A COMPARATIVE MORPHOLOGICAL AND CYTOLOGICAL STUDY OF GELASINOSPORA CALOSPORA (MOUTON) MOREAU ET MOREAU, GELASINOSPORA AUTOSTEIRA ALEXOPOULOS AND SUN, AND GELASINOSPORA CEREALIS DOWDING

By

Sung Huang Sun

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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AN ABSTRACT

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The genus Gelasinospora was erected by E. S. Dowding as a genus of Pyrenomycetes with pitted spores. G. calospora was described by Claude and Mme. Moreau as an octosporous, homothallic species. G. autosteira was described by Alexopoulos and Sun as an octosporous but heterothallic species. The two species are very similar morphologically. A comparative study of G. calospora and G. autosteira was undertaken with a view of shedding some light on the relationship of these species from ontogenetic and cytological evidence. The study also included G. cerealis as a contrasting species which is regarded by everyone as distinct.

A heat treatment was found to be necessary to induce the germination of the ascospores of all three species. Spores of G. autosteira germinate with the formation of a vesicle on one end of the spore. The majority of the ascospores of G. calospora germinate with the formation of two vesicles one on each end of the spore, but about 18 percent germinate only from one end. In G. cerealis all the spores examined germinated from both ends.

In all species perithecial initials appear as coiled lateral branches of vegetative hyphae. In G. calospora the ascogonial coil may develop independently of any copulatory organ or a branch originating from the mother hypha or from the basal cell of the coil may serve as a copulatory organ. In G. autosteira the ascogonial coil needs the stimulus from the hypha of the opposite compatible strain. In G. cerealis a trichogyne-like structure

fuses with a vegetative hypha.

The formation of croziers is similar to that described for many of the Ascomycetes. Fusion of the nuclei takes place in the penultimate cell of the crozier. The young asci are formed by the elongation of the penultimate cells of the croziers. After three successive nuclear divisions, eight nuclei are formed in each ascus. There is another mitotic division in the young ascospore which renders the mature ascospore bi-nucleate. The above discussion is true for all three species of Gelasinospora studied.

The haploid number of chromosomes in G. calospora and G. autosteira appears to be six and that of G. cerealis to be seven.

A rod-like structure was observed in some preparations of all three species of Gelasinospora. The nature and the function of the rod have not been determined.

There are no major morphological and cytological differences between G. calospora and G. autosteira. It is proposed to reduce G. autosteira to a variety of G. calospora under the name: Gelasinospora calospora (Mouton) Moreau et Moreau var. autosteira (Alex. & Sun) n. var.

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CHAPTER I

INTRODUCTION

The genus Gelasinospora was erected by Miss E. S. Dowding (43) as a genus of Pyrenomycetes with pitted spores. It is closely related to two other genera, Fimetaria and Neurospora in having perithecia which are superficial, short-beaked, dark colored, membranaceous, and pyriform, and in that its asci have a crown-like structure at the tip, and a circular pore. The three genera differ in that the spores of Fimetaria are smooth and possess a mucilaginous sheath, those of Neurospora have longitudinal nerve-like markings on the wall, and those of Gelasinospora have pitted walls.

Up to now there are six species of Gelasinospora described:

Gelasinospora tetrasperma was described by Miss Dowding (43) in 1933 as a species with four-spored asci. The normal spore contains four nuclei and produces homothallic mycelium. Dwarf spores containing two nuclei are occasionally produced. The mycelia from dwarf spores are of two compatible groups. Giant spores with six nuclei produce homothallic mycelia. The type material was obtained from ptarmigan droppings in Manitoba about 30 miles north of Fort Churchill on Hudson Bay. A culture labelled Sordaria fimicola bearing

four-spored asci, obtained from Miss Page (84) in England was considered by Dowding to be Gelasinospora tetrasperma.

At the same time Dowding described Gelasinospora cerealis. It has eight-spored asci. Each spore is binucleate, and produces homothallic mycelium. This species has been isolated from diseased crown of oats at Souris, Manitoba, and also diseased crown of Durum wheat at Deloraine.

Claude and Mme. Moreau (71) in 1949 studied a species of Gelasinospora isolated from soil collected at a depth of 40 cm. in palm fields at Akunda, near Etoumbi, Belgian Congo. They found the morphology of this fungus to be similar to that of a specimen collected at Beaufay, Belgium and described in 1897 by Mouton (78) as Rosellinia calospora. The Moreaus changed the name of that fungus to Gelasinospora calospora (Mouton) Moreau and Moreau. Gelasinospora calospora is an octosporous, homothallic species which differs from Gelasinospora cerealis in its larger perithecia and its smaller spores.

Three new species of Gelasinospora were described in 1950. Roy Cain (17) of Toronto, Canada, described Gelasinospora adjuncta which was developed in a moist chamber on dog dung received from Tamsel, Germany. This species has eight-spored asci, and the spores produce heterothallic mycelia. Spermatia are produced in certain media, on upright, aerial and branching spermatiophores. After examining a specimen of Anthostomella destruens which Shear (95) had described in 1907 and subsequently transferred to the genus

Melanospora in 1927 (94), Cain considered it to be similar to his Gelasinospora adjuncta.

