THE CYTOLOGY AND MORPHOLOGICAL DEVELOPMENT OF *TAPHRINA POPULINA* FRIES AND *TAPHRINA CAERULESCENS* (DESM.) TULASNE

by 1264

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INTRODUCTION

The genus, Taphrina has been studied by many workers. Most of the early papers on Taphrina species considered them only from a taxonomic standpoint based on comparative morphology. Detailed developmental and cytological studies have been done for only a few of the approximately 100 recognized species. One of the most recent and comprehensive works is that of Kramer (1961) who studied the developmental morphology and cytology of Taphrina deformans (Berk.) Tulasne, T. carveri Jenkins, T. virginica Sadebeck, T. ulmi (Fkl.) Johanson, and T. populi-salicis Mix.

The objectives of this study were three-fold in nature. The first was to describe the morphological development of Taphrina populina Fries with respect to its intercellular vegetative mycelium and the formation of its ascus from tips of these intercellular hyphae. Morphological development in this species is quite distinctive from those better known such as T. deformans with asci that have stalk cells and that develop from a subcuticular layer of ascogenous cells.

The second objective was to attempt to clarify the cytology of T. populina and T. caerulescens (Desm.) Tulasne with emphasis placed on the nuclear behavior with respect to the parasitic phase of the life cycle.

The third objective was to attempt a correlation of the characteristics of these species with the intention of obtaining a better understanding of the trends and relationships that may exist within the genus. Taphrina populina is the type species

of the genus and a thorough developmental and cytological study of this species is needed before an attempt may be made to discuss possible intrageneric relationships.

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MATERIALS AND METHODS

The essential study of the parasitized tissues and their parasite has been carried out with preserved materials of T. populina and T. caerulescens. Material to be fixed was first sectioned into small fragments in order to facilitate the penetration of the fixative into the tissues. Fixed material was washed, dehydrated, embedded in parafin, sectioned with a rotary microtome (10μ in thickness), fixed to slides with Haupt's adhesive, and stained.

The killing and fixing agent was formalin-propiono-alcohol (F.P.A.) prepared with the following formula: 90 ml of 50% ethyl alcohol; 5 ml of propionic acid; and, 5 ml of formalin (37%).

Heidenhains' iron hemotoxylin (0.5% solution in distilled water) was used as the cytological stain and fast green as a counterstain. The procedure for staining was as follows:

1. Place the sectioned material on a slide to which Haupt's adhesive has been previously applied. This adhesive fixes the sectioned material to the slide.

2. Place slide in xylol for 10 minutes to remove parafin.

3. Remove slide and insert into solution of 50% ethyl alcohol and 50% xylol for 2 to 3 minutes.

4. One can then place slide in the following successive concentrations of ethyl alcohol. These concentrations are 100, 95, 70, 50 and 25%. Two minute intervals for each concentration is sufficient.

5. Rinse slide in water followed by a rinse in distilled

water.

6. The slide should then be placed in a 2-4% aqueous ferric alum, which is a mordant, for 2 hours. This solution must be freshly prepared.

7. Wash for 5 minutes in tap water, and rinse in distilled water.

8. Stain the slide in 0.5% aqueous hematoxylin for 24 hours.

9. Wash 5 minutes in tap water.

10. Destain for 15-30 minutes in 2% ferric alum or 3-4% ferric chloride.

11. Wash for 30 minutes in running water.

12. In an increasing succession at two minute intervals, place the slide in 25, 50, 70, 95, and 100% concentrations of ethyl alcohol.

13. This procedure should be followed by the application of an aqueous fast green counterstain for 1-2 minutes.

14. Rinse slide in equal parts of absolute alcohol and xylene, followed by a 2 minute interval in equal parts of xylene and xylol.

15. Allow slide to remain in 100% xylol for 10 minutes.

16. Mount with balsam using Number 1 coverslips.

The material listed below was used in the study of T. populina. On Populus canadensis Moench.: Haney, British Columbia, September 8, 1960, A. J. Foster. On Populus sp.: Toulouse, France, June, 1967, Alain Schneider. On Populus berolinensis

Dipp.: Akershus Co., As, A. C. of Norway, July 13, 1967, Gjaerum. All species were fixed in F.P.A. This species is distributed widely, although not abundant, in Europe, locally throughout eastern North America and occasionally in western North America.

In the study of T. caerulescens, the following material was examined. On Quercus macrocarpa Michx.: Ft. Leavenworth, Kansas, May 30, 1954, C. L. Kramer. On Quercus rubra L.: Baldwin, Kansas, June, 1952, C. L. Kramer. On Quercus sp.: Blue Mounds, Wisconsin, June 12, 1968, C. L. Kramer. This species is widely distributed throughout the United States and Europe.

Observations of the sectioned material were made by using a Leitz compound microscope with a 1.7 mm 1.30 apochromatic oil immersion objective and 10x and 12x compensating oculars. Pictures were obtained with the aid of a Leitz camera and 4 x 5 inch Polaroid film holder using 55 PN film.

REVIEW OF LITERATURE

History and Synonymy of the Genus Taphrina

The order Taphrinales as established by Gaumann (1926) included two families, the Protomycetaceae and the Taphrinaceae. In the most recent monograph of the family, Mix (1949) recognized the single genus Taphrina in the Taphrinaceae. This concept will be followed in the present study.

The genus Taphrina was first described by Fries (1815) as Taphria but he later dropped the name as Taphria was preoccupied by a genus of insects. In 1825, Fries redescribed the genus as Taphrina, which since then, has been variously interpreted. The type species described by Fries in 1832 is Taphrina populina Fr. and was published in Systema Mycologicum, which is the starting point for the nomenclature of Ascomycetes. Fries' description of the genus is as follows:

"Pseudo-peridia elevata, liberata, rotundato-clavata, in maculas sericeas stipata, materia grumosa (speudo-sporidiis) farcta.

Obs. 1. Taphrina prae ceteris liberatur ut ad Gasteromycetes referendam-censeat Ill. Link (Spec. 1. p. 162) Ejus vero physiologicas rationes easdem esse ac sequentium, nullam adesse autonomam vegetationem, nullam propogationem, certum est. Jam Malpighi in tractatu de excrescentiis et tumoribus exanthematibus adnumerat Taphrinam populinam s. Erineum aureum. Pers.-Non tantum habitu, sed etiam characteribus a sequentibus tantum

recedit, ut genere differat, h.e. in his plantis diversum a sequentibus typum offerat. Vetustum abit in maculus subgelatinosas laevigatas, ex quo analogia quaedam cvm Gymnosporangio-Alias hujus maculae facie satis congruunt cum Spilocaea, et physiologice considerata haec genera havd longe distant.

Obs. 2. Incolunt folia arborum, hactenus quidem in Amentoceis tantum observatae, hujus formationes species quodammodo-dicendae, recedentes situ aequabili et foliis immerso, colore aureo (T. populina Grev. Crypt. Scot. t. 33), badio (T. alnea), purpurescenti-griseo (T. quercina Schmidt, Erin. griseum Pers. - E. minutissimum Grevill.). Sequentium generum rariores et pauciores sunt, at, quod singulare, in eisdem arboribus, in quibus Erinea et Phylleria optime vigent, Taphrinae simul leguntur; quae quidem ratio praecipue me ad genus distinguendum induxit. - Plenius exposvit Cel. Kunze l. c. (E. pallidum l. c. potius ad Erinea referrem.)".

The following is a literal English translation of Fries' generic description:

Pseudoperidia elevated free, rounded- clavate, in silk invested leisons, in compact pustules (pseudo-fructifications).

Obs. 1. Taphrina is free compared to other forms so that Link (Spec. l. p. 162) thinks it must be referred to the Gasteromycetes. It is certain that its physiological characteristics are the same as those of the following, and that there is no vegetative reproduction and no propogation. Malpighi in his treatise on exantematic excresences and tumors, enumerates

Taphrina populina s. Erineum aureum Pers. Not only in appearance but also in characteristics, it differs generically, that is, these plants are of a type different from the following. In old age they appear as lesions which are subgelatinous and smooth, in which there is an analogy with Gymnosporangium. Its other lesions are very similar to Spilocaea and physiologically considered, this genus is not much different from it.

Obs. 2. Formations of this type, are distinguishable for different species, occur in leaves of trees, hitherto certainly observed only in Amentoceis, withdrawing to a suitable site, covered with leaves, of a golden color (T. populina Grev. Crypt. Scot. t. 33.), brown (T. alnea) greyish-purple (T. quercina Schmidt, Erin. Griseum Pers. - E. minutissimum Grevill.). Those of the following genera (Erinea and Phylleria) are more rare and fewer, but what is remarkable, Taphrina species are found simultaneously in the same trees in which Erinea and Phylleria flourish best; this face has especially led me to differentiate this genus. Cel. Kunze explained this more fully in the passage cited (E. pallidum l. c. I rather refer to Erinea).

Following the work of Fries many new species were described with the complex of species variously distributed within a variety of generic concepts. In 1848, Desmazieres described the genus Ascomyces on the basis of T. caerulescens as the type species and characterized it by having ascus-like "sporangia" on the surface of the host leaf. The "sporangia" were thought to constitute the whole fungus, i.e. mycelium was absent. This

concept was followed by Fuckel (1860) who recognized the genus Ascomyces with multispored asci, but erected the genus Exoascus for the remainder of the species characterized by 8-spored asci. He apparently gave little importance to the supposed lack of mycelium, the characteristic upon which Desmazieres erected the genus Ascomyces.

Magnus (1874), in a study of the species Ascomyces tosquinetii Westendorp, assumed that each ascus was a plant in itself that arose in and grew out from an epidermal cell of the host. Based upon observations from his earlier paper, Magnus (1875) was doubtful as to whether the genus Ascomyces as erected by Desmazieres and Montagne, was distinct from Taphrina. If not, he then contended that the species A. tosquinetii represented a true generic type, and proposed the genus name, Endoascus.

Sorokine (1876) proposed a new system of classification, recognizing three genera. They were Exoascus, with intercellular mycelium; Taphrina, with subcuticular mycelium; and Ascomyces, which lacked mycelium. Endoascus was given no consideration.

In 1884, Sadebeck published a monographic account of all species known at that time and placed them under the one genus, Exoascus. His classification included mycelium perennial within shoots, fertile hyphae completely used up in the formation of asci, and stalk cells present. Some species of Exoascus also possessed mycelium perennial beneath the cuticle and confined to that location. The latter included fertile hyphae completely used up in the formation of asci, along with the formation of

asci from some, but not all hyphae.

Johanson (1886) agreed with Sadebeck (1884), but on the basis of priority, used the name Taphrina rather than Exoascus. This concept was followed by Robinson (1887), Magnus (1890), and in Sadebeck's later work of 1890 in which he recognized 32 species of Taphrina.

Brefeld (1891) suggested that the genus be divided into Taphrina, characterized by having four spores per ascus and Exoascus with eight spores per ascus. This was an interesting suggestion because all previous work had shown the species of Taphrina (or Exoascus) to bear normally eight spores in an ascus unless by budding of ascospores, the ascus became filled with blastospores and hence was many spored. Schroeter (1893) suggested two genera, Exoascus with 8-spored asci and Taphrina with asci becoming many spored by budding of the ascospores.

In 1893, Sadebeck again proposed to divide the complex of species into 3 genera, Exoascus, Taphrina, and Magnusiella, separated primarily by the type of mycelium and origin of the asci. Exoascus was described as having perennial mycelium with the cells of its subcuticular hyphae separating without previous differentiation into ascogenous cells and deforming shoots and leaves. Taphrina lacked perennial mycelium, with its subcuticular hyphae differentiated into fertile and sterile cells, the former becoming mother cells or the ascogenous cells, the latter degenerating. Necrotic spots were caused only on leaves. Magnusiella formed no ascogenous layer; the asci arose at the

ends of branches of the intercellular mycelium.

Giesenhagen (1895) accepted Sadebeck's genus Magnusiella but united all other forms into the one genus, Taphrina. Giesenhagen criticized Brefeld (1891) because Atkinson (1894) and Sadebeck (1895) had shown that in several species the asci could be 4-spored and sometimes 8-spored. He also did not consider the possession or lack of perennial mycelium used by Sadebeck (1893), a good basis for generic distinction.

Giesenhagen (1895) accepted Sadebeck's genus Magnusiella but united all other forms into the one genus, Taphrina. He recognized four basic morphological forms among the Taphrinaceae based on the shape of the ascus with respect to its host relationship. These are: 1, the Felicina-type with slender asci, narrowed above and below and confined to the ferns; 2, the Betula-type with cylindrical asci, more or less truncate at the apex and confined to the Amentiferae; 3, the Pruni-type with clavate or cylindrical asci often rounded at the apex and found on Rosaceae; and 4, the Magnusiella-type with ovate or elliptic asci confined to the genus Aesculus.

In 1901, Giesenhagen further divided the genus Taphrina into 4 sub-genera on the basis of his earlier classification: the Felicina-type becoming Taphrinopsis; the Betula-type, Eutaphrina; the Pruni-type Euexoascus; and the Aesculus-type, Sadebeckiella. Giesenhagen recognized 49 species of Taphrina and 5 species of Magnusiella.

The next 25 years seemed to be a period of collecting in

which many new species of Taphrina were described. Cytological studies were begun and there was considerable research undertaken with respect to the plant disease aspects of these organisms.

Mix (1949) did the most recent comprehensive account of this genus and following Giesenhagen's (1895) interpretation placed all known species, of which there were 98, under the one genus, Taphrina.

