasidium and this later becomes the original nucleus of the spore. Two nuclei remain in the hypobasidium and degenerate there.

All three varieties produce conidia on the mycelium. These are globose, elliptical or oval as produced by D. deliquescens var. deliquescens, var. ellisii, and var. minor respectively. It is suggested that mycelial conidia are carried away by water rather than ejected by some mechanism as proposed by Bulat (1954).

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PLATE I

Dacrymyces deliquescens var. *deliquescens*

Figs. 1-3 Mature basidiospores with four nuclei

Figs. 4-5 Mature basidiospores with three nuclei

Fig. 6 Mature basidiospore with two nuclei

Fig. 7 Germination of basidiospore by conidia - one conidium produced by each of the four cells of the spore.

Fig. 8 Germination of basidiospore by conidia - one conidium produced by three of the cells of the spore.

Figs. 9-10 Germination of basidiospores by conidia - one conidium produced by each of the four cells of the spores.

Fig. 11 Germination of basidiospore by both conidia and germ tubes - more conidia produced than germ tubes; one conidium and one germ tube produced by one of the cells of the spore.

Fig. 12 Globose conidia produced by germinating basidiospores.

Note: Scale is approximate.

PLATE I

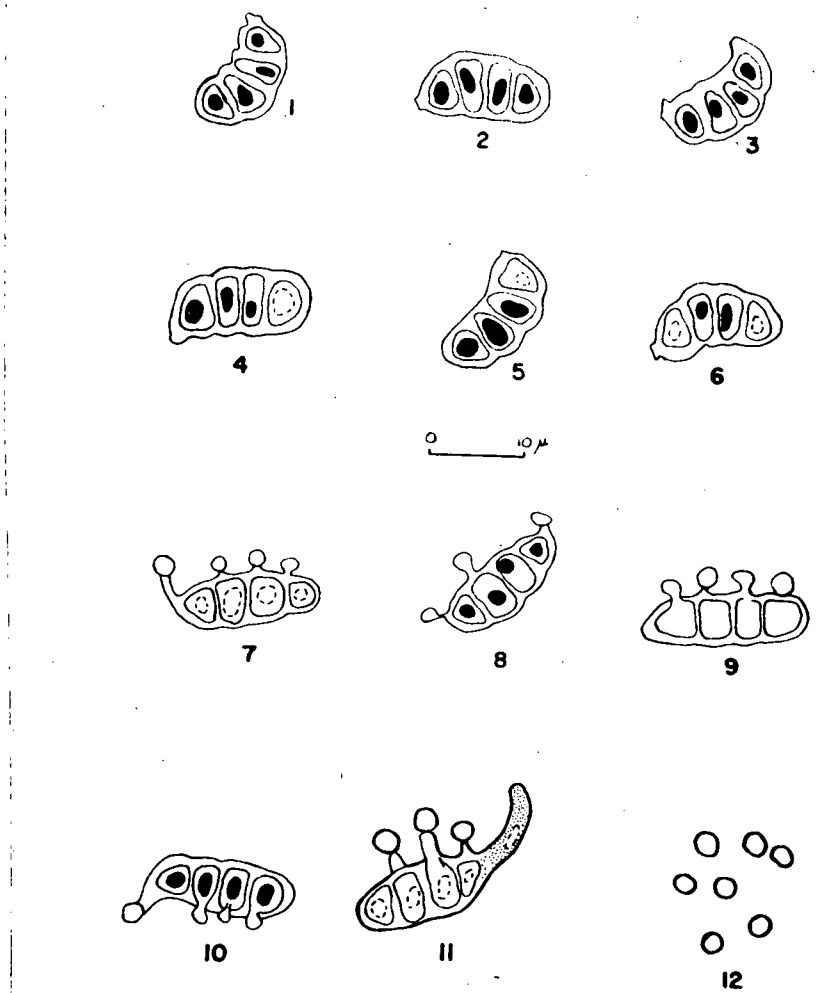

PLATE II

Dacrymyces deliquescens var. *deliquescens*

Fig. 13 Basidiospore in dormant condition with four nuclei

Fig. 14 Beginning of spore germination by germ tubes

Fig. 15 Basidiospore with two germ tubes

Figs. 16-17 Basidiospores producing the first germ tube at the end of the spores on germination

Fig. 18 Basidiospore with three germ tubes, two originating from the same cell

Fig. 19 Basidiospore with three germ tubes, all originating from different cells

Fig. 20 Basidiospore with four germ tubes, two originating from the same cell, two from separate cells

Fig. 21 Basidiospore with both germ tubes and conidium - more germ tubes than conidia; two germ tubes on one cell, and one conidium and no germ tube on one cell.

Note: Scale is approximate.