Cain (17) described another species, Gelasinospora retispora, which was cultured from Swiss chard seed imported from Holland. This species has much larger pits in the wall of the ascospore than the others.

Alexopoulos and Sun (1) described another eight-spored, heterothallic species of Gelasinospora collected at Natchez, Mississippi, on Tillandsia usneoides in 1949. They named it Gelasinospora autosteira and pointed out its morphological similarity to both Gelasinospora calospora (Mouton) Moreau and Moreau and Gelasinospora adjuncta Cain. These three species were described at about the same time independently, none of the authors knowing of the work of the others at the time they were preparing their respective publications.

In 1951 Moreau (72) combined Gelasinospora adjuncta, Gelasinospora autosteira and Gelasinospora tetrasperma with Gelasinospora calospora. He considered Gelasinospora tetrasperma to be a four-spored form of Gelasinospora calospora.

Below are appended the original descriptions of the six known species of Gelasinospora:

Gelasinospora tetrasperma spec. nov.: Perithecia sparsa, erumpentai, atra, opaca, pyriformia, membranacea, 0.3 x 0.6 mm., basi hyphis radiantibus cincta; rostro glabro, conoideo; asci plerumque tetraspori, interdum 1-, 2-, vel 3-spori, cum giganteis sporis, raro 5-spori cum nanis sporis, 8 x 230 u aparaphysati, permanentes, cylindracei, apice

asci truncato, perforato, ore circumvento crasso annulo; ascospori plerumque 13.2-16.0 u x 20-28 u, interdum 15.0-19.8 u x 38.0-42.9 u vel 10.0-13.2 u x 16.5-18.0 u, visi a dorso ellipsoidei vel ovoidei, visi a latere plani vel fabiformi, hyalini primo, viridi-atri serius, foveolati; species homothallica.

Gelasinospora cerealis spec. nov.: Perithecia sparsa, erumpentia, fusca, translucida, subglobosa, membranacea, 0.3-0.4 mm. x 0.6-0.7 mm., basi sparsis hyphis cincta, rostro glabro, cylindeico; asci octospori, 29.7-33.0 u x 214.5-260.0 u, aparaphysati, permanentes, cylindeacei; apice asci truncato, perforato, incrassato radiatim in dubbus locis; ascospori 23.1-25.0 u x 26.4-32.0 u visi a dorso late ellipsoidei vel sub globosi, visi a latere plani vel fabiformi, interdum apiculati, hyalini primo, atri serius, foveolati; species homothallica.

Gelasinospora calospora comb. nov. (Mouton) Moreau et Moreau Syn. Rosellinia calospora Mouton: Perithecia sparsa, emergentia, globosa, breve papillata, glabra, 3/10 mm., contextu coriaceo brunneo. Asci cylindracei in stipitem (30-40 longum) attenuati, p. s. 150=15, sursum truncati. Paraphyses pauci articulati. Sporidia monosticha, elliptica, fusda, demum atro-opaca, 20-24=15, episporio eleganter et regulariter foviolato.

Gelasinospora adjuncta sp. nov.: Peritheciis superficialibus, sparsis vel gregariis, piriformibus, 700-1000

x 450-600 u, nigris, superne denudatis, pilis longis, flexuosis, pallide-brunneis, in parte inferiore vestitis; collo papilliformi vel conico, levi, nigro, denudato, 200-400 x 200 u, periphysibus praedito; membrana perithecii mediocriter crassa, interdum coriacea, fere opaca, e cellulis atrobrunneis, angulatis 10-20 u, constituta. Ascis octosporis, cylindraceis, 200-260 x 18-21 u (interdum 400 u longis), superne truncatis, apice distincte perforatis, basi in stipitem brevem attenuatis, qui post maturitatem elongandam. Sine paraphysibus sed cellulis magnis, hyalinis, vesiculiformibus praeditis. Ascosporis oblique monostichis, ellipsoideis 22-27 x 12-15 u, nigris, opacisque, foraminibus obtectis. Spermatiis ovatis, 2.5 x 2.0 u. Speciebus heterothallicis.

Gelasinospora retispora sp. nov.: Perithecis superficialibus, separatis vel gregariis, piriformibus, 700-1000 x 400-600 u (interdum 1200 u longis), nigris, levibus, superne denudatis, pilis longis, flexuosis, hyalinis, septatis, in parte inferiore vestitis; collo conico interdumve longiore, nigro, denudato, 200-300 u longo, periphysibus praedito; membrana perithecii mediocriter crassa, interdum coriacea, opaca, e cellulis atro-brunneis, angulatis, 10-15 u, constituta. Ascis octosporis, cylindraceis, 250-300 x 20-24 u, apice truncatis distincte perforatisque basi in stipitem longum 60-100 u attenuatis. Sine paraphysibus sed cellulis magnis, hyalinis, vesiculiformibus

praeditus. Ascosporis oblique monostichis, ellipsoideis, 28-33 x 14-17 u, initio hyalinis dein olivaceo-brunneis usque olivaceo-nigris opacisque, foraminibus angulatis, 2-5 u in diam. et jugis 1-2 u latis praeditis. Spermatiis et conidiis non observatis. Speciebus homothallicis.