The Morphology of Taphrina

The Vegetative Mycelium. Three types of vegetative mycelium are known to occur in the genus Taphrina. These are the intercellular, subcuticular, and "wall habit" types. The first two types are the more common.

Intercellular mycelium develops between the interior cells of leaf, stem, or fruit and subsequently forms a subcuticular layer of ascogenous cells. deBary (1865) produced the first account of the description of the mycelium of these fungi when he studied the intercellular mycelium of Taphrina pruni Tulasne in enlarged fruits of the plum. In the young stages of development, the mycelium was observed between the cells of the vascular bundles and extended throughout the entire length of the vessels, sometimes penetrating into the petiole. An intercellular network was formed as the branches from this mycelium in the vascular bundles grew outward. In later development, the mycelium formed beneath the epidermis and hyphal branches protruded upward between the epidermal cells to give rise to the asci.

Sadebeck (1882) studied two forms on alder, Taphrina tosquinetii (Westendorp) Tulasne and T. Sadebeckii Johanson and found that their asci developed from thickly branched mycelium which grew between the epidermis and the cuticle. A similar form of mycelial development was later described for the species Taphrina ulmi (Fuchal) Johanson, T. betulae (Fuckel) Johanson, T. bullata (Berk.) Tulasne, and T. carpini (Rostr.) Johanson.

In T. deformans, the vegetative mycelium is at first intercellular but prior to sporulation, branches of this mycelium grow outward between the epidermal cells and form a highly branched system of subcuticular mycelium. The cells of this mycelium eventually fragment to give rise to separate ascogenous cells (Sadebeck, 1893; Martin, 1940; Kramer, 1961).

Mix (1939) described the third mycelial type as the "wall-habit mycelium" indicating that the mycelium occurs entirely within the outer epidermal wall of the host. Mix studied Taphrina maculans Butler and observed that in the early stages, the mycelium frequently grew above the radial walls of the epidermal cells. As the haphae broaden and thicken, the separated layers of the wall spread apart and the mycelium comes to lie in a locule formed within the cell wall. The locule is finally filled with close-packed, elongated ascogenous cells. Other species known to possess the "wall-habit mycelium" are Taphrina californica Mix, T. rhomboidalis Sydow and Butler, T. tonduziana P. Henn., T. thaxteri Mix, T. amplicans Mix, and T. laurencia Giesenhagen.

Haustoria have been reported for only two species of Taphrina, both of which have the "wall-habit mycelium". These are T. laurencia with unspecialized haustoria (Giesenhagen, 1892) and T. maculans with specialized haustoria composed of intertwined hyphal branches (Butler, 1911).

Origin of the Asci. Asci develop from the ascogenous layer which in turn is derived from the mycelium. There are various ways in which the mycelium gives rise to the asci. Kramer (1961) summarized them as follows:

1. Intercellular mycelium that does not form a subcuticular layer, but instead bears asci at the tips of hyphal branches which grow outward between the epidermal cells. Species which exemplify this type of mycelial development are T. johansonii, T. caerulescens and T. carnea Johanson.

2. Mycelium which is at first intercellular, but becomes subcuticular to form a hymenium of ascogenous cells that is similar to that of the next type. Species which exemplify this type of mycelial development are T. deformans and T. pruni.

3. Subcuticular mycelium that is richly branched and septate. This mycelium fragments into separate ascogenous cells which enlarge and form a rather compact hymenium. Species which exemplify this type of mycelial development are T. betulae (Fkl.) Johanson and T. carveri.

4. Subcuticular mycelium which remains intact throughout the development and maturation of the asci. Only certain cells enter into ascus formation while others remain sterile. Species

which exemplify this type of mycelial development are T. ulmi and T. celtii Sadebeck.

5. Subcuticular mycelium that functions only in the capacity of distributive hyphae and the hymenium is formed by a budding yeast-like colony in which the cells enlarge to form a compact ascogenous layer. Taphrina virginica is a species that exemplifies this type of development.

6. Subcuticular mycelium which is in general, sparingly branched and septate. The hyphae remain intact during the early development of the ascogenous cells. A species which exemplifies this type of development is T. populi-salicis. The ascogenous cells and asci are extremely large in this species.

7. Mycelium that grows within the wall of the epidermal cells, forms locules in which the ascogenous cells and asci develop. Species which exemplify this type of development are T. californica, T. laurencia and T. maculans.

The ascogenous cells may form in one of two ways. As the mycelium matures, the cells of the hyphae become separated, round up and enlarge to become the typical irregular shaped ascogenous cells which in turn elongate and push up through the cuticle. Finally, by cross-wall formation, the ascogenous cells are converted into an ascus and a stalk cell. The second method of development occurs when the mycelium does not separate or round up but instead elongates under the cuticle and there, often becomes divided by cross walls. The asci elongate as papillae from the middle of the ascogenous cells.

Development of the Ascus. There have been two concepts reported in the literature regarding the ascis development from the germinating ascogenous cell that is sometimes referred to in the older literature as the chlamydospore. Juel (1921) found that in T. epiphylla and in T. sadebeckii a pore formed in the outer wall (exospore) of the ascogenous cell, and the ascus emerged as a thin walled endospore through the widening spore. Mix (1949) found in his work on T. epiphylla and T. sadebeckii that the ascus developed from the rupture of the ascogenous cell wall rather than by the formation of a pore to allow the inner membrane to emerge. In other species such as T. betulina and T. carnea, Juel (1921) found that the chlamydospore itself elongated to form the ascus; its wall becoming thinner in the process, did not rupture.

Martin (1940) writing on T. deformans states that, "after the nuclear fusion, the ascogenous cell elongates vertically and its thick wall is stretched to become the thinner wall characteristic of the ascus. The wall of the ascus does not break for the ascus to emerge as described earlier by Pierce (1900) and Juel (1921)".

In discussion of the cytological studies of the development of the ascus, Dangeard (1895) gave a comprehensive account of T. deformans. Cells of the intercellular mycelium and young ascogenous cells were binucleate, and nuclear fusion occurred in the ascogenous cell. This was the first report of karyogamy in the genus Taphrina, which is now known to occur in all species thus

far studied. The ascogenous cells then put forth a papilla which elongated to become the ascus. The fusion nucleus migrated to the center of the ascus and divided by three successive divisions to form the nuclei for the spores. This concept was supported by later studies of Mix (1935), Martin (1940) and Kramer (1961).

There have been two concepts reported in the literature regarding the division of the fusion nucleus. Most early workers (Mix, 1935) advocated that as the septum separated the ascus from the stalk cell, the stalk cell was void of any contents. Hence, the ascospore nuclei were formed by the fusion nucleus proceeding directly to divide by meiosis. The second theory advocated by Ikeno (1903) and supported by Martin (1940), and subsequently supported by Kramer (1960) reported that the fusion nucleus underwent a vegetative division prior to the two meiotic divisions. As a result of this first equational division, one nucleus migrated to the distal portion of the papilla and the other nucleus remained in the stalk cell. A septum was then formed that cut off the stalk cell, which then becomes in all reality a vegetative diploid generation. The nucleus and cytoplasm contained in the stalk cell soon disintegrated.

Mix (1949) thought that the occurrence of a vegetative diploid cell was a rather significant feature. The occurrence of this stage in fungi is rare and with the exception of Allomyces, seems to be limited to certain yeasts or yeast-like organisms. It is apparent that there is a rather close relationship between Taphrina and the Saccharomycetales.

Spore Formation. The process of ascospore formation may conclude with four, eight, or many ascospores (Martin, 1940; Mix, 1949; Kramer, 1961). In addition, ascospores may bud to produce an ascus with several to numerous blastospores (Mix, 1949; Kramer, 1961). One common characteristic of spore formation is that the ascospores are uninucleate and haploid as are the blastospores derived from them.

Kramer (1961) reported that spore formation seemed to occur through the fusion of vacuolar membranes. Portions of cytoplasm containing a single nucleus become surrounded by small vacuoles and are delimited when the inner membranes of the vacuoles unite. After formation of a wall, this portion of cytoplasm becomes an ascospore. Some unused epiplasm remains in the ascus after spore formation.

Asci described with four ascospores seem to be a result of non-utilization or disintegration of four ascospore nuclei before spore formation. Eight ascospored asci are obtained by three nuclear divisions. The first division of the fusion nucleus is apparently reductional, giving rise to four haploid nuclei. Following soon after, a second equational division occurs to give rise to the normal eight ascospore nuclei.

Budding of the eight ascospores within the ascus may lead to a multispored ascus. This has been reported in a number of species. However, Kramer (1961) was the first to report that multispored asci in T. populi-salicis were obtained by successive mitotic divisions after the second division. Cytoplasm is

delimited by the fusion of vacuoles and eventually divides into uninucleate portions which produce a cell wall and become the ascospores in all cases described above.

RESULTS

Taphrina populina Fries

The Vegetative Mycelium. Although actual penetration by the germ tube of a spore of T. populina has not been observed, it is assumed that the nucleus of the germinating spore undergoes equational division to produce the first conjugate pair of nuclei. This initiates the binucleate condition or dikaryophase which exists throughout the development of the vegetative phase in the life cycle of this organism.

The vegetative mycelium is intercellular (Fig. 1). Infection is thought to occur while the host tissues are in a meristematic condition, when the fungus is able to invade the healthy tissues. However, as the tissues mature, a point is reached where the fungus is no longer able to encroach upon healthy tissues of the host plant. The fungus makes a limited amount of growth through the cuticle, but soon begins to penetrate the host tissue sending down a slender hypha which forms its way between the epidermal cells until it reaches the less compacted parenchymatous tissue. From there, the hyphae branch out and ramifies in all directions filling the intercellular spaces.

In the early stages of the growth of the fungus, little damage is noticeable in the host tissue (Figs. 2 and 3). In the intermediate stage of growth, considerable change occurs in the host tissue. Hyphae have penetrated through the outer layers of epidermal cells into the palisade parenchyma. Large

hypertrophied host cells are prevalent with some damaged cells being filled with a substance that causes the hypertrophied cells to become brown in color (Figs. 4 and 5). Damage to the host tissue has reached a climax when the fungus becomes mature, evidenced by old empty asci (Figs. 6 and 7). It appears that penetration of mycelium into the host tissue does not go any deeper than the palisade parenchyma. The spongy parenchyma shows no sign of hypertrophy or disorganized, damaged cells.

Origin of the Asci. The intercellular mycelium does not form a subcuticular layer of ascogenous cells, but instead bears asci at the tips of hyphal branches which grow outward between the epidermal cells. As this mycelium matures, cells of the hyphae do not separate but instead portions of the very long cells begin to round up to form typically irregular ascogenous cells (Figs. 8-13). The hyphal fragments which may in some cases include whole cells and in others only portions of cells remain attached to the ascogenous cells as they mature. These hyphae form an anastomosing branched network of mycelium (Fig. 13). The ascogenous cells are closely alligned between the epidermal cells of the host tissue and as they enlarge outward toward the surface of the leaf, they cause a partial collapse of the epidermal cells. In cross section, they appear to have a tapered, narrow base wedged between the epidermal cells. There is no ascogenous layer or hymenium such as is found in T. deformans.

The ascogenous cells are at first dicaryotic (Fig. 14) as are the cells of the vegetative mycelium. However, as the

ascogenous cells begin to develop into the asci, karyogamy occurs initiating the diplophase (Fig. 15).

Development of the Ascus. Since the stalk cells are not formed in this species, the elongating diploid ascogenous cell may now be referred to as the young ascus (Figs. 16-18). Development of the young ascus in this species occurs by the stretching and elongation of the wall of the ascogenous cell. As the ascus grows to maturity, the wall becomes thinner especially at the tip due to the stretching process (Fig. 18). At no time was the outer wall seen to rupture to allow the inner membrane to emerge as a papilla in the formation of the ascus as in the case in some other species of Taphrina. As the ascus emerges to the outside, the cuticle is ruptured.

Spore Formation. The fusion nucleus migrates outward as the elongation process extends the ascus from within the host tissue. A mitotic division of the fusion nucleus preceding meiosis has been reported only in species with stalk cells. Such a mitotic division does not appear to occur in this species, thus it is felt that the fusion nucleus undergoes meiosis to form four haploid nuclei. The prophase of the first division was easily discernible under the microscope (Fig. 19), but other phases of division were difficult to interpret.

The four nuclei resulting from meiosis undergo equational division to form eight haploid nuclei (Fig. 20). In most species thus far studied, these are the ascospore nuclei, however,

in this species these nuclei undergo repeated division forming from 32 to 128 nuclei before spore formation begins (Fig. 21). This results in an ascus filled with small ascospores as opposed to species such as T. deformans that often become filled with small blastospores resulting from repeated budding of the eight original ascospores.

Each nucleus with an adjacent portion of cytoplasm is encompassed by a vacuolar membrane and develops into a mature ascospore. Spore formation begins at the tip of the ascus (Fig. 22) and progresses downward until the entire ascus is filled with many ascospores (Fig. 23). Some unused epiplasm is apparent in the ascus after spore formation is completed. In old asci that are void of ascospores, large yellow globules are apparent (Fig. 24). Their purpose or function is unknown at this time.

Four chromosomes are evident in the nucleus of the ascus (Fig. 25). This indicates a chromosome number of four in the diploid stage. Eight chromatids are faintly visible in Figure 26 which further substantiates the chromosomal number in this organism.