PLATE II

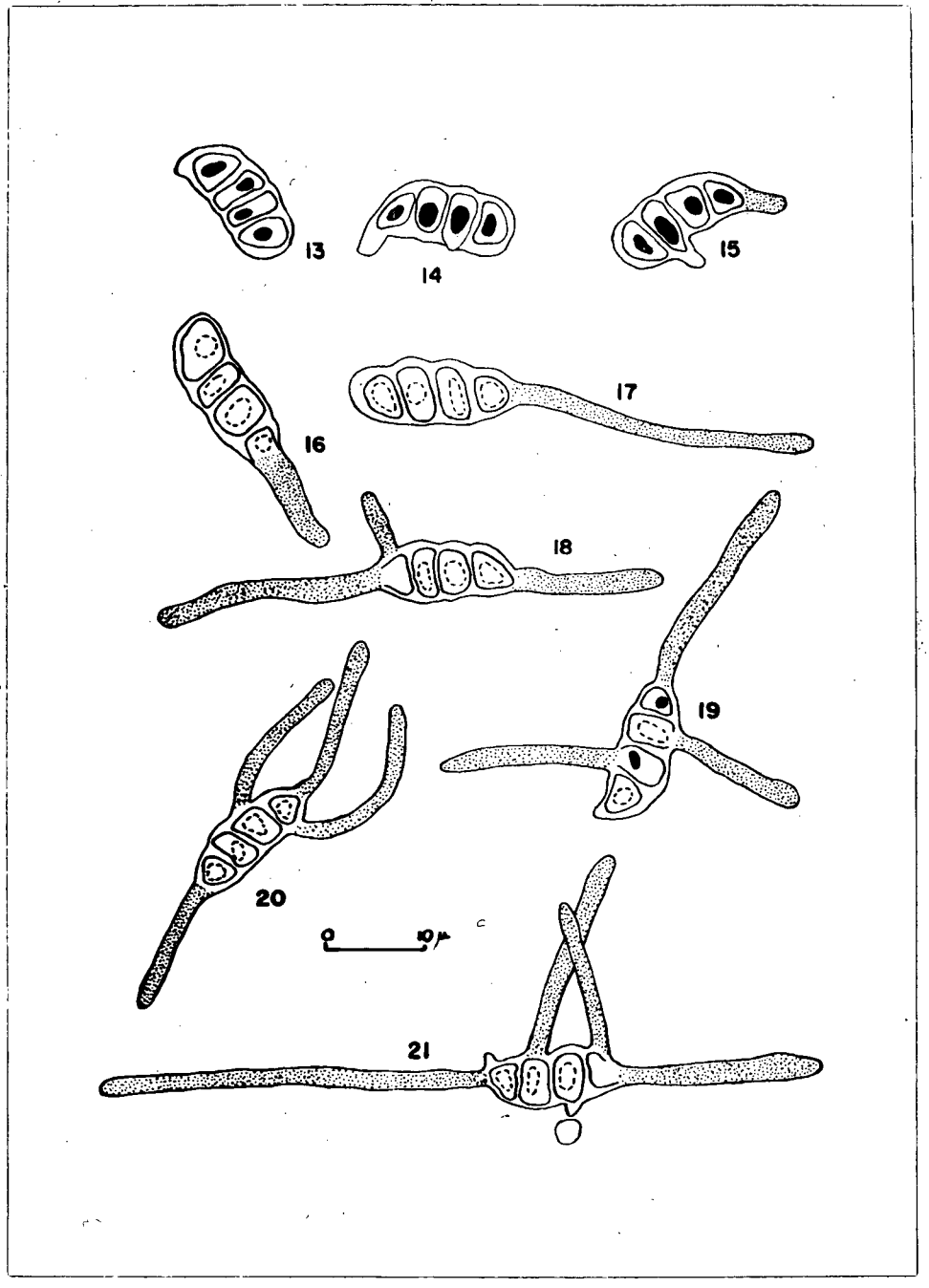

PLATE III

Dacrymyces deliquescens var. *deliquescens*

Fig. 22 Basidiospore with both germ tubes and conidia - one cell with two germ tubes, one cell with one conidium; conidium on germ tube

Fig. 23 Basidiospore with germ tubes which bear conidia

Fig. 24 Basidiospore with germ tubes and conidia - some of the conidia were removed

Figs. 25-27 Basidiospore with germ tubes and conidia - conidia also on germ tubes