Gelasinospora autosteira spec. nov.: Mycelium ramosius, hyphae 3-14.6 u diam.; perithecia superficialia, subglobosa, membranacea, 0.42-0.61 mm. diam., 0.65-0.71 mm. altra, glabra, rostro cylindrico et prominente; asci octospori, aparaphysati, cylindrici, 14.1-18.3 u x 218.6-244 u; ascospori primo hyalini, deinde fusco-brunnei vel atri, opaci, faveolati, 10.6-14.1 u x 16.7-26.5 u, binucleati; conidia et spermatia incognita. Thalli auto-incompatibiles; species consistens ex duabus lineis designatis A et B.

Table I summarizes the chief taxonomic characters of all species of Gelasinospora thus far described.

It is obvious from Table I that:

1. Gelasinospora tetrasperma is different from the others by having four ascospores in one ascus.
2. Gelasinospora retispora is a distinct species because the spore wall is provided with low ridges which form a reticulate pattern around the angular pores.
3. Gelasinospora cerealis has larger and broader asci and ascospores.
4. Gelasinospora calospora, Gelasinospora adjuncta and

Gelasinospora autosteira are very close morphologically, except that Gelasinospora calospora is a homothallic form, Gelasinospora adjuncta has spermatia and is heterothallic, and Gelasinospora autosteira is heterothallic but forms no spermatia.

In view of the above a comparative study of Gelasinospora calospora and Gelasinospora autosteira was undertaken with a view of shedding some light on the relationship of these species from ontogenetic and cytological evidences. The study also included Gelasinospora cerealis as a contrasting form which is regarded by everyone as distinct.

TABLE I

TAXONOMIC CHARACTERS OF SIX SPECIES OF GELASINOSPORA

Characteristics	Gelasinospora species					
	tetrasperma	cerealis	calospora	adjuncta	retispora	autosteira
Number of spores to the ascus	4	8	8	8	8	8
Size of perithecia in microns	600 x 300	600-700 x 300-400	550-750 x 350-600	700-1000 x 460-600	700-1000 x 400-600	650-710 x 420-610
Size of asci in microns	150-180 x 16-18	214.5-260 x 29.7-33	160-250 x 16-25	200-260 x 18-21	250-300 x 20-24	218.6-244 x 14.1-18.3
Size of ascospores in microns	20-28 x 13-16	26.4-32 x 23.1-25	23-28 x 13-16	22-27 x 12-15	28-33 x 14-17	16.7-26.5 x 10.6-14.1
Shape of ascospores	ellipsoid, rounded at ends	oblong	ellipsoid, narrow toward ends	ellipsoid, narrow toward ends	ellipsoid, wall with reticular ridges	ellipsoid, rounded at ends
Spermatia	none	none	none	present	none	none
Sexual compatibility	homothallic	homothallic	homothallic	heterothallic	homothallic	heterothallic
Ascospore germination	one vesicle	two vesicles	mostly two vesicles	?	?	one vesicle

CHAPTER II

MATERIAL AND METHODS

1. Source of Cultures

A culture of *Gelasinospora cerealis* was furnished by Dr. Keeping (nee Dowding). Cultures were made by isolating single ascospores under the dissecting microscope at a magnification of 90x.

A culture of *Gelasinospora calospora* was obtained from Dr. Moreau and was likewise single-ascospored.

Gelasinospora autosteira was isolated from Spanish moss kept in a moist chamber. Four ascospores, which had been ejected from perithecia and were lying on the filter paper at the bottom of the dish were thus obtained from them. The cultures remained sterile, but formed perithecia when the compatible strains were paired. The eight ascospores in each of several asci were isolated in series from the newly formed perithecia. After several transfers, paired cultures lost their ability to form perithecia. Consequently, it was necessary to make continuous isolations of single ascospores throughout the progress of this work, in order to induce formation of perithecia.

2. Media Used

Difco Corn Meal Agar was used for most cultures, because it is a fairly clear agar and because it discourages formation of aerial mycelium which interferes with observations. Perithecia of Gelasinospora calospora and Gelasinospora cerealis begin to form in from three to five days and mature within seven to twelve days after inoculation. Gelasinospora autosteira generally requires a full month or more of incubation on corn meal agar before it will mature its perithecia. This medium is, therefore, not very satisfactory, and a search was made for a medium which would hasten perithecial production.

Several media were tried in order to hasten the initiation and maturation of perithecia of Gelasinospora autosteira. No perithecia were found after a month's incubation on soybean meal agar, rice agar and 3:1 malt peptone agar. Abundant perithecia were found on Czapek's agar, wheat agar, carrot agar and on corn meal agar to which two percent malt extract had been added. However, all these media soon developed a dark pigment which seriously interfered with observations on perithecial development.