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Taphrina populina Fries

Figs. 1-26

Fig. 1. Asci embedded between epidermal cells thus vegetative mycelium is intercellular. 925X.

Fig. 2. Immature stage of infection in host tissue. 233X.

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Fig. 1.

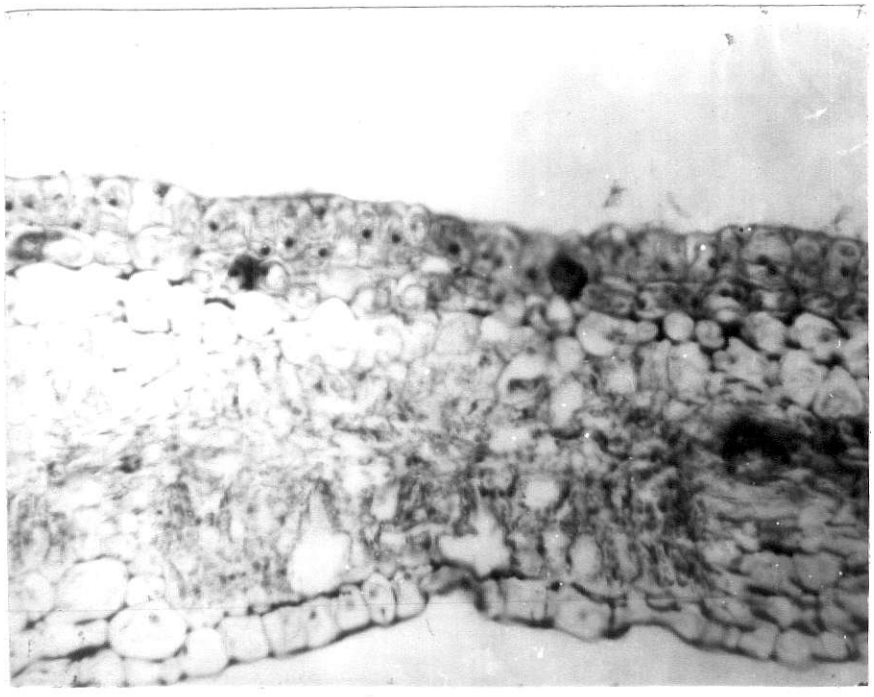

Fig. 2.

Fig. 3. Immature stage of infection in host tissue. 925X.

Fig. 4. Intermediate stage of infection with large hypertrophied palisade parenchyma cells of host tissue. 233X.

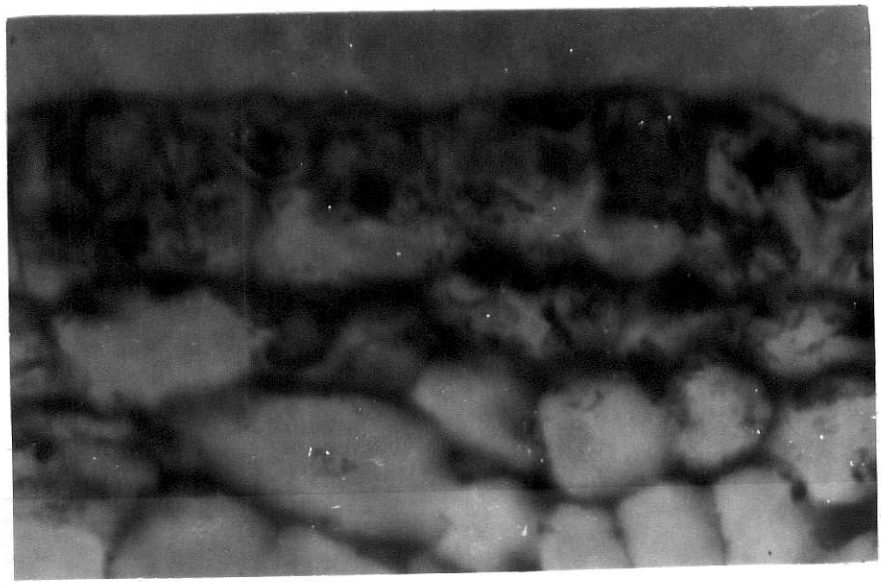

Fig. 3.

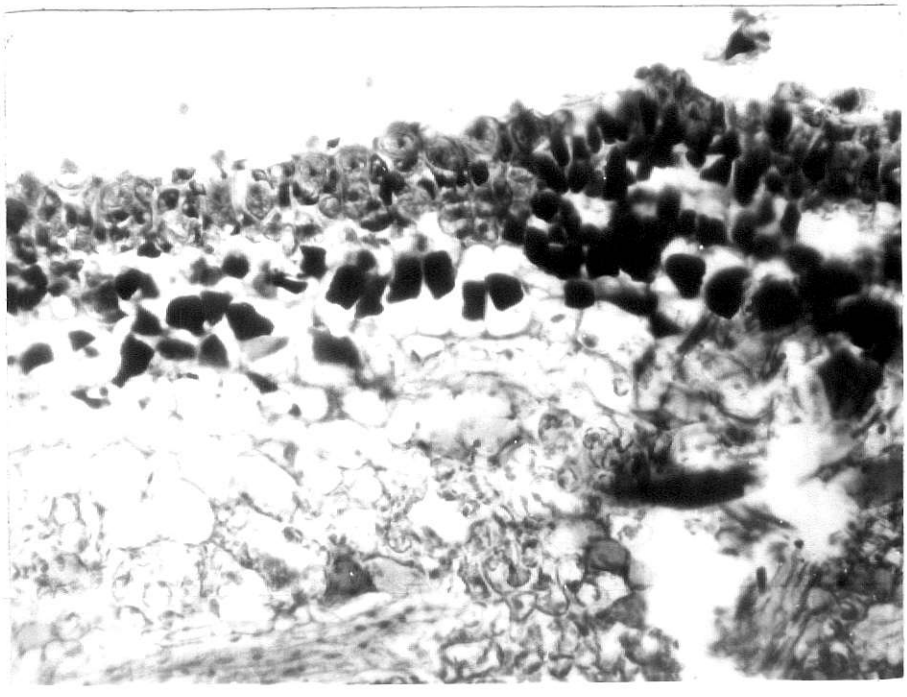

Fig. 4.

Fig. 5. Intermediate stage of infection with large hypertrophied palisade parenchyma cells of host tissue. 925X.

Fig. 6. Mature stage of the fungus showing hypertrophied, disorganized broken parenchyma cells of host tissue. 233X.

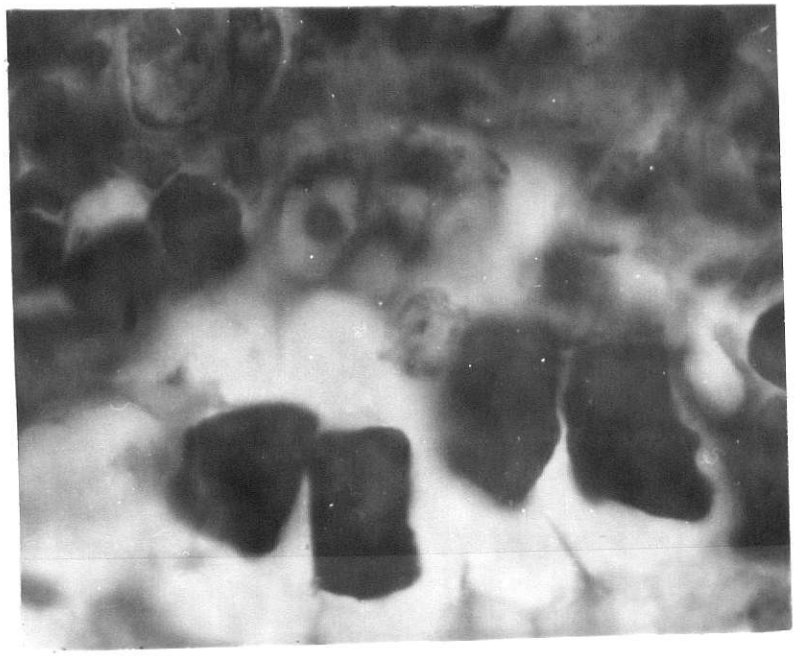

Fig. 5.

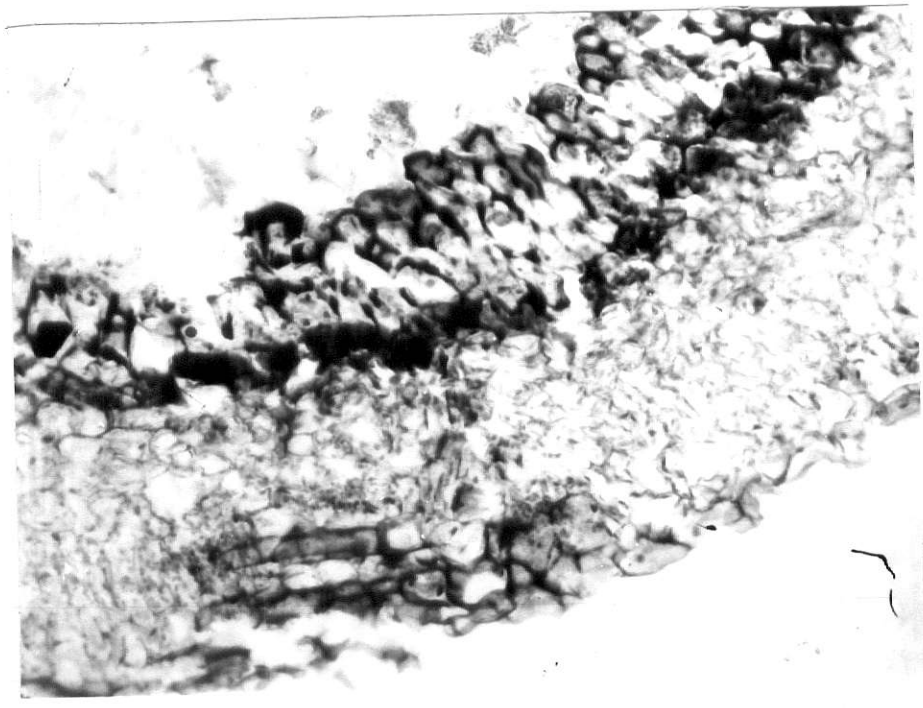

Fig. 6.

Fig. 7. Mature stage of the fungus showing hypertrophied, disorganized, broken parenchyma cells of host tissue. 925X.

Fig. 8. Hyphae of the young vegetative mycelium undergoing formation of ascogenous cells. 2100X.

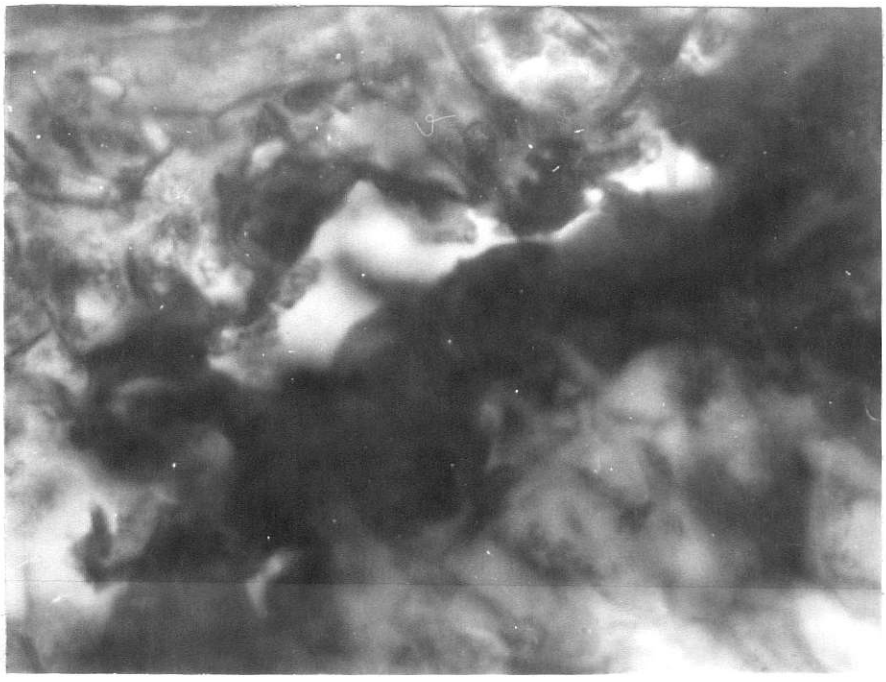

Fig. 7.

Fig. 8.

Fig. 9. Hyphae of the young vegetative mycelium undergoing formation of ascogenous cells. 2100X.

Fig. 10. Hyphae of the young vegetative mycelium undergoing formation of ascogenous cells. 2100X.