Fig. 28 Basidiospore with conidia-bearing germ tubes

Fig. 29 Basidiospore with germ tubes and conidia - conidia also on germ tube

Note: Scale is approximate

PLATE III

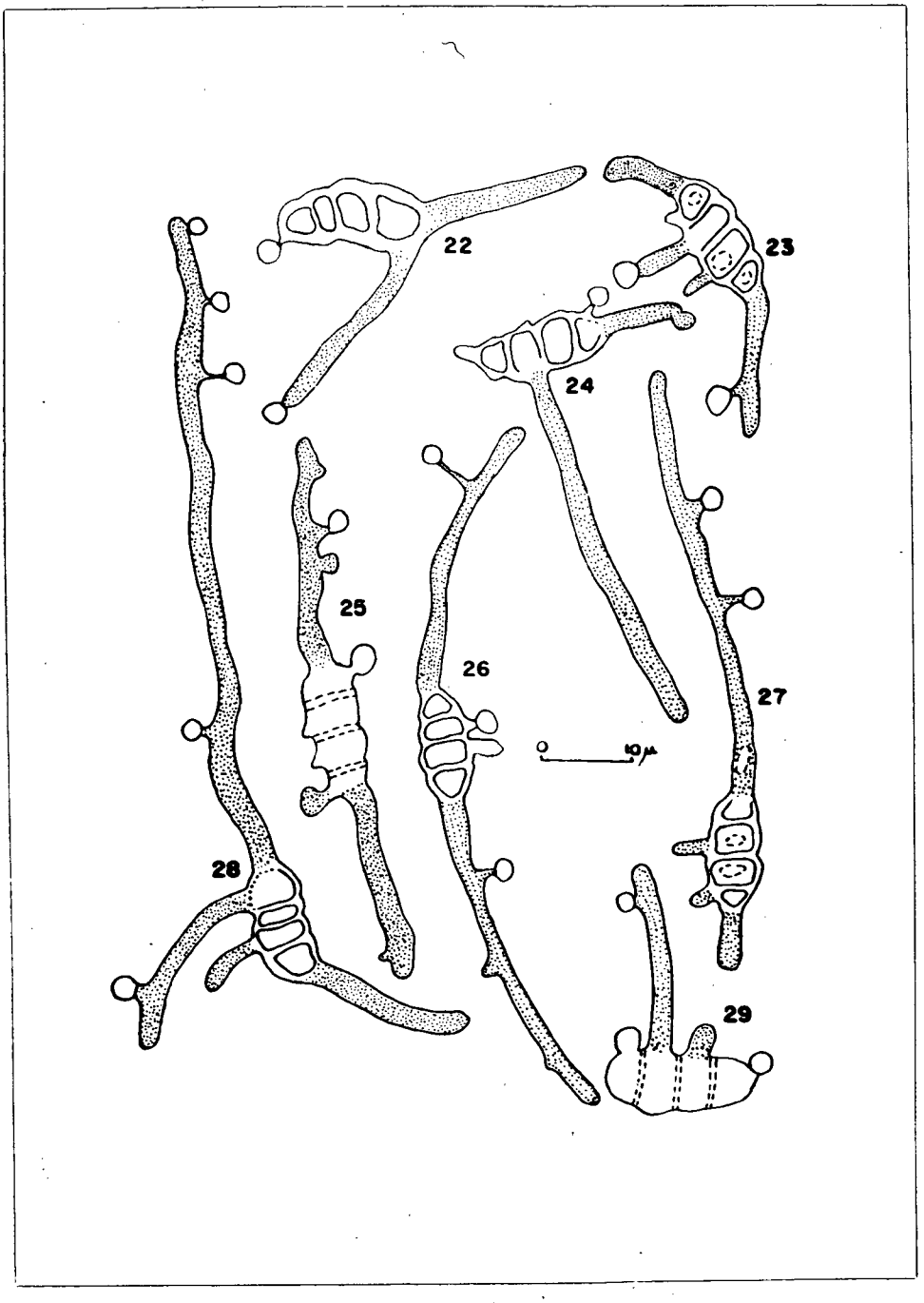

PLATE IV

Dacrymyces deliquescens var. *deliquescens*

Fig. 30 Chain of oblong arthrospores with binucleate cells

Fig. 31 Germinating binucleate arthrospores

Fig. 32 Chain of binucleate arthrospores of different shapes

Figs. 33-34 Germinating arthrospores

Fig. 35 Chain of two celled, binucleate arthrospores

Figs. 36-37 Uninucleate, septate hyphae with conidiophores and conidia

Fig. 38 Binucleate hyphae from subhymenial portion of basidiocarp

Note: Scale is approximate

PLATE IV

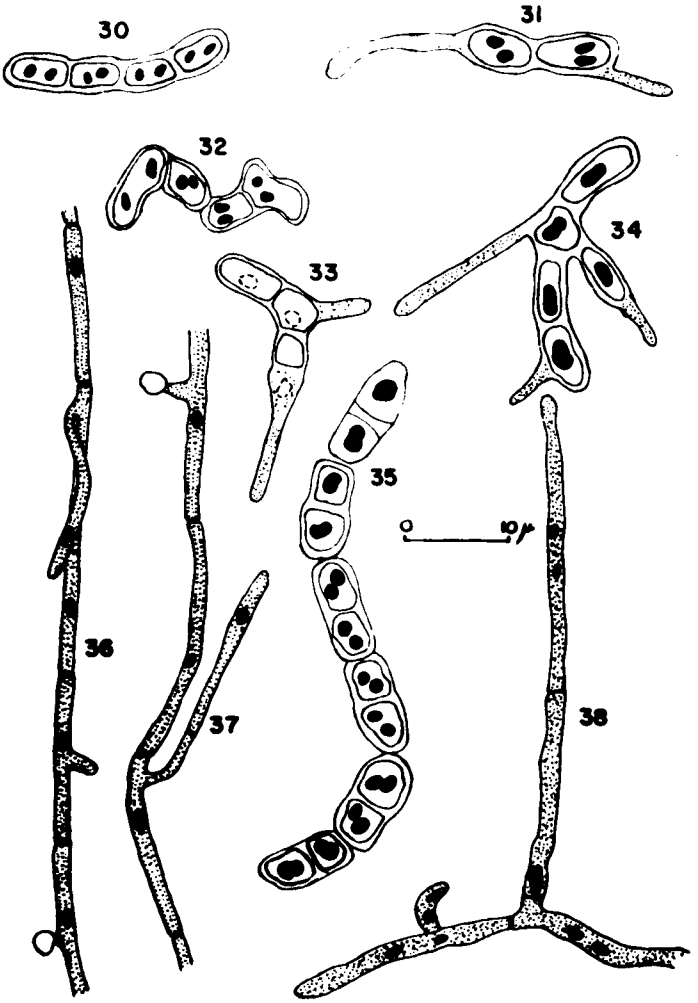

PLATE V

Dacrymyces deliquescens var. *deliquescens*

Fig. 39 Probasidium - two nuclei before fusion

Fig. 40 Probasidium - with fusion nucleus

Fig, 41 Probasidium - with four daughter nuclei

Fig. 42 Probasidium with four daughter nuclei - the flat top of probasidium is the first step of epibasidial development

Fig. 43 Well expressed flattening of probasidium with four daughter nuclei

Fig. 44 Beginning of the formation of epibasidia

Fig. 45 Fusion nucleus in hypobasidium with well developed epibasidia

Fig. 46 Four-nucleate stage in basidium with developing epibasidia (stichobasidial arrangement)