The most satisfactory medium found for studying Gelasinospora autosteira is Spanish moss agar. It is prepared by steaming 15 g. of Spanish moss in 500 ml. of distilled water, filtering through filter paper, adding 1.5 percent agar, and sterilizing in the autoclave at 15 pounds for 20 minutes. On this medium, mycelial growth is very faint

and perithecia will often form within a week.

3. Method of Observing Spore Germination and Perithecial Development

A large cover glass 50 x 43 mm. was placed in a Petri dish and sterilized. A thin film of agar, about 1 mm. in thickness, was then poured on the cover glass and allowed to solidify. For spore germination of all species and for perithecial development of Gelasinospora calospora and Gelasinospora cerealis corn meal agar was used. For studying perithecial development of Gelasinospora autosteira Spanish moss agar was used.

Spores were isolated by the use of sterile steel needles with the aid of a dissecting microscope. They were placed on a block of agar on a sterile slide. As will be explained in the next chapter, it was necessary to heat the spores in order to induce germination. After being subjected to the heat treatment, the spores were transferred onto the film of agar over the center of the cover glass. At the same time a piece of filter paper was cut to fit the bottom of another Petri dish, and a glass ring 40 mm. in diameter was placed on the filter paper. The Petri dishes thus prepared were sterilized and the filter paper was moistened with distilled water. The cover glass with the agar film bearing spores was cut and inverted on the ring to form a Van Tieghem cell.

Spores were examined for germination four hours after sowing and photographs were taken every half hour after the

spores began to germinate, for a period of four hours.

For perithecial development of the homothallic species, a bit of agar with mycelium was transferred onto the film of agar over the center of the cover glass. When the mycelium had covered the agar over the whole cover glass (about 36-48 hours after inoculation), the cover glass with its agar film was used to prepare a Van Tieghem cell. Twenty-four hours later the Van Tieghem cell was searched for perithecial initials. In studying the heterothallic species, two comparible strains were inoculated on opposite sides of the cover glass. After the mycelia had met, a Van Tieghem cell was prepared. The search for perithecial initials began three to five days later along the line of contact of the two mycelia.

4. Staining Procedures

For cytological studies of the developing asci, a modification of Olive's (98) iron-propiono-carmin smear method was used. Just after the perithecial beaks were formed, a block of agar 10 x 20 mm. approximately, bearing perithecia was cut and processed in accordance with the following directions:

1. Fix in 3:1 absolute alcohol and propionic acid for 24-48 hours.
2. Pass through a series of alcohol to distilled water.
3. Mordant with four percent iron alum for four to ten minutes.
4. Wash with several changes of distilled water and

then leave in slightly acidified water overnight.

5. Dissect the perithecia under the dissecting microscope with the aid of two steel needles.
6. Pick up the groups of young asci and lay on a slide.
7. Add a drop of the stain, place a No. 1 cover glass on the mount, and tap gently.
8. Heat the slide gently by passing through an alcohol lamp flame quickly several times.
9. Drain and blot off the excess stain.
10. Seal the edges of the cover glass with glycerine jelly.
11. Permit the mount to age at least 48 hours before observing.

Dodge (38) found in Gelasinospora tetrasperma that from 7:30 to 11:30 P.M. the young asci gave the most division figures. Therefore, perithecia were fixed every two hours during the day and night in order to find the period in which most of the divisions occur. It was found that division figures were about equal for all periods in the species studied.

During the course of the work many other fixatives were tried but no others were as satisfactory as the one mentioned above. Chloroform 3 parts:absolute alcohol 2 parts: glacial acetic acid 1 part was used as recommended by Hirsch (62). It is a good fixative since it removes from the asci the oily material which interferes with the staining. This fixative was not extensively used, however,

because the cytoplasm of the asci turned dark within a week.

Other stains like aceto-carmine, and aceto-orcein were used but were found to be inferior to propiono-carmine. The Feulgen technique was repeatedly tried but was never successful possibly because of the presence of the oily material in the asci.

For sectioned preparations, perithecia were fixed in weak chrome-acetic fixing solution which was made by mixing:

10% aqueous chromic acid	3 ml.
10% aqueous acetic acid	7 ml.
Distilled water	100 ml.

After 12-24 hours' fixation the material was washed thoroughly with running water and dehydrated successively with increasing concentrations of a mixture of ethyl and tertiary butyl alcohol. Paraffin sections 4-8 u thick were cut by a rotary microtome. Crystal violet-iodine, Heidenhain's hematoxylin, triple stain, safranin and anilin blue, tannic acid and iron chloride, and chlorazol black E were all tried for staining. They were all good for staining cell walls, but not the nuclei or chromosomes. It was found that staining with chlorazol black E is the simplest method and therefore is the best one to use.

CHAPTER III

ASCOSPORE GERMINATION

1. Heat Treatment

When the ascospores of Gelasinospora autosteira were first isolated from Spanish moss, they germinated readily. The ascospores resulting from crossings of single spore cultures however will not germinate. A heat treatment described by Dodge (29) was employed to induce germination. The method consists of heating the spores in an oven slowly up to 70° C taking about 30 minutes to reach that temperature, cutting off the heat and letting the spores stay in the oven until the oven cools to room temperature.