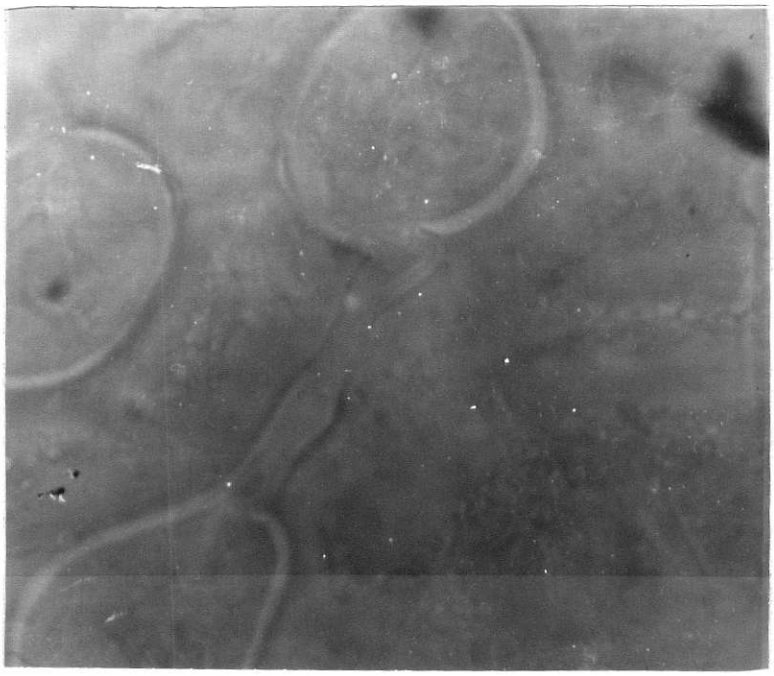

Fig. 9.

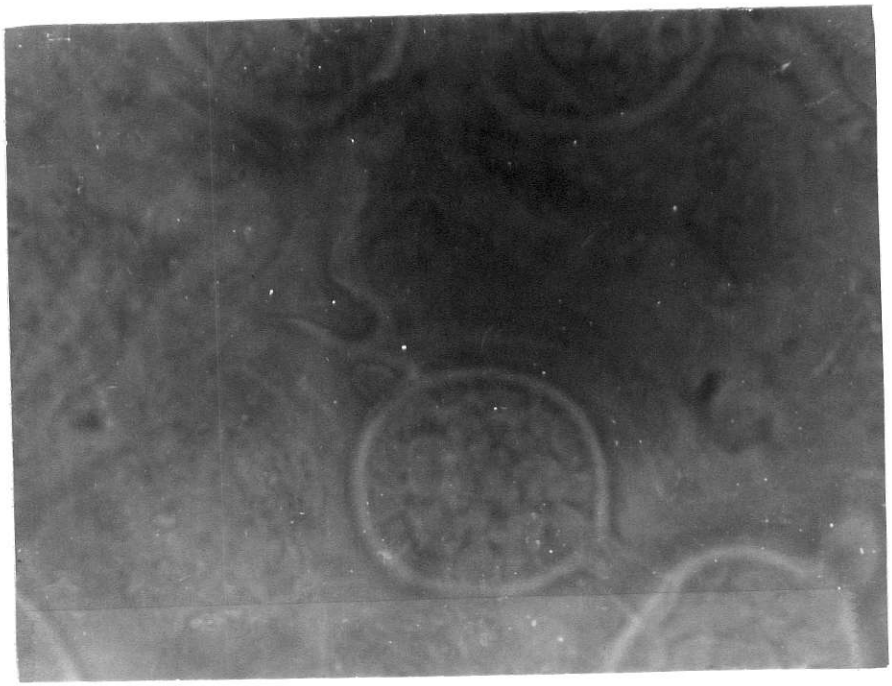

Fig. 10.

Fig. 11. Hyphae of the young vegetative mycelium undergoing formation of ascogenous cells. 2100X.

Fig. 12. Hyphae of the young vegetative mycelium undergoing formation of ascogenous cells. 2100X.

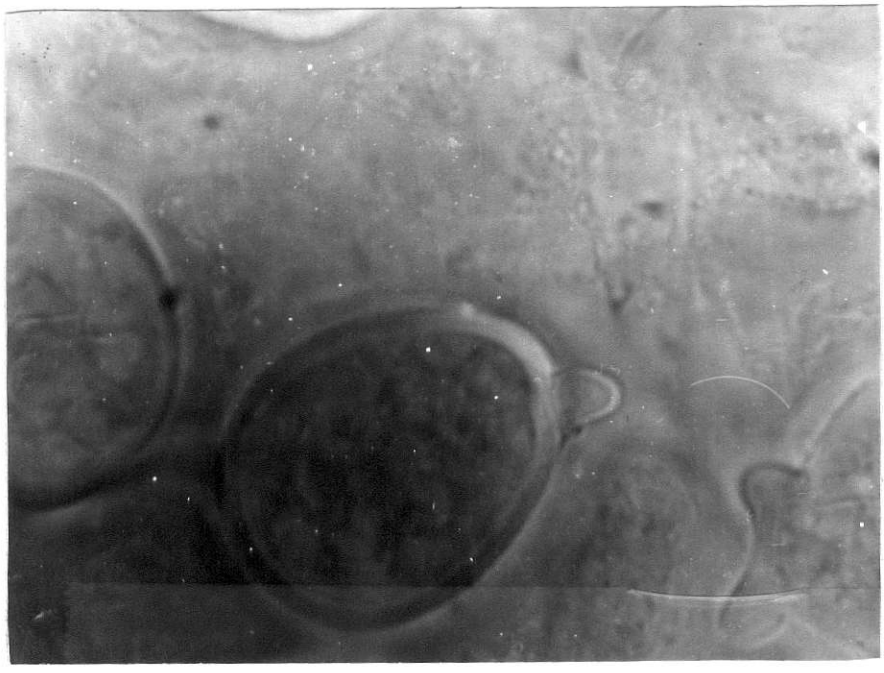

Fig. 11.

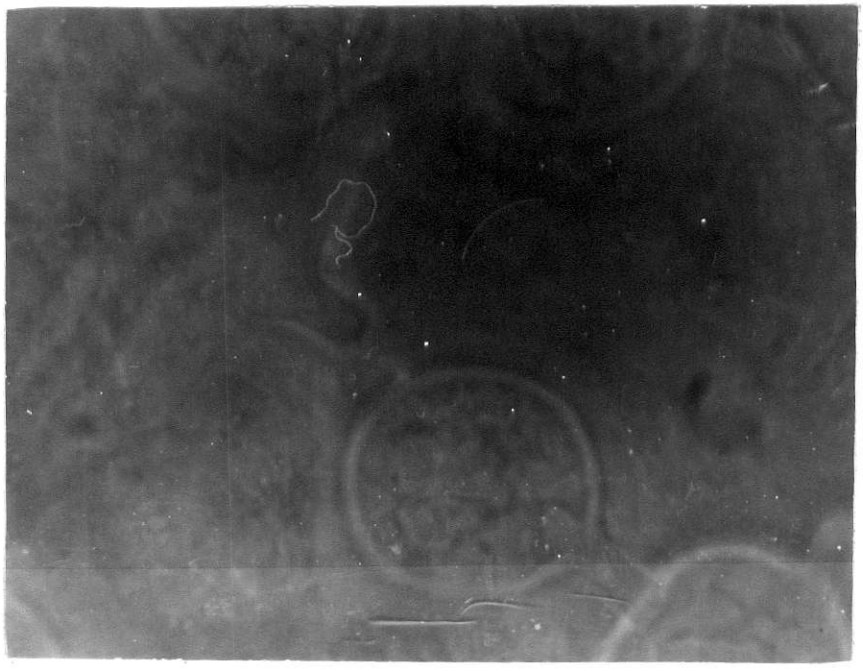

Fig. 12.

Fig. 13. Vegetative mycelium showing anastomosing branched network of hyphae. 2100X.

Fig. 14. Binucleate young ascogenous cell. 2100X.

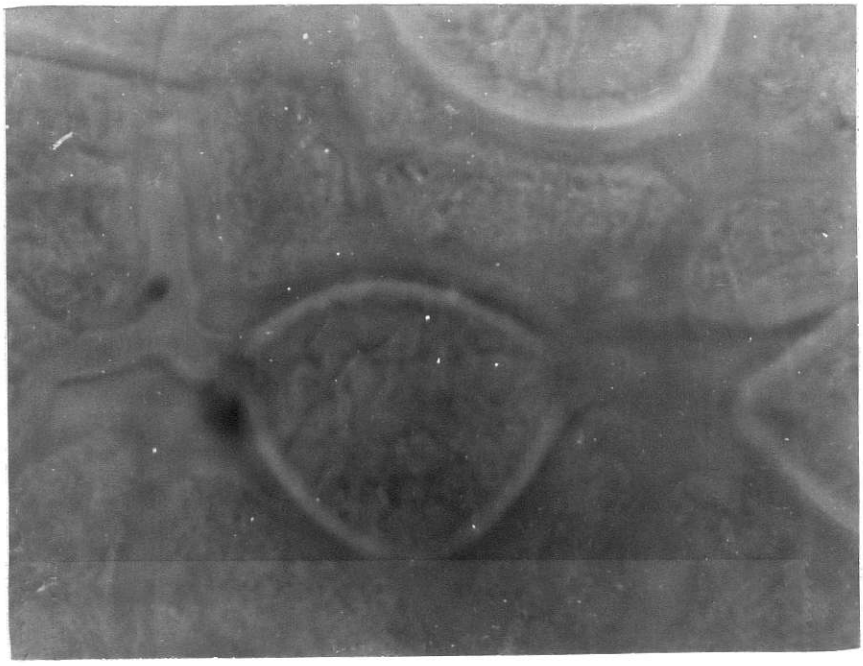

Fig. 13.

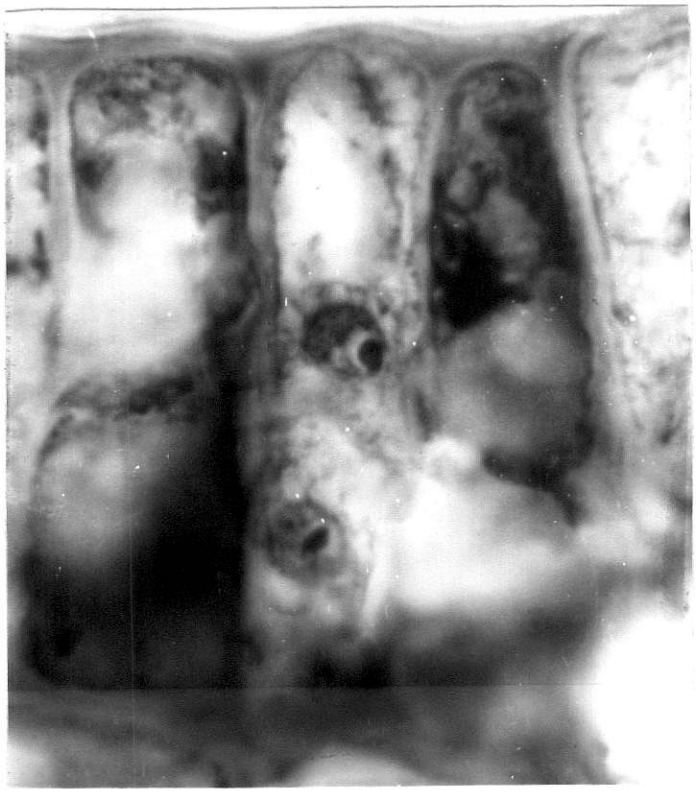

Fig. 14.

Fig. 15. Ascogenous cells with large fusion nucleus. 2100X.

Fig. 16. Ascogenous cell elongating into ascus, two membranes make up ascus cell wall. 2100X.

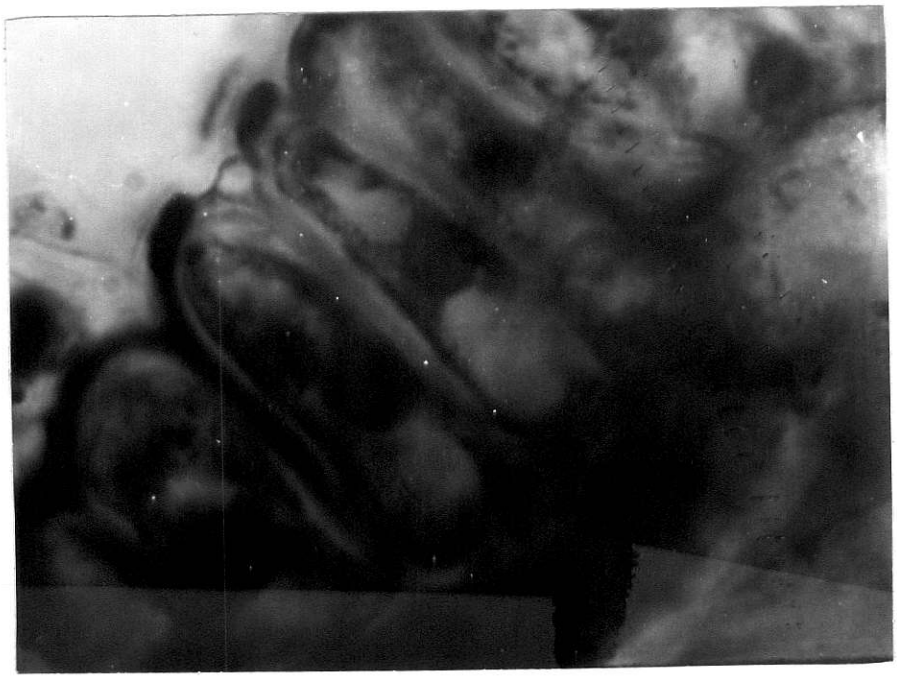

Fig. 15.