Note: Scale is approximate

PLATE V

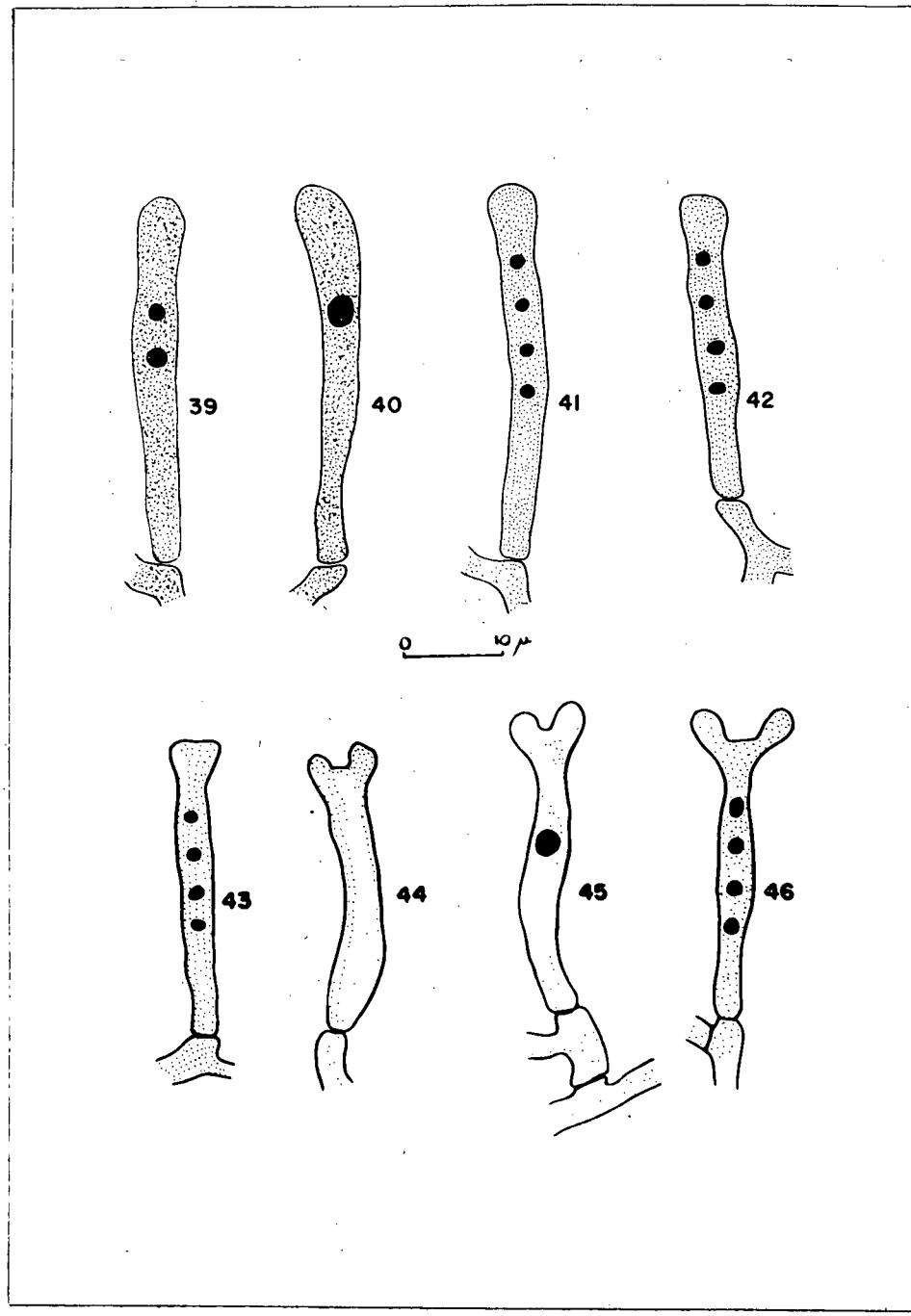

PLATE VI

Dacrymyces deliquescens var. *deliquescens*

Fig. 47 One nucleus already in the epibasidium, another moving toward the other epibasidium

Figs. 48-49 The beginning of basidiospore formation; basidiospores on sterigmata, one nucleus in each epibasidium, two nuclei at the top of hypobasidium

Fig. 50 Basidium after spore discharge; one of the nuclei unusually far from the top of hypobasidium

Fig. 51 Developing basidiospores after the nuclei have passed through the sterigmata. Nuclei in hypobasidium are unusually far from the top