It was found that spores sown in mass germinated more easily than single-spores, and the three species of Gelasinospora responded differently to the heat treatment.

When spores were sown singly the following percentages of germination were obtained:

	Maximum temperature reached			
	72-75°	70°	65-68°	50°
G. calospora	0	18	50	36
G. autosteira	0	0-75*	0-75*	0
G. cerealis	95	88	20	0

*In Gelasinospora autosteira the spore germination

appears to be governed by an hereditary factor, as has been shown to be true for Neurospora by Lindegren (68). It appears that ascospores from certain crosses respond to the heat treatment better than those from other crosses.

When spores were sown in mass the following percentages of germination were obtained:

	Maximum temperature reached			
	72-75°	70°	65-68°	50°
G. calospora	2	20	94	80
G. autosteira	3	63	86	18
G. cerealis	96	95	28	0

2. Differences in Germination

Four and one-half to six hours after the heat treatment, spores start to germinate. Ascospores of Gelasinospora autosteira germinate with the formation of a vesicle on one end of the spore (Pl. I, Fig. K). Plate V, Figure Q shows several young ascospores with a germ pore on one end of each spore only.

The majority of ascospores of Gelasinospora calospora germinated with the formation of two vesicles one on each end of the spore (Pl. I, Fig. H). About 18 percent of the germinated spores showed single end germination. Plate I, Figure J shows four germinating spores of which one has germinated from one end only.

In Gelasinospora cerealis the spores examined, germinated from both ends with three percent of them having a

third germ tube. Dowding (43) reported that some of the spores produced one vesicle, but this was not observed in this work. Plate VI, Figure J shows young ascospore of Gelasinospora cerealis with two germ pores.

Under the same treatment, spores of Gelasinospora cerealis germinate about one-half to one hour later than those of Gelasinospora calospora and Gelasinospora autosteira.

CHAPTER IV

DEVELOPMENT OF PERITHECIA

1. Initiation and Early Development of Perithecia

In Gelasinospora calospora perithecial initials appear as coiled lateral branches of vegetative hyphae (Pl. II, Figs. A-H). Under the conditions of these experiment, they form approximately at the same time over the culture, but not all develop simultaneously. Usually the ones close to the edges of the cover slip will develop first. This may be an oxygen effect. Some of the coils will not develop any further, possibly because of failure to receive the stimulus from the male element. Three different initials were found in this species. The first one is shown in Plate II, Figure A. A side branch with dense contents is developed on a hypha. As it twists to form a double ascogonial coil, septa are formed. The basal cell divides to form a compact mass around the coil. A young perithecium is formed within 24 hours.

The second type of perithecial initial is shown in Plate II, Figures B-D. The ascogonial coil is formed at the terminus of the hypha (Pl. II, Fig. B). From the mother hypha a branch is sent out toward the coil (Pl. II, Fig. C). Soon the entire mass grows quickly. After 24 hours it becomes too opaque to study.

The third type is shown in Plate II, Figures E-H. An ascogonial coil is developed (Pl. II, Fig. E). A branch is formed from the basal cell of the coil, and another one from the mother hypha (Pl. II, Fig. F). They meet and both grow toward the coil. Then the development of the perithecium proceeds to a pseudoparenchymatous ball.

Whether or not the branches that grow to the ascogonial coil are antheridial structures cannot be determined unless the actual transfer of nuclei is observed. There are no other branches or vegetative hyphae involved in the development of the perithecia. Neither conidia nor spermatia were found. There is no structure that can be interpreted as a trichogyne.

In Gelasinospora autosteira ascogonial coils are formed in certain strains. They make several turns and then develop to a spherical opaque mass which is visible by the naked eye. These appear to be archicarps which never grow any further. When the archicarps are crushed, there are no signs of asci; but only a few thin-walled cells and some oil droplets.

In mated cultures, the ascogonial coils formed close to the line of contact between two mycelia of compatible strains are these which develop into perithecia. Before the coil develops to a compact mass (Pl. II, Fig. I), a hypha comes from the direction of the opposite strain, contacts, and presumably enters the coil (Pl. II, Fig. J). Then the development continues, and a mature perithecium

is formed within a week. This hypha which enters the ascogonial coil must carry the male element, because coils which do not receive the hypha remain sterile. There are no other branches or hyphae involved in the development. Conidia or spermatia have not been observed.

During the course of this study, archicarps were always found in one of the two compatible strains. However, there are not enough statistical data to prove that the strain which forms the archicarps is female and forms only ascogonial coils whereas the other strain is male and forms only antheridial branches. This phase of the problem should be investigated to discover if Gelasinospora autosteira may be a dioecious, heterothallic species.

In Gelasinospora cerealis ascogonial coils are formed abundantly over the cover glass. The coil twists (Pl. II, Fig. L) and forms a knot-like structure (Pl. II, Fig. M). Eventually a trichogyne-like structure is developed (Pl. II, Fig. N), grows and contacts another hypha (Pl. II, Fig. O) from which possibly it receives the nuclei necessary for diploidization. In certain cases many trichogynes are sent out from the ascogonium. The further development is more or less similar to that of the other two species discussed, except there are no branches of hyphae, other than the coil itself, involved in the development. Here again no conidia or spermatia were found.