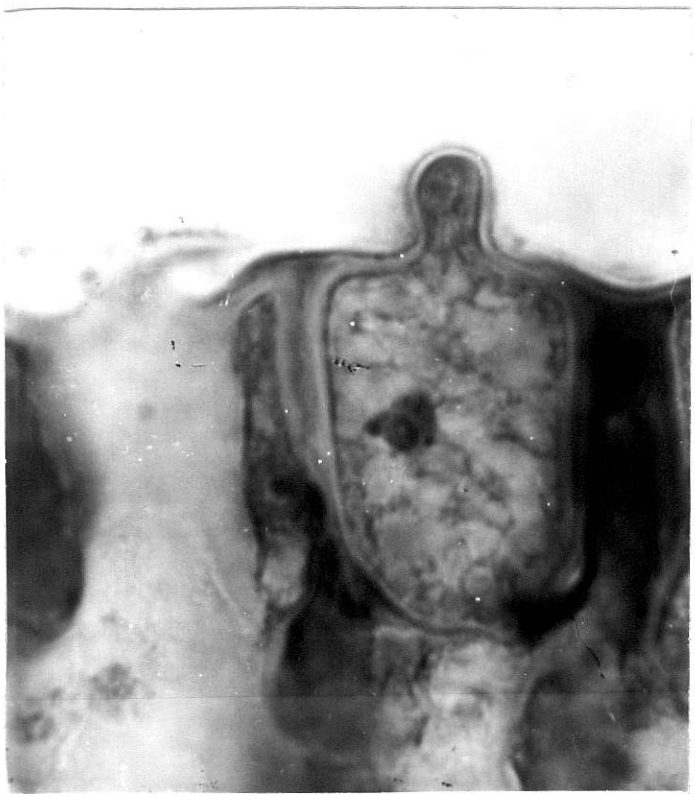

Fig. 16.

Fig. 17. Young, highly vacuolated, diploid ascus. 2100X.

Fig. 18. Vacuolated diploid ascus emphasizing no stalk cell. 2100X.

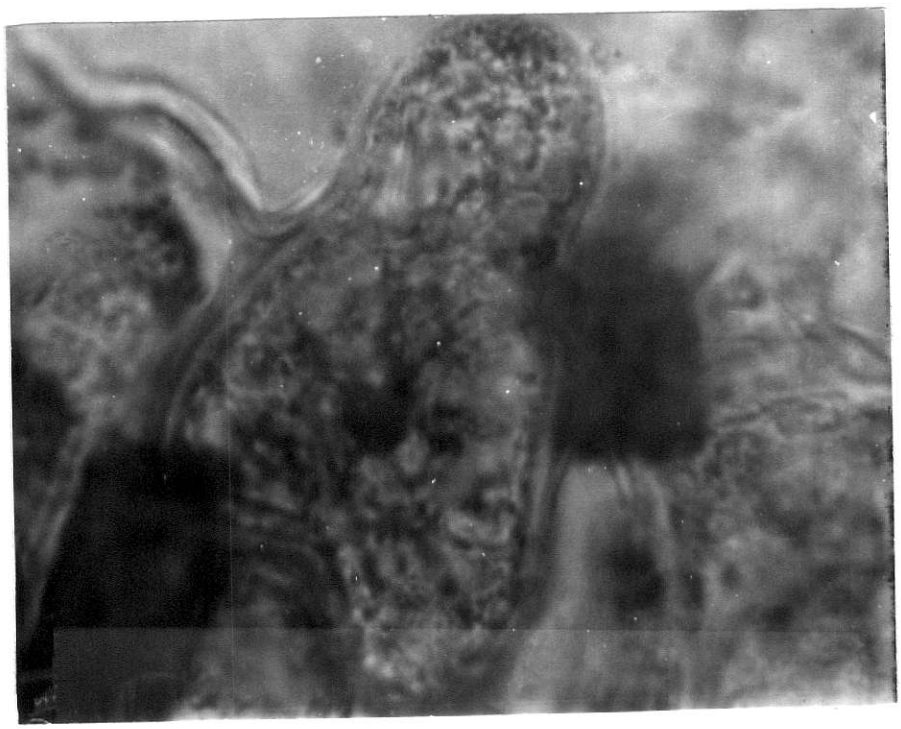

Fig. 17.

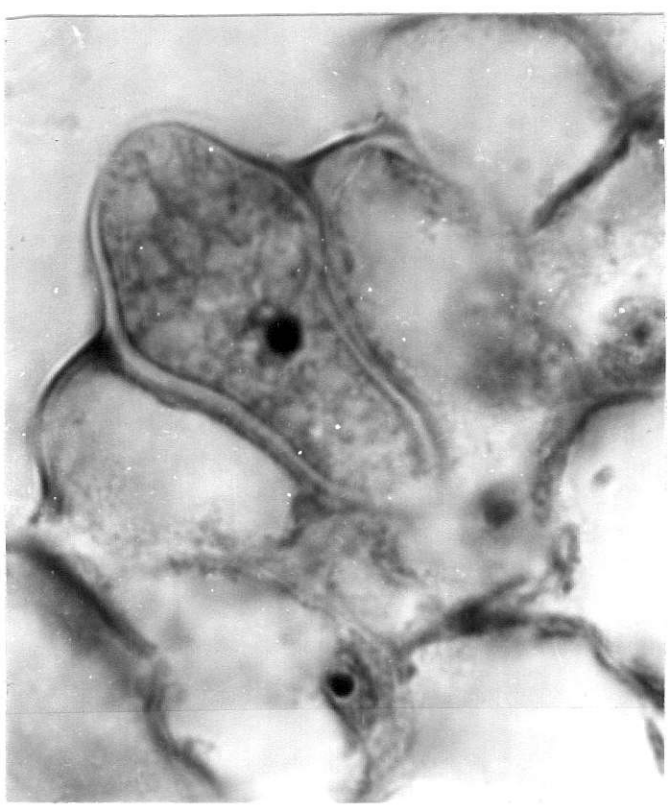

Fig. 18.

Fig. 19. Interpreted as prophase of meiotic division. 2100X.

Fig. 20. Eight nuclei in ascus prior to ascospore formation. 2100X.

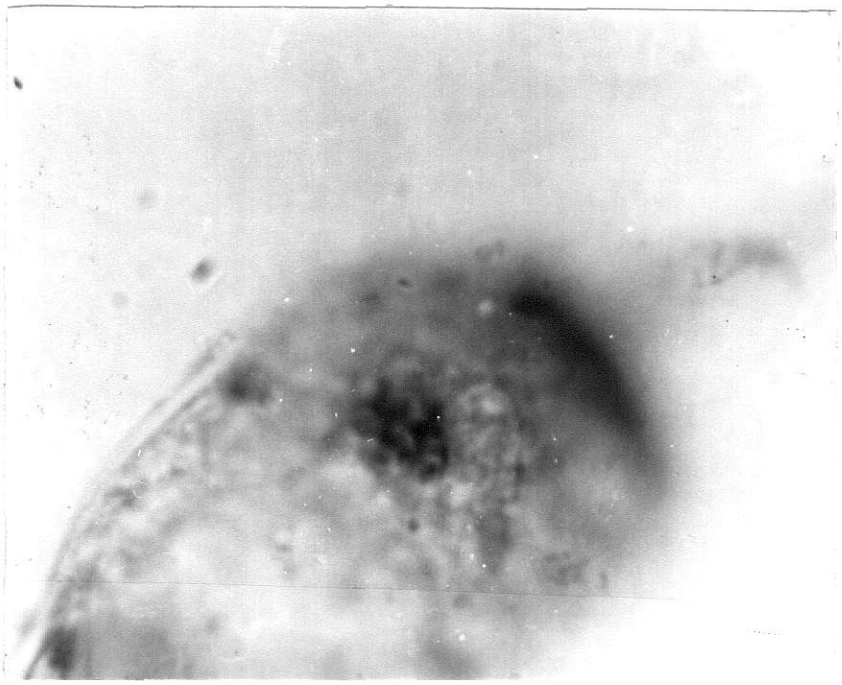

Fig. 19.

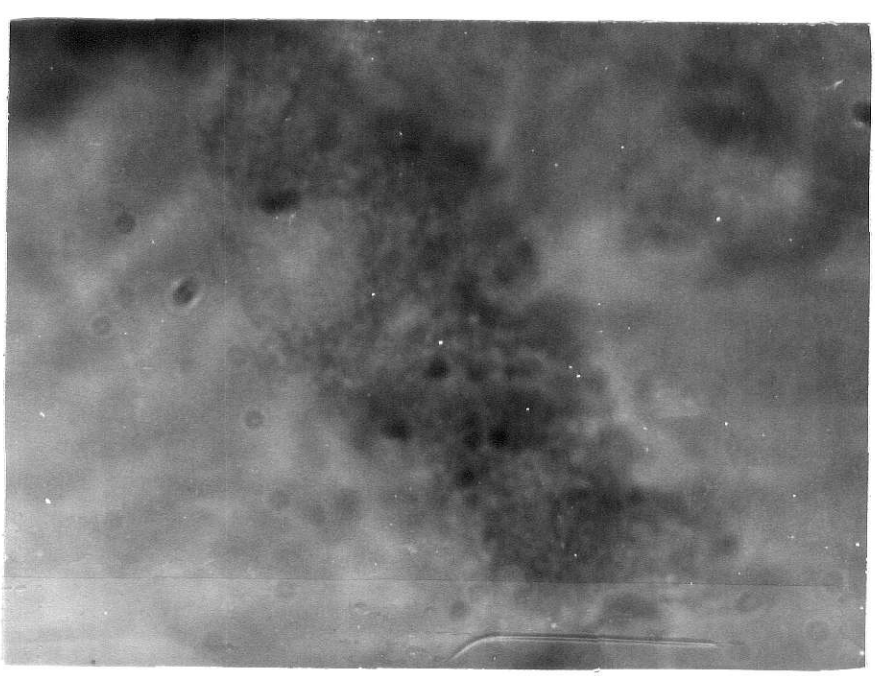

Fig. 20.

Fig. 21. Numerous nuclei in ascus prior to ascospore formation. 2100X.

Fig. 22. Ascus with spores forming at the tip first. 2100X.

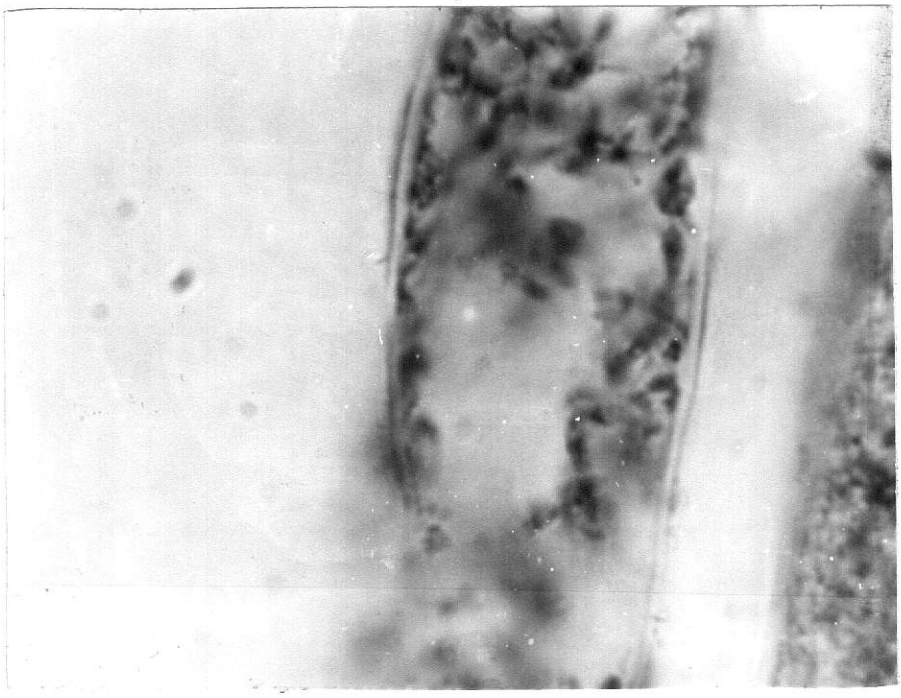

Fig. 21.

Fig. 22.

Fig. 23. Mature ascus filled with ascospores. 2100X.

Fig. 24. Old asci void of spores but containing large yellow globules. 2100X.

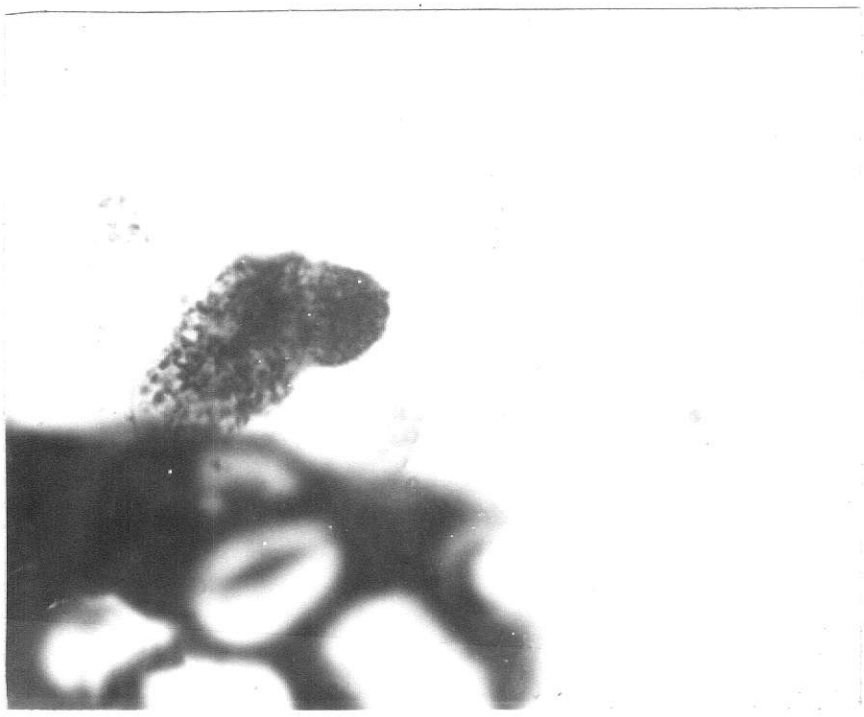

Fig. 23.