Fig. 52 Deteriorating "empty" hyaline basidium

Note: Scale is approximate

PLATE VI

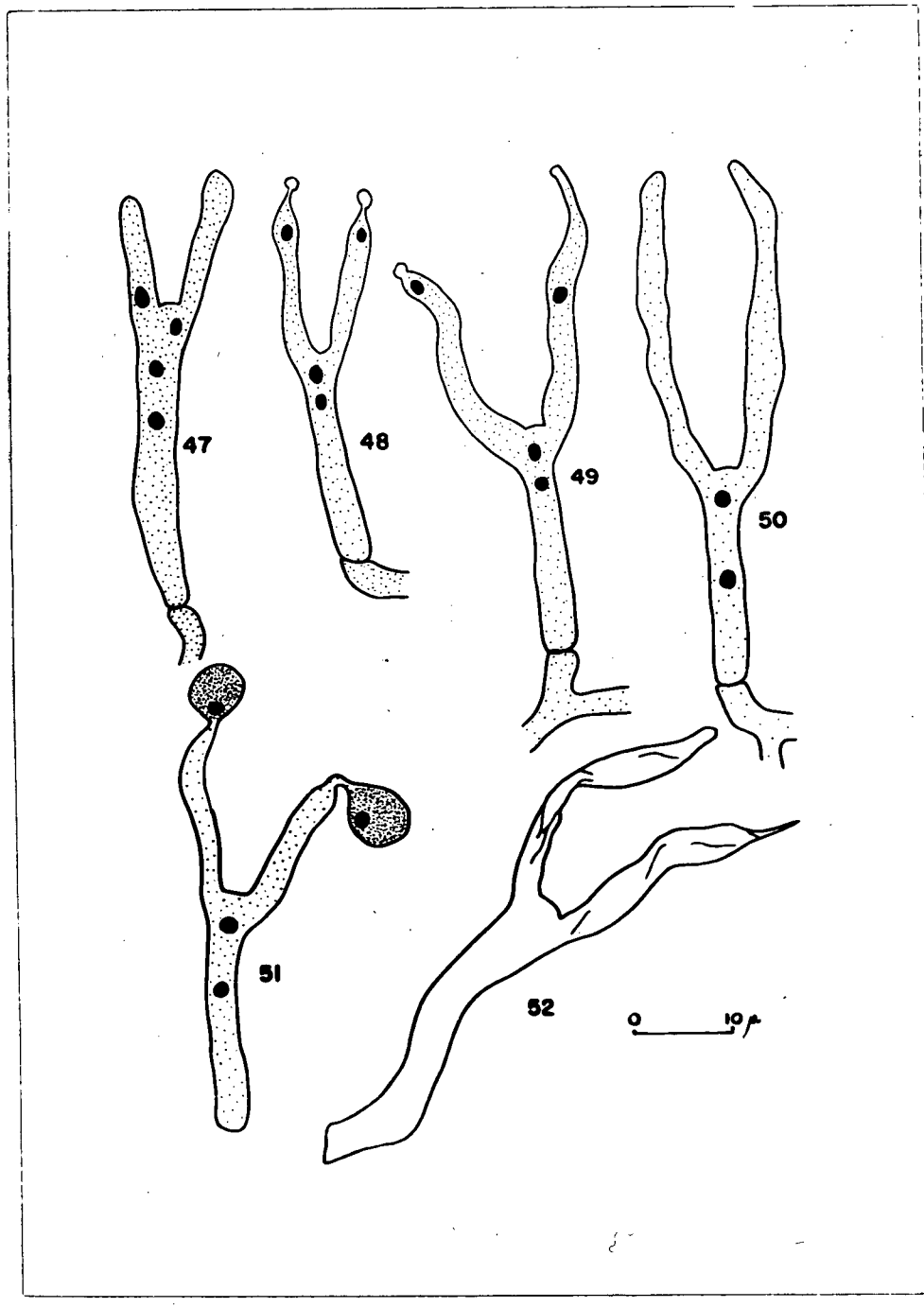

PLATE VII

Fig. 53 Mature basidiospore of D. deliquescens var. deliquescens. Note the nuclei and the definite separation of cells by septa. 3500 x

Fig. 54 1-septate mature basidiospore of D. deliquescens var. minor. 3500 x

Fig. 55 Mature basidiospore of D. deliquescens var. deliquescens. Note the nuclei and the definite separation of cells by septa. 3200 x

PLATE VII

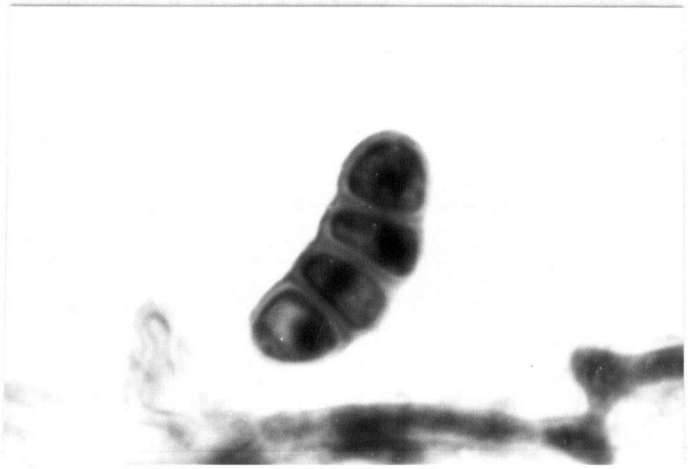

Fig. 53

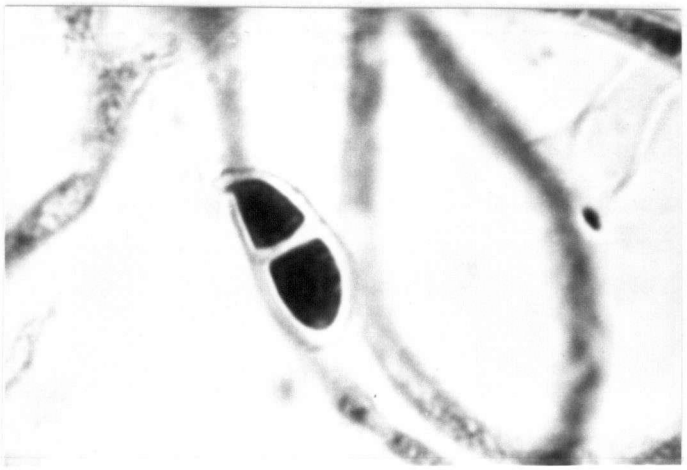

Fig. 54

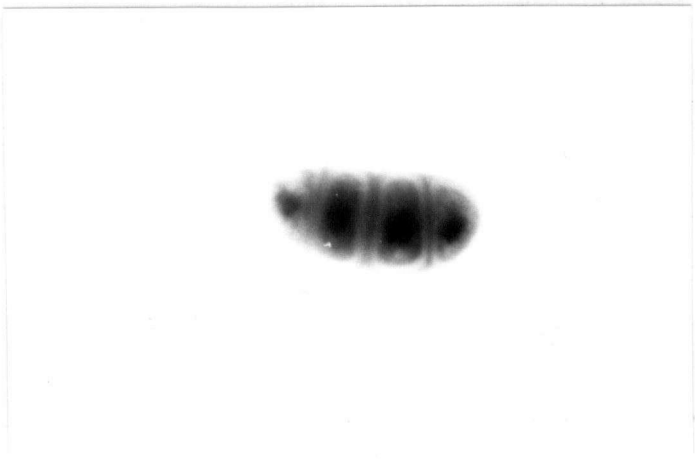

Fig. 55

PLATE VIII

Dacrymyces deliquescens var. *deliquescens*

Fig. 56 Spore deposit on potato dextrose agar. Note the differences in septation. Non-, 1-, and 3-septate spores are present. Some 3-septate spores are already germinating. 500 x

Fig. 57 Mature (3-septate) and immature (non-septate) spores from spore deposit on potato dextrose agar. Mature spore germinating by germ tube. 3200 x

Fig. 58 Germination of basidiospore by germ tubes. Spicule on which conidium was formed also present. 2000 x