2. The Ascogenous Hyphae

After the perithecial initials have developed into a

pseudoparenchymatous ball, it is no longer possible to follow the subsequent cellular development in the living material. Microtome sections were made, but were not very successful; only a ball filled with thin-walled cells could be observed. When such balls were crushed and stained with propiono-carmine, all that could be observed was a mass of thin-walled cells and innumerable oil droplets. The large quantity of oil interferes with the staining and no ascogonial coils can be differentiated. It is evident, however, that the development of ascogenous hyphae is much delayed. In perithecia, which externally appear to be nearly mature, croziers and young asci can usually be found. In addition, there are usually chains of very thin-walled cells, which contain four to six groups of chromatic material (Pl. I, Fig. B). These chains of cells may represent ascogenous hyphae, but the proliferation of croziers from such hyphae, has never been observed. The above discussion refers to all three species studied.

3. Structure of the Perithecium

When the perithecium is mature, a longitudinal section (Pl. I, Fig. D) will show well differentiated layers of the perithecial walls. The outermost layer is one-cell thick and heavily pigmented on the outer side of the cells. The next layer is several cells thick and consists of very thin-walled cells. The innermost layer is a few cells thick and lines the perithecial cavity. At the top of the perithecium, the inner wall grows out and usually upward to form the peri-

physes. There are no paraphyses or pseudoparaphyses in the perithecium.

CHAPTER V

CYTOLOGY OF ASCI

1. Stages in Development

The cytology of the ascus in the three species of Gelasinospora under discussion follows the typical pattern of Ascomycetes in general. The following discussion refers to all three species unless otherwise specified.

a. The croziers.

The formation and development of croziers is similar to that described for many of the Ascomycetes. It starts with the formation of a hook from a binucleate cell (Pl. III, Fig. A; Pl. V, Fig. C). One of the two nuclei moves to the hook and the other remains near the base of the cell (Pl. III, Fig. B). A conjugate division takes place (Pl. V, Figs. A and B; Pl. VI, Fig. A), and thus a crozier with four nuclei is formed (Pl. III, Fig. C). Septa are formed immediately, so that a uninucleate hook cell, a binucleate penultimate cell and a uninucleate basal cell are produced (Pl. III, Figs. A and D). Soon the penultimate cell elongates (Pl. III, Figs. C and D) and the two nuclei fuse (Pl. III, Fig. E) in the penultimate cell. Young asci, each with a large fused nucleus, still attached to the crozier are frequently found (Pl, III, Fig. B; Pl. V, Fig. D, and Pl. VI, Fig. C).

The hook cell often fuses with the basal cell of the crozier and forms a binucleate cell. From this newly formed binucleate cell another crozier is formed (Pl. III, Fig. E). This procedure continues, so when a nearly mature perithecium is dissected, different stages of the croziers and asci can be found.

b. The young asci

After the fusion of the two nuclei, the penultimate cell may be regarded as the young ascus. At this stage the young ascus has a fiarly large nucleolus and a group of chromosomes so entangled that the individual chromosome strands can not be traced (Pl. III, Fig. B; Pl. V, Figs. C and D; Pl. VI, Fig. C). The nuclei appear to remain in this phase for a long time, but further development is quite rapid. This is concluded from the fact that in most preparations the two most prevalent stages are those of the uninucleate young asci, prior to meiosis, or of asci in which the ascospores are already differentiated. The meiotic and mitotic nuclear divisions appear, therefore, to be of short duration.

Following this stage the chromosomes become shorter and thicker. Synapsis takes place at this time as evidenced by the fact that some chromosomes appear to be double. It is in diakinesis (Pl. V, Figs. E and F), first metaphase (Pl. III, Figs. F-O; Pl. VI, Figs. D and E, and F-G asci on the right) and first anaphase (Pl. IV, Figs. A and B; Pl. V, Figs. G and H), that the individual chromosomes can

best be recognized and the number of chromosomes counted. The spindle apparatus is always parallel to the long axis of the ascus as shown in Plate III, Figures N and O and Plate V, Figure I.

After the first division, the two daughter nuclei reorganize themselves and immediately go into the second division. The chromosomes in the second division are smaller than those in the first division. However, in metaphase II (Pl. IV, Figs. C and D; Pl. V, Figs. J and K) and anaphase II (Pl. IV, Figs. E and F; Pl. V, Figs. L and M) the individual chromosomes are still visible and can be clearly counted. Actual spindles have not been stained in the second division, but from the arrangement of the daughter nuclei, it is evident that the position of the spindles is either parallel or oblique to the long axis of the ascus.