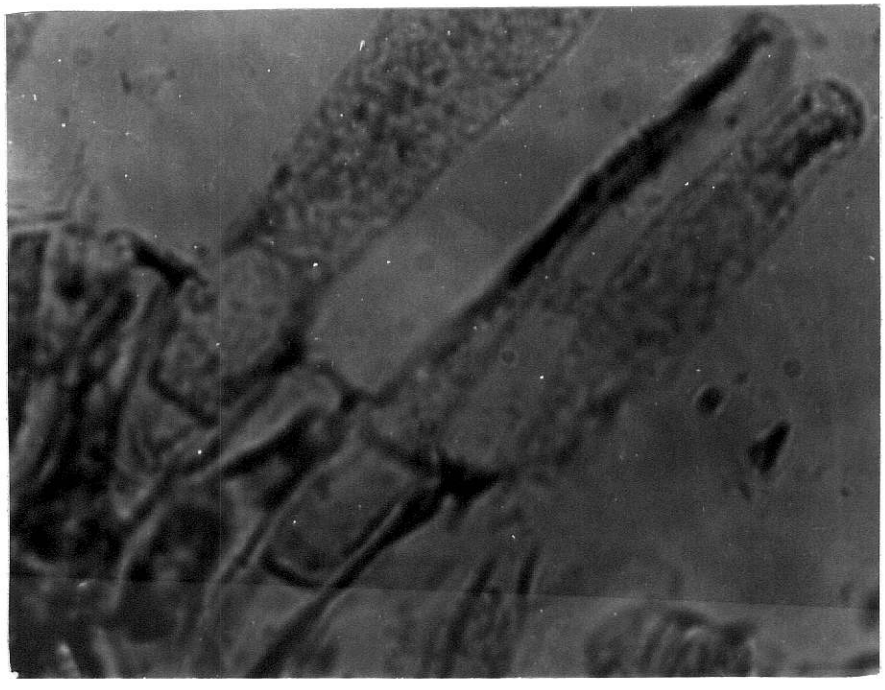

Fig. 24.

Fig. 25. Early division phase of first meiotic division showing four chromosomes. 2100X. Line drawing accompanies figure to give a more vivid description.

Fig. 26. Eight chromatids faintly evident in dividing nucleus. 2100X. Line drawing accompanies figure to give a more vivid description.

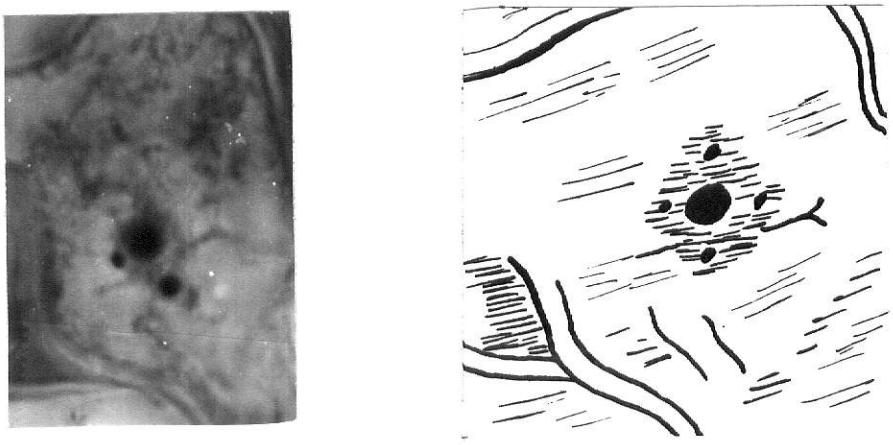

Fig. 25.

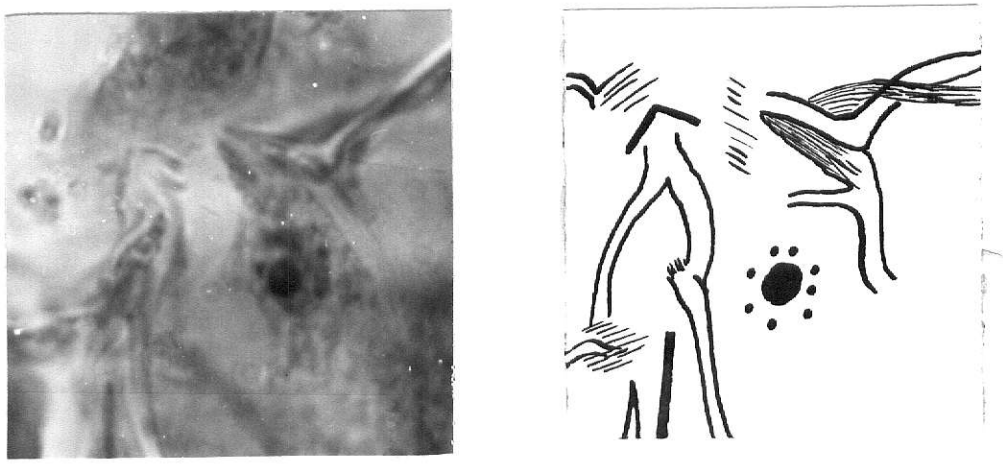

Fig. 26.

Taphrina caerulescens (Desm.) Tulasne

Formation of the Ascus. Ascus formation in T. caerulescens resembles that in T. populina in that the ascus emerges from an intercellular mycelium. The ascus containing both an inner and outer wall that appears to be slightly thicker than the ascus of T. populina (Fig. 27). At no time does the outer wall rupture but instead the entire ascus stretches and elongates to form the mature ascus. The cuticle of the host tissue ruptures as the ascus elongates and exerts pressure on the surface of the host tissue. The base of the ascus is slightly inserted in the host tissue (Fig. 28). No stalk cell is formed in this species.

Spore Formation. A mitotic division preceding meiosis does not occur in T. caerulescens. The fusion nucleus undergoes meiosis to form four haploid nuclei. These four nuclei undergo subsequent equational divisions to form between 16 and 32 nuclei. These nuclei are randomly distributed throughout the ascus (Fig. 29).

Early stages of spore formation produce a rather interesting occurrence in this species. The ascus protoplast seems to break up into long strands of protoplasm of various lengths and shape (Figs. 30-34). Nuclei are encompassed by these strands of protoplasm which divide into shorter segments with accompanying nuclear division (Figs. 35 and 36).

Spore formation does not appear to occur in a particular area of the ascus. In some cases spores are aggregated at the

tip (Fig. 36) and in other instances they are congregated at the base (Fig. 37). A rather interesting occurrence is evident in Figure 37. The lower two-thirds of the ascus is filled with spores but in the upper third a long strand of protoplasm is evident. A concise answer for this occurrence is not available, but it appears that spore formation is still occurring in some areas of the ascus while it is complete in other areas. Completion of spore formation results in an ascus filled with spores (Figs. 38 and 39).

Taphrina caerulescens (Desm.) Tulasne

Figs. 27-39.

Fig. 27. Developing ascus with thick double membrane. 2100X.

Fig. 28. Mature ascus filled with ascospores, revealing no stalk cell and slightly inserted in host tissue. 2100X.

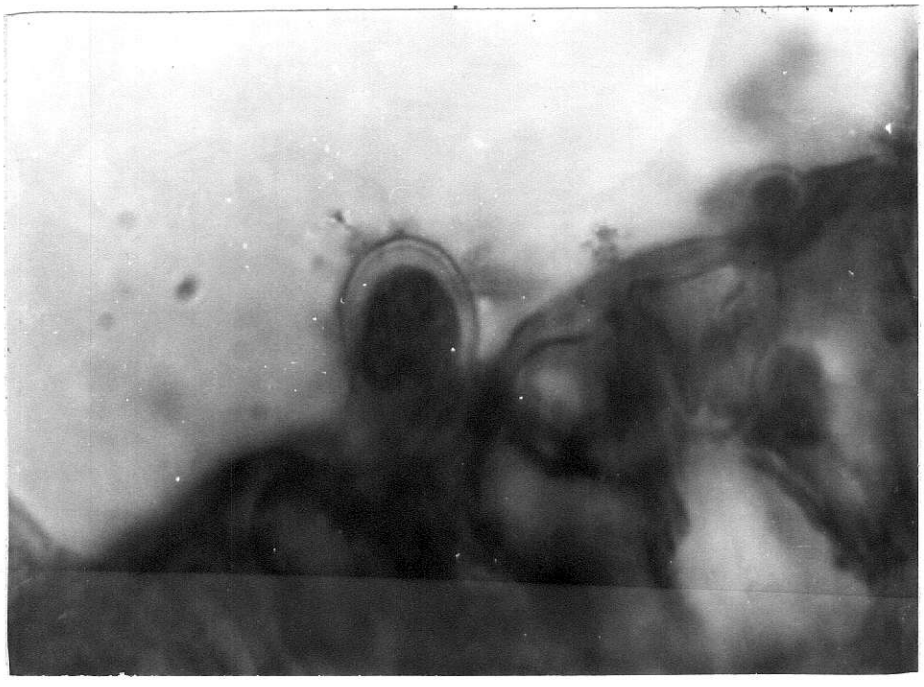

Fig. 27.

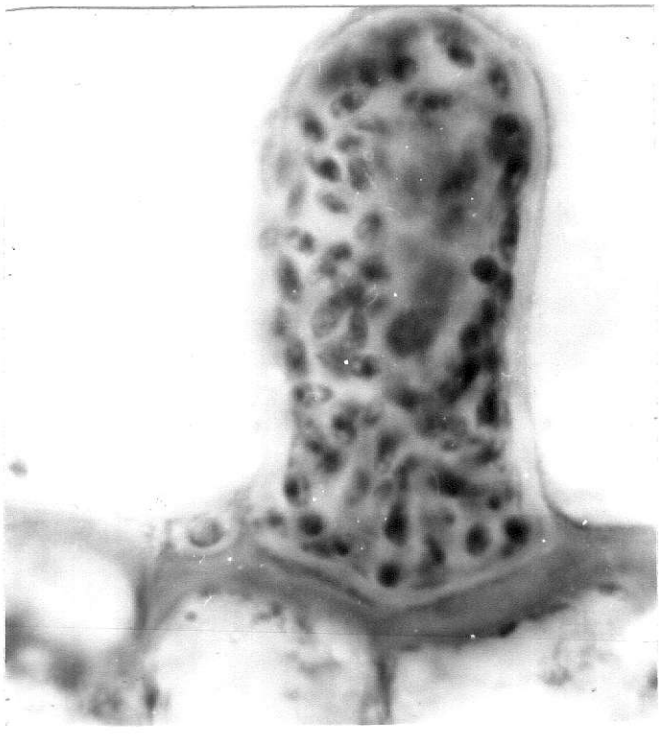

Fig. 28.

Fig. 29. Numerous nuclei that are to become spore nuclei. 2100X.

Fig. 30. Very early stages of spore formation — long segments in the ascus protoplast. 2100X.

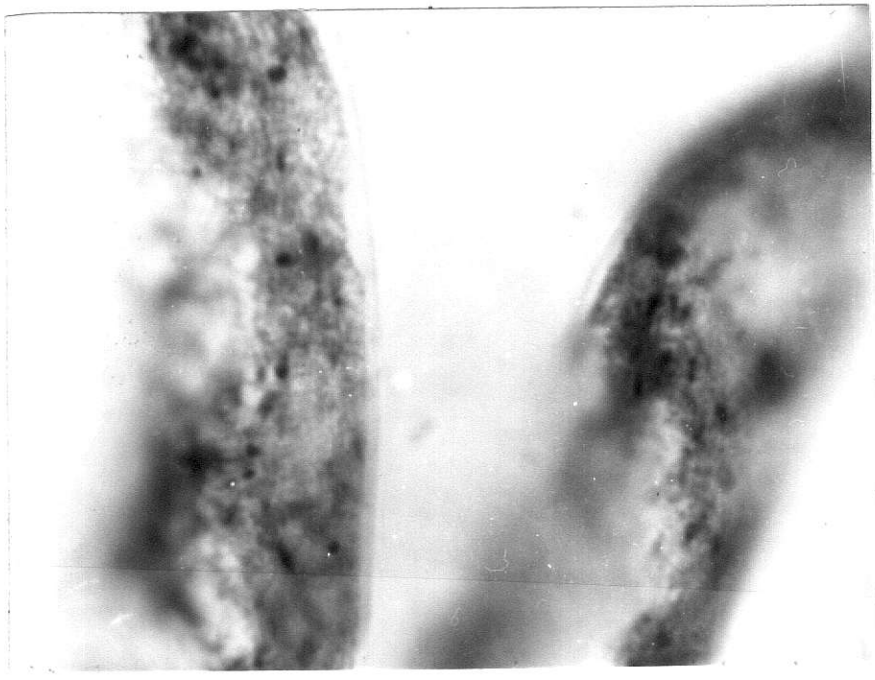

Fig. 29.