PLATE VIII

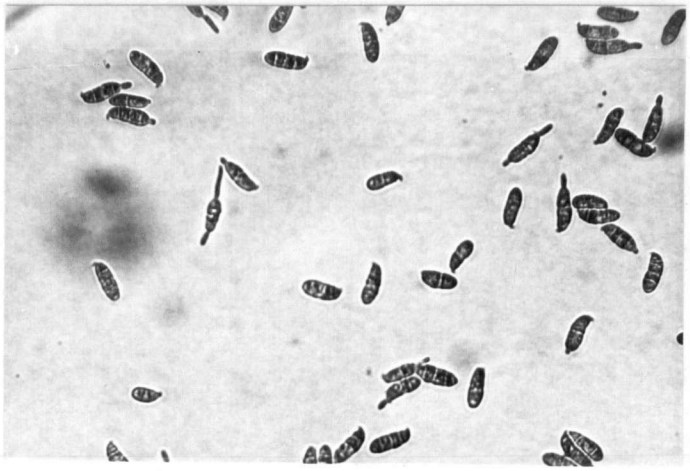

Fig. 56

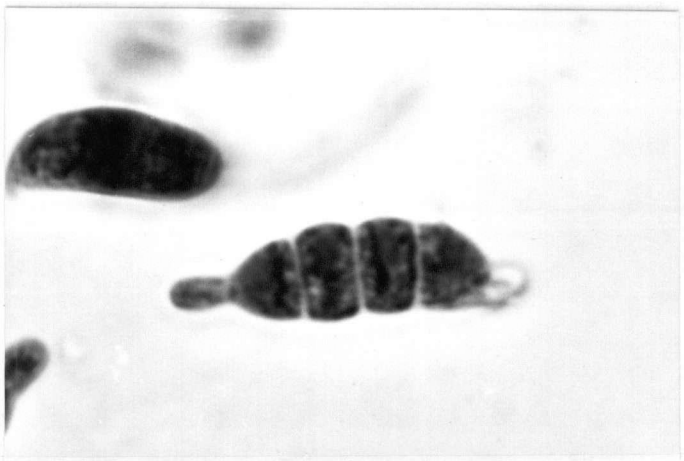

Fig. 57

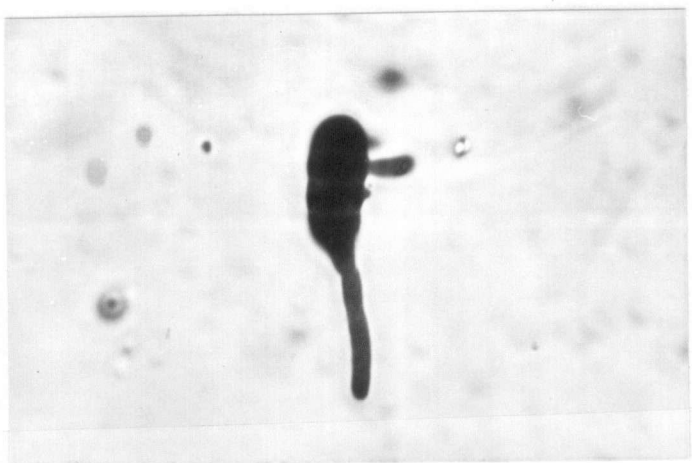

Fig. 58

PLATE IX

Dacrymyces deliquescens var. *deliquescens*

Fig. 59 Germination of basidiospores by germ tubes in glass tube. 500 x

Fig. 60 Two probasidia in the early stage of development. 1500 x

Fig. 61 Different stages of basidial development. Beginning of basidiospore development. 750 x

PLATE IX

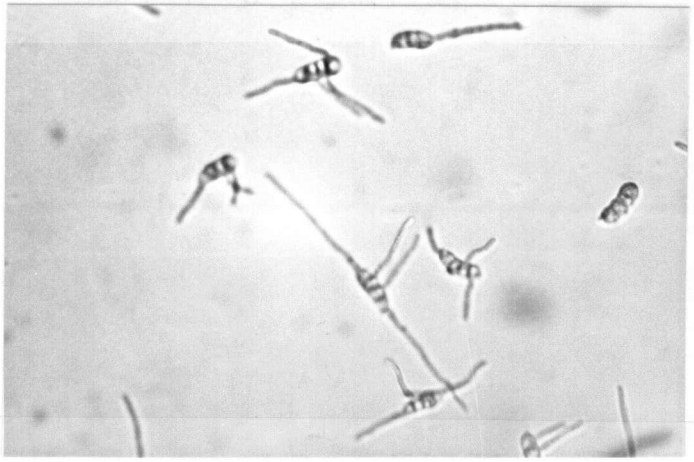

Fig. 59

Fig. 60

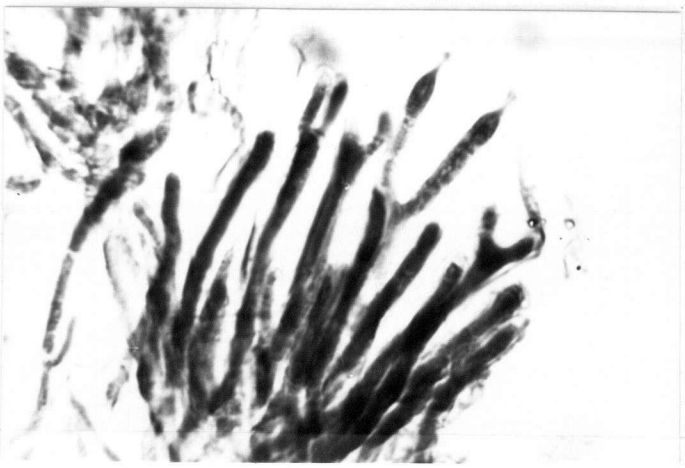

Fig. 61

PLATE X

Dacrymyces deliquescens var. *deliquescens*

Fig. 62 Binucleate arthrospores. 1800 x

Fig. 63 Chain of 2-celled arthrospores. Irregular shaped arthrospore in lower right corner. 1200 x