Prophase III must be very short because throughout the study, it was found only once. At third metaphase and anaphase, the chromosomes are extremely thin, and it is very hard to photograph them. As seen in Plate IV, Figures G and H, and Plate V, Figure N, the spindles lie either perpendicular or oblique to the long axis of the ascus. Plate IV, Figure I; Plate V, Figure O; and Plate VI, Figures H and I show the eight-nucleated stage.

c. The ascospores

When the eight nuclei reorganize after the third division, delimitation of ascospores begins. The young

ascospores are hyaline and each contains one nucleus (Pl. V, Fig. P). Mitotic division takes place in the young ascospore while it is still hyaline and homogenous (Pl. IV, Fig. J). Soon the ascospores become highly vacuolated and filled with oil droplets, but the two nuclei still can be recognized (Pl. IV, Fig. K; Pl. VI, Fig. J). Later the ascospores change to yellow and finally dark brown to black with pits forming on their walls (Pl. IV, Fig. L; Pl. V, Fig. R; Pl. VI, Fig. L).

2. The Nucleolus

The fused nucleolus in the young ascus is very large. At the first division it reduces to from one-half to one-fourth of the original size. The nucleolus persists during the first division, and even in the second division the nucleolus is visible in certain preparations (Pl. IV, Figs. C-F; Pl. V, Figs. J and K). It has never been observed in the third division. The nucleolus reappears in the young ascospores in the one-nucleated stage.

The number of nucleoli varies from one (in most preparations) to two (Pl. III, Figs. N-O; Pl. V, Figs. G-H), or three (Pl. III, Figs. H-I).

In Gelasinospora calospora and Gelasinospora autosteira the nucleolus is often situated far away from the group of chromosomes, but in Gelasinospora cerealis it is closely associated with the chromosomes. In the last species there is the nucleolus, always found attached to a chromosome

which presumably contains the nucleolus organizing region.

3. The Rod

The frequent appearance of a rod-like structure has drawn attention to the study of its behavior and function. It has been observed in all three species of Gelasinospora and in all stages of nuclear divisions in the asci. It is not found in the croziers (Pl. V, Figs. A-B; Pl. VI, Figs. A-B), therefore it must be developed after that stage.

It is not a chromosome because:

1. It is not always present (Pl. III, Figs. F-G; Pl. IV, Figs. C-F; Pl. V, Figs. A-B; Pl. VI, Figs. A-E, F and G asci on the right).

2. It does not behave as the chromosomes:

In Plate III, Figures L-O the other chromosomes have synapsed, but the rods remain unpaired. In Plate V, Figures E-F, J-K, the rods are separated away from the rest of the chromosomes. In Plate IV, Figure I; Plate V, Figure O; Plate VI, Figures H-I, when the chromosomes are reorganizing, the rods remain distinct. In Plate IV, Figures G-H; Plate V, Figure N, the rods go to the ends of the spindles.

3. It has a different consistency and stain reaction to propiono-carmine. The rod appears beaded and more or less stiff, and it is fainter in color than the chromosomes.

It does not appear to be a centrosome because, although, as shown in Plate V, Figure N, the two rods behave like centrosomes, in the ascus depicted in Plate III, Figures N-O

they are not at the ends of the spindles; furthermore, there is a centrosome-like body visible at one end of the spindle. In Plate V, Figures E-F, J-K, there are structures which can be called centrosomes.

It is not a bridge, because it has nothing to do with any other chromosomes and it is present in stages other than anaphase.

The nature of the rods has not been determined. They await further study.

4. The Chromosome Numbers

Six univalent chromosomes can be counted in the croziers of Gelasinospora autosteira (Pl. V, Figs. A-B). Seven univalent chromosomes are observed in the croziers of Gelasinospora cerealis (Pl. VI, Figs. A-B). In Gelasinospora calospora no good division figures of the crozier could be found. Consequently, the chromosome number before fusion has not been counted.

From diplotene to metaphase I, six bivalents can be counted in Gelasinospora calospora (Pl. III, Figs. F-O) and Gelasinospora autosteira (Pl. V, Figs. E-F); and seven bivalents in Gelasinospora cerealis (Pl. VI, Figs. D-E, F and G asci on the right). In anaphase I, 12 chromosomes are recognizable in Gelasinospora calospora (Pl. IV, Figs. A-B), and Gelasinospora autosteira (Pl. V, Figs. G-H); and 14 chromosomes in Gelasinospora cerealis.

Similar counts can be made in the second metaphase (Pl. IV, Figs. C-D; Pl. V, Figs. J-K), second anaphase

(Pl. IV, Figs. E-F; Pl. V, Figs. L-N), and third metaphase (Pl. IV, Figs. G-H; Pl. IV, Figs. F-G asci on the left).

The haploid number of chromosomes in Gelasinospora calospora and Gelasinospora autosteira appears to be six and that of Gelasinospora cerealis seven.

CHAPTER VI

DISCUSSION

1. Sexual Compatibility

Blakeslee (14) did the pioneer work on sexual incompatibility in members of Mucorales. Species in which a single thallus is capable of reproducing sexually he called "homothallic" and species which require two compatible thalli for sexual reproduction he called "heterothallic". Since then much work has been done on homothallic and heterothallic forms of fungi. Among the Ascomycetes, Neurospora (30, 35, 41, 90, 100), Schizothecium (4, 5, 42), Glomerella (47, 70), Ophiostoma (83), and Hypomyces (58, 59, 62) have been studied most extensively.