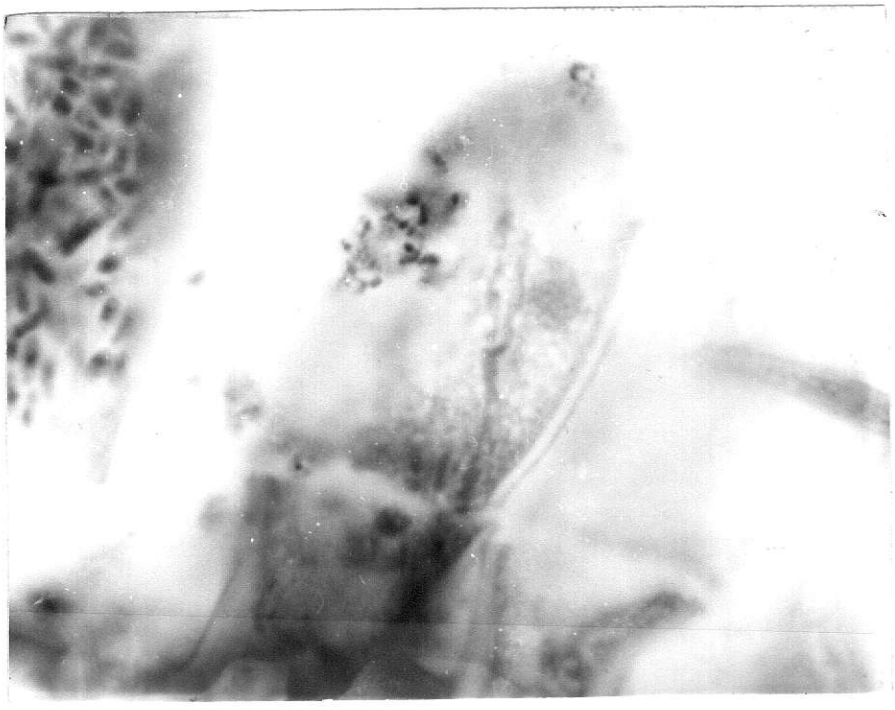

Fig. 30.

Fig. 31. Very early stages of spore formation — long segments in the ascus protoplast. 2100X.

Fig. 32. Very early stages of spore formation — long segments in the ascus protoplast. 2100X.

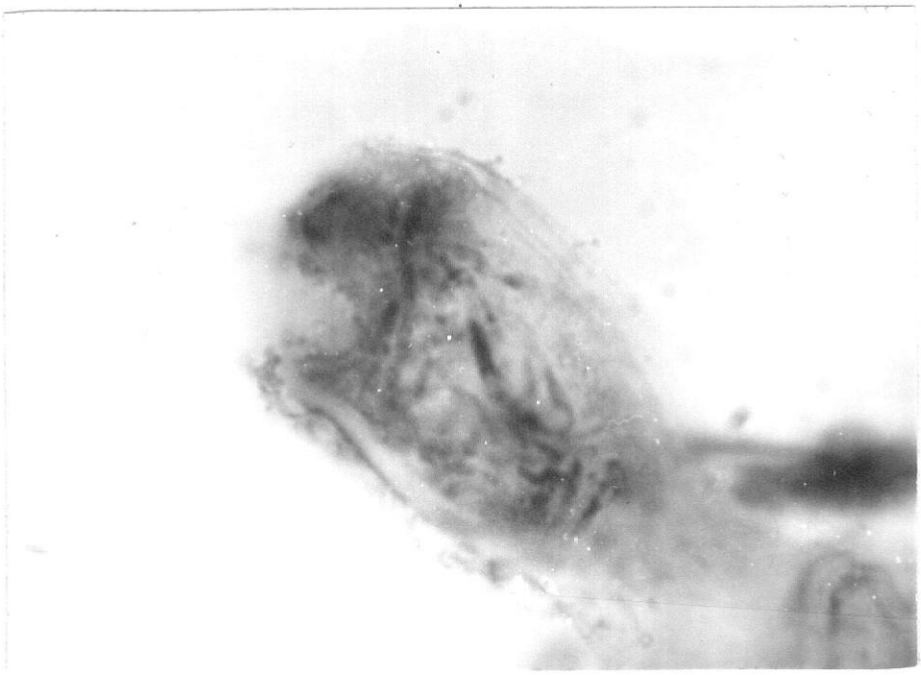

Fig. 31.

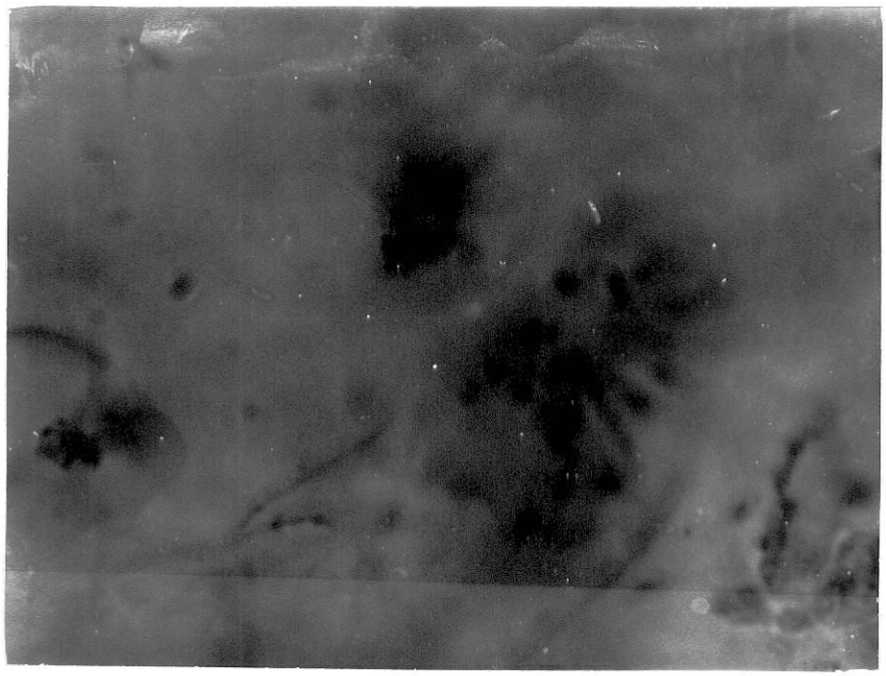

Fig. 32.

Fig. 33. Very early stages of spore formation — long segments in the ascus protoplast. 2100X.

Fig. 34. Very early stages of spore formation — long segments in the ascus protoplast. 2100X.

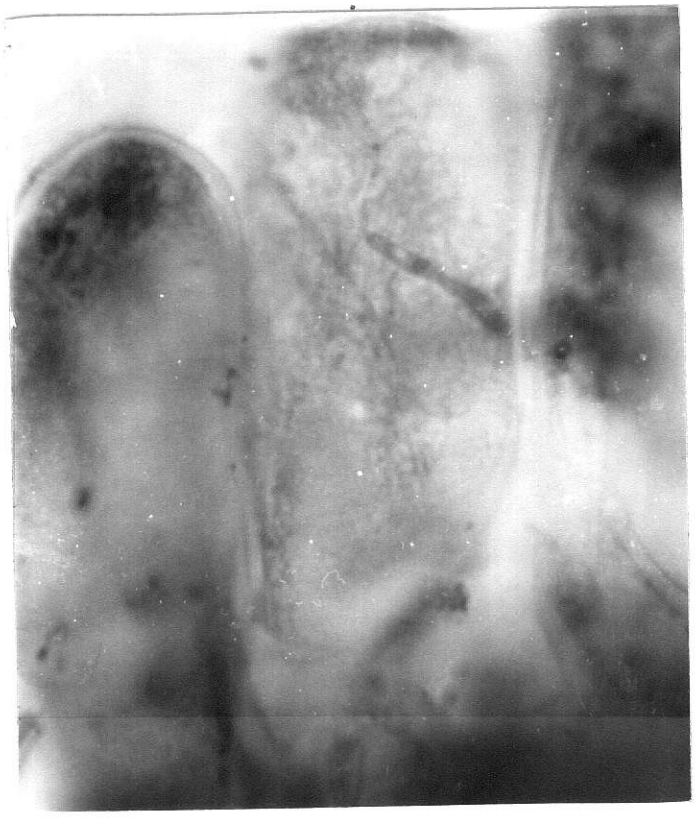

Fig. 33.

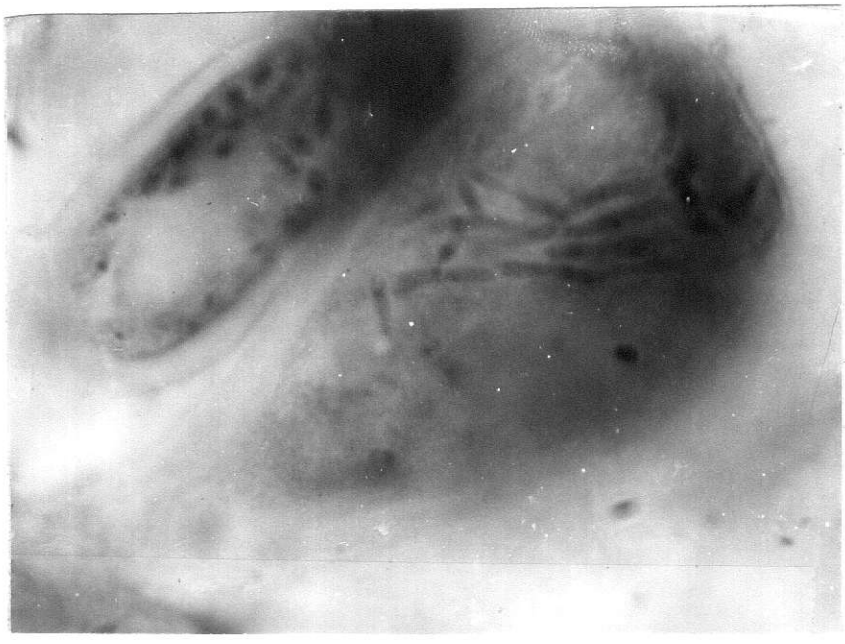

Fig. 34.

Fig. 35. Very early stages of spore formation — long segments in the ascus protoplast. 2100X.

Fig. 36. Very early stages of spore formation — long segments in the ascus protoplast. 2100X.

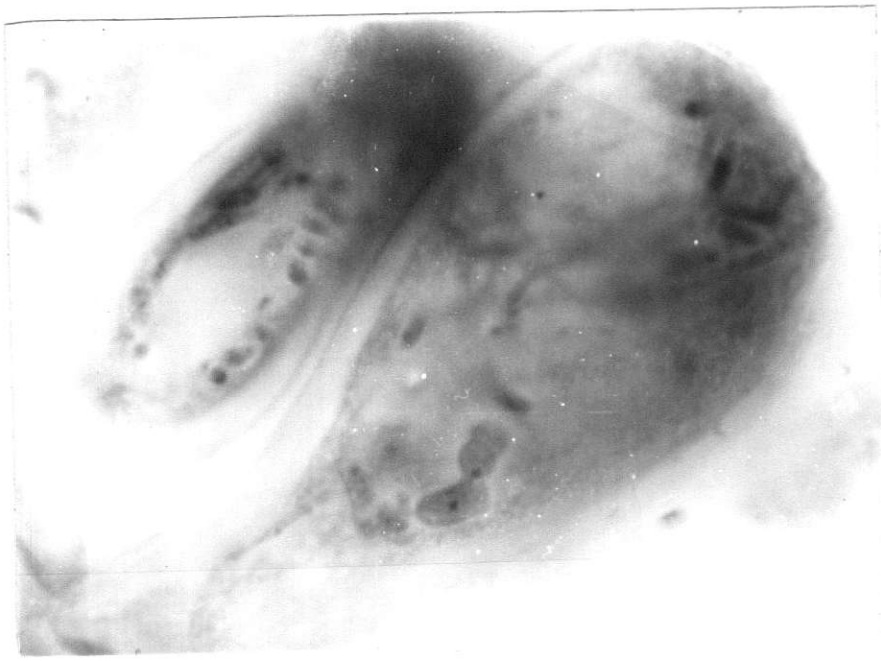

Fig. 35.

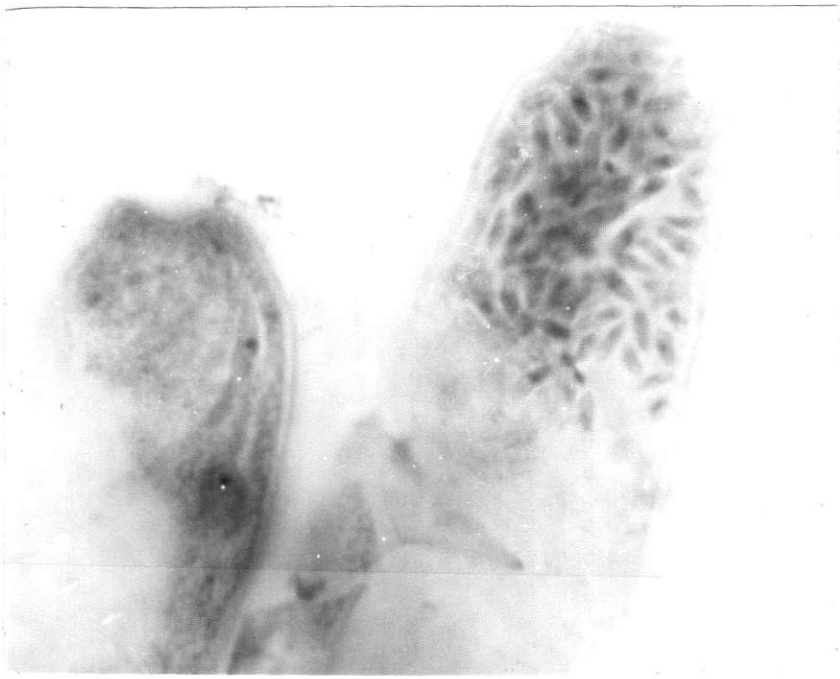

Fig. 36.