Fig. 64 Two binucleate arthrospores. 900 x

PLATE X

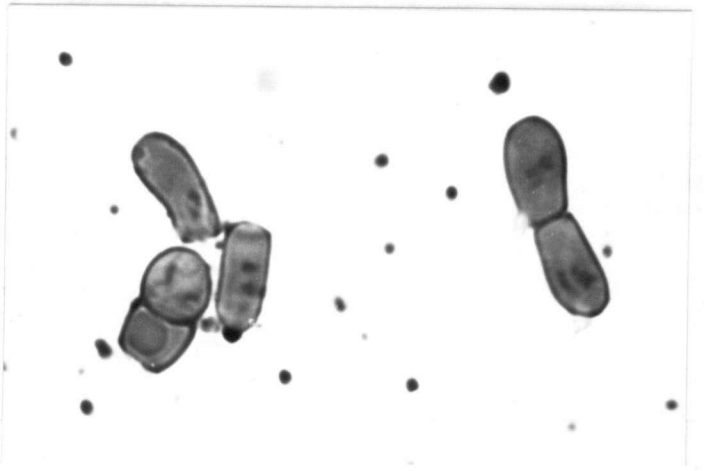

Fig. 62

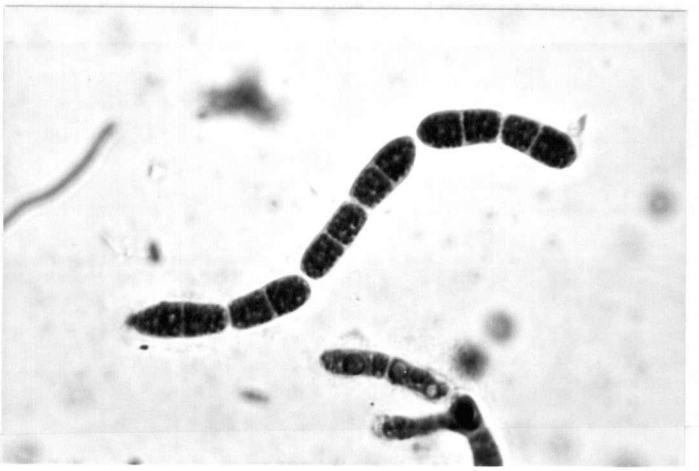

Fig. 63

Fig. 64

APPENDIX

Preparation I., modified LIlly and Barnett (1951) formula (BMA)

Sucrose	10.0 g
$(NH_2)SO_4$	2.24 g
KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Fe^{+++}	0.2 mg
Zn^{++}	0.2 mg
Mn^{++}	0.1 mg
Thiamine	100.0 µg
Biotin	5.0 µg
Dist. water to make	1000.0 ml

For solid medium agar was added at the rate of 15 g/ 1000 ml. of medium.

Preparation II. Freshly prepared potato dextrose agar (FPDA)

Distilled water	1000 ml
Dextrose	20 g
Peeled and sliced potatoes	200 g
Agar	17 g

Potatoes were cooked in 500 ml of distilled water for 40 minutes at 15 psi. in the autoclave. The agar was treated the same way in the other 500 ml of distilled water. The liquid from the potatoes was filtered through cheese cloth into the agar. Dextrose was added and the preparation sterilized.

The amounts of agar used in different media

PDA	39.0 g/1000 ml Difco potato dextrose agar
FPDA	17.0 g/1000 ml agar agar
MA	45.0 g/1000 ml Difco malt agar
MEA	33.6 g/1000 ml Difco malt extract agar
BMA	15.0 g/1000 agar agar
WA	15.0 g/1000 agar agar

Preparation of Belling's iron - acetocarmine stain

Glacial Acetic Acid	45 ml
Distilled water	55 ml

Add these two together. Heat the mixture to boiling then immediately add 0.5 g certified carmine dye. Shake to mix. Let the mixture cool and filter it through filter paper.

Photography

Photomicrographs were taken through an Olympus (Tokyo(microscope. The microscope was fitted with three objectives of magnification, 10x, 40x and 100x. The latter was used for oil immersion. The eyepiece, which was also used as the lens for the camera, had a magnification of 10x. An exakta 35 mm was attached to the microscope by means of an Ihagee camera adapter. Light was supplied by a laboratory microscope lamp. The amount of light was controlled by a transformer and by adjustment of the diaphragm on the lamp. A blue filter was used to improve contrast. The photographs were taken on Kodak High Contrast Copy film.

List of specimens used in the study 1.

Collection number	Name of variety	Substratum	Location	Date
BC - 351	minor	coniferous wood	Coldwater River	25 - IV - 59
BC - 408	minor	Acer macrophyllum	Spanish Banks	21 - V - 59
BC - 433	deliquescens	Tsuga heterophylla	Mt. Seymour Park	2 - VII - 59
BC - 436	ellisii	Alnus sinuata	UBC Campus	8 - VI - 59
BC - 444	deliquescens	Rubus spectabilis	UBC End. Lands	10 - VI - 59
BC - 451	deliquescens	Rubus sp.	UBC End. Lands	10 - VI - 59
BC - 457	deliquescens	? coniferous wood	UBC End. Lands	10 - VI - 59
BC - 525	deliquescens	coniferous wood	Mt. Revelstoke	14 - VII- 59
BC - 602	deliquescens	Rubus spectabilis	Spanish Banks	23 - IX - 59
BC - 612	ellisii	Populus trichocarpa	UBC End. Lands	25 - IX - 59
BC - 637	ellisii	Rubus sp.	Squamish	26 - IX - 59
BC - 894	deliquescens	coniferous wood	UBC End. Lands	26 - III- 60
BC - 895	deliquescens	coniferous wood	UBC End. Lands	26 - III- 60
BC - 922	deliquescens	Sambucus pubens	UBC End. Lands	15 - IV - 60
BC - 923	deliquescens	? coniferous plank	UBC End. Lands	15 - IV - 60
BC - 987	? minor	coniferous wood	Nanaimo	19 - IV - 60
BC -1000	ellisii	Alnus sp.	UBC End. Lands	7 - V - 60

List of specimens used in the study 2.

Collection number	Name of variety	Substratum	Location	Date
BC - 1005	deliquescens	coniferous wood	Wreck Beach	7 - V - 60
BC - 1037	ellisii	Salix sp.	UBC End. Lands	17 - V - 60
BC - 1096	deliquescens	coniferous wood	Monashee Pass	29 - V - 60
BC - 1134	deliquescens	decaying wood	Arrow Lake	30 - V - 60
BC - 1159	minor	Betula sp.	Revelstoke	31 - V - 60
BC - 1176	deliquescens	coniferous wood	Revelstoke	31 - V - 60
BC - 1314	minor	Pinus ? contorta	Lulu Island	16 - VI- 60
BC - 1317	minor	Pinus contorta	Lulu Island	16 - VI- 60
BC - 2185	ellisii	Alnus sp.	Harrison H.S.	17 - V - 61
BC - 2358	ellisii	Cytisus sp.	Shirley	24 - IV- 62
BC - 2378	ellisii	Alnus sp.	Jordan River	24 - IV- 62
BC - 2385	ellisii	Alnus rubra	Point-No-Point	24 - IV- 62
LM - 63	deliquescens	Sambucus sp.	UBC End. Lands	28 - I- 61
LM - 72	? ellisii	Sambucus sp.	UBC End. Lands	28 - I- 61
LM - 74	deliquescens	Acer macrophyllum	UBC End. Lands	23 - II- 61
LM - 77	deliquescens	Acer macrophyllum	Mt. Seymour Park	I -VII- 61
LM - 81	deliquescens	Acer macrophyllum	Mt. Seymour Park	I -VII- 61

List of specimens used in the study 3.

Collection number	Name of variety	Substratum	Location	Date
LM - 82	? minor	decayed wood	Mt. Seymour Park	1 - VII - 61
LM - 84	minor	Thuja plicata	Mt. Seymour Park	1 - VII - 61
LM - 88	deliquescens	coniferous wood	Pt. Atkinson	8 - VII - 61
LM - 92	deliquescens	? Thuja sp.	Pt. Atkinson	8 - VII - 61
LM - 95	deliquescens	coniferous wood	Pt. Atkinson	8 - VII - 61
LM - 98	deliquescens	Thuja plicata	Pt. Atkinson	8 - VII - 61
LM - 100	deliquescens	Thuja plicata	Pt. Atkinson	8 - VII - 61
LM - 128	deliquescens	coniferous wood	Pt. Atkinson	5 - IX - 61
LM - 131	deliquescens	coniferous wood	Pt. Atkinson	5 - IX - 61
LM - 135	deliquescens	coniferous post	Stanley Park	16 - X - 61
LM - 138	? deliquescens	coniferous post	Stanley Park	16 - X - 61
LM - 140	deliquescens	Acer macrophyllum	Stanley Park	16 - X - 61
LM - 141	deliquescens	coniferous post	Stanley Park	16 - X - 61
LM - 142	deliquescens	coniferous wood	Stanley Park	16 - X - 61
LM - 144	deliquescens	Acer macrophyllum	Stanley Park	16 - X - 61
LM - 145	deliquescens	Sambucus sp.	UBC End. Lands	15 - XI - 61
LM - 146	deliquescens	Sambucus sp.	UBC End. Lands	15 - XI - 61

List of specimens used in the study 4.

Collection number	Name of variety	Substratum	Location	Date
LM - 153	deliquescens	Tsuga heterophylla	UBC End. Lands	18 - III - 62
LM - 154	deliquescens	Sambucus sp.	UBC End. Lands	18 - III - 62
LM - 155	deliquescens	Tsuga heterophylla	UBC End. Lands	18 - III - 62
LM - 156	deliquescens	Tsuga heterophylla	UBC End. Lands	18 - III - 62
LM - 157	deliquescens	Tsuga heterophylla	UBC End. Lands	18 - III - 62
LM - 158	deliquescens	Sambucus sp.	UBC End. Lands	18 - III - 62
LM - 160	deliquescens	Sambucus sp.	UBC End. Lands	18 - III - 62
LM - 163	deliquescens	coniferous wood	UBC End. Lands	18 - III - 62
LM - 164	deliquescens	coniferous wood	UBC End. Lands	18 - III - 62
LM - 165	deliquescens	Sambucus sp.	UBC End. Lands	18 - III - 62
LM - 166	deliquescens	Acer macrophyllum	UBC End. Lands	23 - V - 62
LM - 167	deliquescens	Tsuga heterophylla	UBC End. Lands	23 - V - 62
LM - 168	deliquescens	Tsuga heterophylla	UBC End. Lands	23 - V - 62
LM - 169	deliquescens	Tsuga heterophylla	UBC End. Lands	23 - V - 62
LM - 170	deliquescens	Tsuga heterophylla	UBC End. Lands	23 - V - 62
LM - 172	deliquescens	Acer macrophyllum	UBC End. Lands	23 - V - 62
LM - 173	? deliquescens	Alnus rubra	UBC End. Lands	23 - V - 62

List of specimens used in the study 5.

Collection number	Name of variety	Substratum	Location	Date
LM - 174	deliquescens	Rubus spectabilis	UBC End. Lands	23 - V - 62
LM - 175	? deliquescens	Acer macrophyllum	UBC End. Lands	23 - V - 62
LM - 176	deliquescens	Rubus sp.	UBC End. Lands	23 - V - 62
LM - 177	deliquescens	Alnus rubra	UBC End. Lands	23 - V - 62
LM - 179	deliquescens	Rubus sp.	UBC End. Lands	23 - V - 62
LM - 180	deliquescens	Sambucus sp.	UBC End. Lands	23 - V - 62
LM - 181	deliquescens	Sambucus sp.	UBC End. Lands	23 - V - 62
LM - 188	deliquescens	coniferous log	Wreck Beach	23 - V - 62
LM - 190	deliquescens	coniferous log	Wreck Beach	23 - V - 62
LM - 192	deliquescens	Sambucus sp.	UBC End. Lands	23 - V - 62
LM - 198	deliquescens	coniferous post	Pt. Atkinson	5 -VII- 62
LM - 199	deliquescens	coniferous wood	Pt. Atkinson	5- VII- 62
LM - 200	deliquescens	coniferous twig	Pt. Atkinson	5 -VII- 62
LM - 201	deliquescens	coniferous stump	Stanley Park	7 -VII- 62
LM - 202	deliquescens	coniferous log	Stanley Park	7 -VII- 62
LM - 203	deliquescens	coniferous post	Stanley Park	7 -VII- 62
LM - 204	deliquescens	coniferous post	Stanley Park	7 -VII- 62

List of specimens used in the study 6.

Collection number	Name of variety	Substratum	Location	Date
LM - 205	deliquescens	coniferous log	Stanley Park	7 - VII - 62
LM - 206	deliquescens	coniferous log	Stanley Park	7 - VII - 62