Fig. 37. Long strands of protoplasm evident in older asci that have spores. 2100X.

Fig. 38. Mature asci filled with spores. 925X.

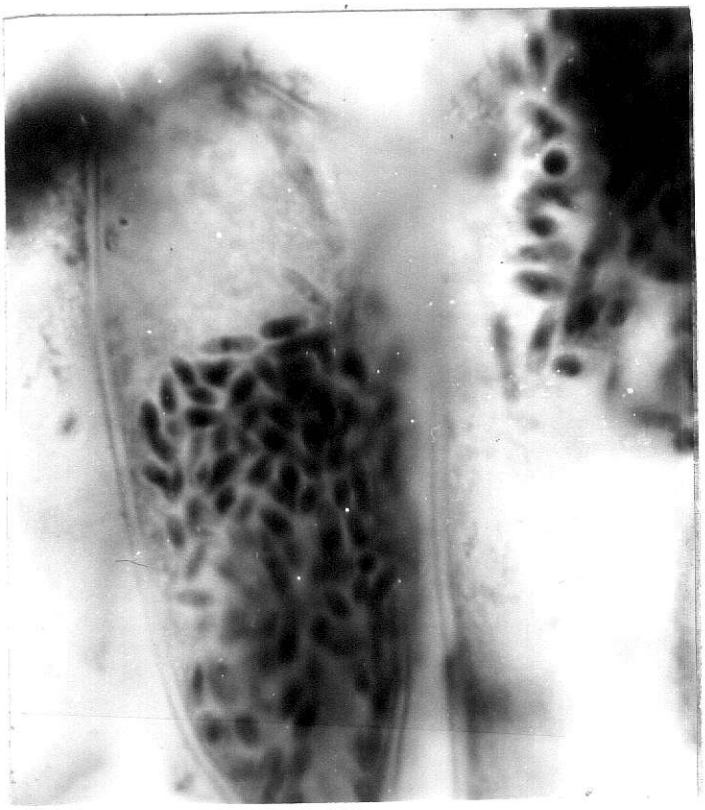

Fig. 37.

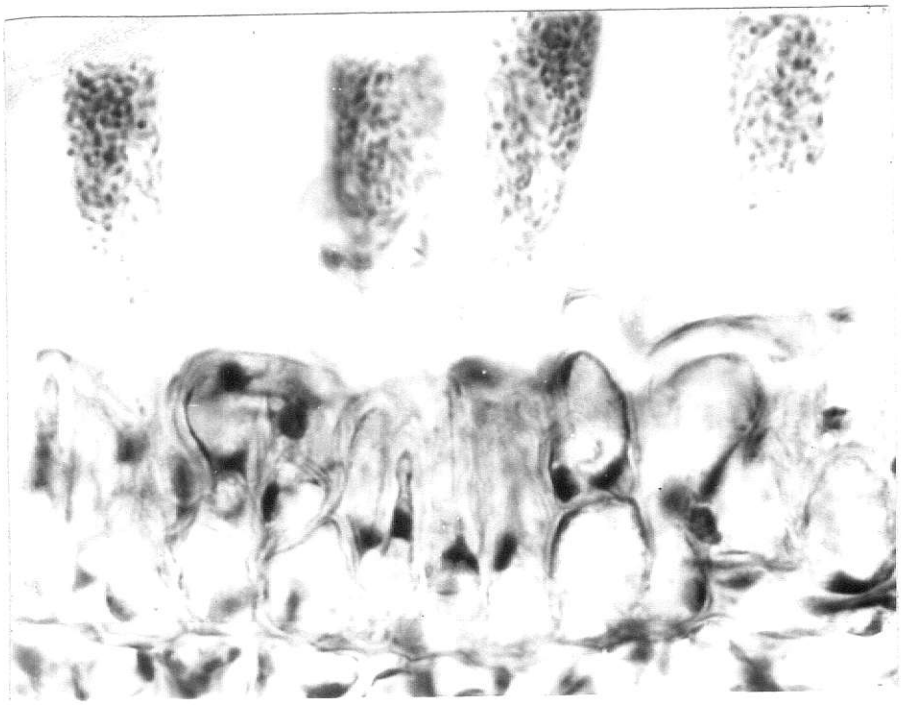

Fig. 38.

Fig. 39. Mature asci filled with spores. 925X.

Fig. 39.

DISCUSSION

Most of the comprehensive studies of the genus Taphrina have been taxonomic in nature based on comparative morphology and host relationships. Characteristics that were considered important were size and shape of the ascus, presence or absence of a stalk cell, type of mycelial development, and to a lesser extent geographical location and symptoms that were caused by the organism on its host. As modern methods progressed, cytological aspects were given some consideration.

Because of the emphasis placed on limited sets of characteristics, a need was felt for a complete study of the developmental morphology and cytology of these organisms in order to clarify a number of little known or disputed theories. Kramer (1961) did extensive work on five species of Taphrina with the above point his primary concern. Only by the completion and correlation of such studies on all members of this genus, will it be possible to obtain an understanding of the intrageneric relationships of these organisms and thereby establish a sounder basis on which to found a species concept.

Development of the Vegetative Mycelium. Within the genus, three distinct levels of mycelial development have been reached. These are the intercellular, subcuticular, and "wall-habit mycelium". However, there are certain species which have a combination of the intercellular and subcuticular types of mycelial habit such as T. deformans. In T. deformans, the subcuticular

mycelium is formed from the intercellular mycelium. In this type of development, the vegetative mycelium could become entirely subcuticular simply by the loss of the intercellular phase. This theory is based entirely on the chronological sequence that exists in T. deformans. For this reason a progression in the opposite direction seems less likely to have occurred.

When the genus as a whole is considered, there is no evidence of a general trend of mycelial habit throughout its species because of the distinct levels and sub-levels of mycelial development evident in the genus. Thus, if there are transitions they would appear to be confined within the host groups. For example, this would entail the species which occur on closely related members of the Aceraceae, Fagaceae, Salicaceae, etc.

Development of the Stalk Cell. Although the majority of species in the genus Taphrina possess stalk cells, the two species studied in this paper lack them. Taphrina populina is one species that reportedly may or may not possess stalk cells (Mix, 1949), however, asci with stalk cells were not seen in any of the observations reported in this paper. It does seem likely that there may be some species that may or may not have them, indicating a transition from one form to the other. However, it is felt that Mix (1949) who reported asci of both types, had mistaken the bases of adjoining asci to be stalk cells.

Development of the Ascus from the Ascogenous Cell. Asci which lack stalk cells are usually formed by the stretching of

the ascogenous cell. There does not appear to be any correlation between the method of ascus development and the type of mycelial habit. However, data are not complete enough with regard to these characteristics to make any definite comparisons with respect to species of Taphrina that lack stalk cells.

Cytology in Taphrina. Certain aspects of the cytology of this genus have been controversial for some time. The major dispute has centered around the division of the fusion nucleus in the young ascus.

Many workers, including Juel (1921), Eftimiu (1927), and Mix (1935) believed that the first division of the fusion nucleus was the first of two meiotic divisions. Under this assumption, they also believed that the stalk cell when present was cut off without contents. However, Kramer (1961) reported in species with stalk cells, a mitotic division of the fusion nucleus preceding the meiotic divisions. This equational division is an important feature, since it is actually a vegetative diploid cell. The occurrence of this stage in fungi is rare.

Several similarities occur in this study of T. populina and T. caerulescens which correspond to the study by Kramer (1961) of T. virginica. Cytoplasm in the young ascogenous cells of all three species is very dense, but becomes somewhat vacuolated as the ascogenous cell wall begins to elongate. The extension of the ascogenous cell wall to form the ascus is identical in the species mentioned above. All three species lack a stalk cell, and their fusion nucleus undergoes immediate meiosis, thus, there

is no mitotic division preceding meiosis. Successive mitotic divisions follow meiosis to produce numerous spore nuclei that are destined to become ascospores in T. populina and T. caerulescens, however, only one mitotic division follows meiosis in T. virginica. Budding of the ascospores brings about a multispored ascus in T. virginica.

A question arises at this point which needs further investigation. In the taxon, T. populina, asci reportedly may or may not have stalk cells (Mix, 1949). If this in fact is true, it would be extremely desirable to know whether a mitotic division precedes meiosis in those examples of T. populina which possess stalk cells as in the case of T. deformans or if the stalk cell is cut off empty with no prior mitotic division of the fusion nucleus. Asci with both types were not found in any of the material studied at the time.

Spore Formation. Most species of this genus normally have eight ascospores. In contrast to this, a few species have many ascospores per ascus. Previous to the work of Kramer (1961) with T. populi-salicis, it was believed that those species with more than eight spores acquired them by budding of the ascospores to produce secondary spores, blastospores.

The author found ascospores in T. populina to be developed in the same manner as reported by Kramer (1961) for T. populi-salicis. Instead of eight nuclei being formed, there is a prolonged series of mitotic divisions to produce a large number of nuclei that eventually enter into spore formation. Taphrina

caerulescens forms numerous nuclei by repeated mitotic divisions but the final number of nuclei is less than in T. populina, indicating less divisions.

In T. populina, spore formation usually begins at the apex of the ascus and progresses downward filling the entire ascus with ascospores. Taphrina caerulescens appears to have no certain area in the ascus where spore formation begins. Other species in which spore formation begins anywhere in the ascus are T. deformans, T. carveri, and T. ulmi.

In nature the ascospores of Taphrina species bud readily, either within the ascus or after spore expulsion. There is reason to believe (Fitzpatrick, 1934; Mix, 1935) that propogation by budding in the "yeast stage" may continue indefinitely, the fungi surviving in this fashion on various plant surfaces and probably also in the soil. In other words, species of Taphrina are yeasts in the broad sense during their asexual cycle. In fact, if a species of Taphrina were brought into culture without knowledge of its previous parasitic existence, it would probably be refered to the genus, Torulopsis. In most cases, presumably because host organs are susceptible only when young and tender, a period of oversummering and overwintering is undergone by the blastospore before infection. The occurrence of the "yeast stage" seems to be important in accomplishing survival during this prolonged period apart from the host tissues. For this reason, a complete study of the saprophytic phase of all species in the genus is needed as a criterium for classification.

Taphrina organisms are closely specialized to their hosts. Henderson (1954) asserted "that there seemed to be little justification for the view that two morphologically similar fungi on different but closely related hosts should be considered distinct species — they should be specialized forms at most".

In order to prove Henderson's idea right or wrong, the genus must be studied from more than a morphological and cytological approach. Features for classification in the future must include seriological, nutritional, biochemical and cross inoculation reactions. When a thorough study of each species of this genus by the above methods is complete, the author feels that the number of valid species in the genus, Taphrina, will be further reduced.

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THE CYTOLOGY AND MORPHOLOGICAL DEVELOPMENT OF *TAPHRINA POPULINA* FRIES AND *TAPHRINA CAERULESCENS* (DESM.) TULASNE

by

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The objectives of this study were threefold in nature. The first was to describe the morphological development of Taphrina populina Fries with respect to its intercellular vegetative mycelium and the development of the ascus from the tips of these intercellular hyphae. The second objective was an attempt to clarify the nuclear behavior of T. populina and T. caerulescens (Desm.) Tulasne with respect to the parasitic phase of their life cycle. The third objective was an attempt to corrulate all the characteristics of these species with the intention of obtaining a better understanding of the trends and relationships that may exist in the genus.

Taphrina populina and T. caerulescens are similar in that they possess intercellular vegetative mycelium with the subsequent development of asci from the tips of the intercellular hyphae. Both organisms are parasitic on vascular plants. Taphrina populina is parasitic on the genus Populus and T. caerulescens is parasitic on the genus Quercus. Both species obtain their asci by the stretching and elongation of the entire ascogenous cell. The ascogenous cell is at first dikaryotic but later undergoes karyogamy to produce a diploid cell. Since both species lack a stalk cell, the diploid ascogenous cell may be referred to as a young ascus.

Similarities are also evident in the nuclear behavior of these organisms with respect to the parasitic phase of their life cycle. There is no mitotic division preceding meiosis in those species that lack stalk cells, thus the diploid fusion nucleus

directly undergoes meiosis to produce four haploid ascospore nuclei. These nuclei in turn each undergo a mitotic division to produce the eight original ascospore nuclei. At this point dissimilarities become evident in each organisms life cycle.

In Taphrina populina, the eight ascospore nuclei undergo successive mitotic divisions to produce from 32-128 spore nuclei. These nuclei undergo spore formation by the encompassing of cytoplasm around the nucleus and the delimitation of vacuolar membranes to produce the mature ascospores that fill the ascus. In the diploid fusion nucleus, the chromosomal number is four.

The eight original spore nuclei of T. caerulescens undergo one or two successive mitotic divisions to produce from 16-32 spore nuclei. In spore formation, long protoplasmic strands are evident. The strands break up into shorter segments. Each of these segments contain a spore nucleus. The final stage of spore formation involves the rounding up of the short segments into the mature spores.