Dodge (36) gave the name "facultative heterothallism" to the phenomenon exhibited by forms like Neurospora tetrasperma in which normal ascospores yield self-fertile mycelia, whereas dwarf spores yield self-sterile mycelia; and "obligate heterothallism" the phenomenon exhibited by such forms as Neurospora sitophila in which all the ascospores are self-sterile. He wrote that "the main difference between facultative and obligate heterothallism is that in the former the nuclei of opposite sex have a certain rather strong attraction for each other throughout the life cycle. In obligate heterothallic species this attraction is strong

only at a certain stage of maturity."

Whitehouse (99) applied the term "morphological heterothallism" to the behavior of fungi in which reproduction occurs through interaction of two thalli bearing morphologically different gametangia; and "physiological heterothallism" to the behavior of fungi in which reproduction occurs through interaction of two physiologically different thalli bearing no morphological differences. The term "primary homothallism" indicates the condition in a homokaryotic individual, that is, one in which all the nuclei have the same genotype; and "secondary homothallism" indicates that in a heterokaryotic individual, that is, one in which the nuclei are of two or more compatible mating types.

Gelasinospora tetrasperma has been studied by Dowding (43, 44), Dodge (38, 35), and Campbell (18). They found its sexuality to be essentially similar to that of Neurospora tetrasperma, Pleurage (Schizothecium) anserina, and the four-spored form of Sordaria (Fimetaria) fimicola. In accordance with the terminology discussed above, Gelasinospora tetrasperma is a "secondary homothallic" form, but the dwarf spores produce "facultative" or "physiologically heterothallic" mycelia. Gelasinospora calospora and Gelasinospora cerealis are "primary homothallic" species and Gelasinospora autosteira is an "obligate" or "physiologically heterothallic" species. Whether Gelasinospora autosteira is "morphologically heterothallic" as well, remains to be verified.

Whitehouse (99) considered the unisexual strains of Bombardia lunata, Hypomyces solani, Neurospora sitophila, Sordaria (Fimetaria) fimicola, and Ceratostomella (Ophiostoma) fimbriata as mutant forms of fungi which are normally monoecious. Since the morphology and cytology of Gelasinospora calospora and Gelasinospora autosteira are closely similar, Gelasinospora autosteira might be a mutant form of Gelasinospora calospora.

2. Perithecial Development

It seems very probable that when a more detailed study of the perithecial development in all species of Gelasinospora is made, a very similar situation to that described and summarized by Gäumann (50) for the perithecial development of the Fimetariaceae will be found.

In Sordaria (Fimetaria) fimicola (52, 53, 84, 86) the ascogonia appear as normal, coiled branches, but the antheridia are often less differentiated, hypha-like or entirely lacking. The ascogonium usually copulates with a neighboring hypha or, in a homothallic race, with a neighboring cell of the mother hypha. In certain cases these somatic copulations do not occur, and the ascogonium continues to develop by itself. In Sordaria (Fimetaria) macrospora Anersw. (28) there are no morphologically recognizable antheridia present, but the ascogonia copulate regularly with vegetative hyphae. A similar condition is found in Gelasinospora calospora where the ascogonium appears to copulate with a vegetative hypha or develop without somatic copulation.

In the case of Bombardia lunata (89, 103, 104) and Pleurage (Schizothecium) anserina (2, 3, 4, 42, 87, 88) both ascogonia and spermatia are present. The long, up to ten-celled, trichogyne contacts the spermatia of the other compatible group and copulates with them. If no spermatium is present then the trichogyne copulates with a phialide which may even be in the process of discharging, or it copulates with a vegetative hyphal branch. There are no spermatia present in Gelasinospora cerealis, but the trichogyne-like structure does come in contact with other hyphal branches. Brooks (14) found a trichogyne-like structure in Gnomonia erythrostoma, but believed its function to be respiratory. Blackman and Wellsford (12) considered the trichogyne in Polystigma rubrum to be vegetative in function. In Glomerella (80) trichogyne-like outgrowths have been found, but fusion with other structures have not been observed. In Neurospora sitophila (8, 11, 30, 31, 32, 33, 35, 36, 41, 67, 77, 75, 76, 90, 93, 100, 102) the trichogynes or receptive hyphae of the ascogonial coil have a special affinity for opposite components. If one places micro- or macro-conidia or ascospores of the opposite compatibility group onto a culture with young ascogonial coils, the initials develop into perithecia. The receptive hyphae will copulate with the germ tubes of micro- or macro-conidia of the compatible strains. Such copulations sometimes occur somatically and entirely independent of the presence of an ascogonium. The act of copulation has lost the need of

specific organs. Any cell may give or receive nuclei, thus behaving as male or female in the presence of another compatible cell.

Two perithecial initials were found in Glomerella (80). The inner one develops into the fertile portion and the outer one develops into the sterile portion of the perithecium. McGahen and Wheeler stated that "the two initials could be mistaken easily for antheridial and ascogonial structures, if their development had not been followed step by step in living cultures, and if the uninucleate condition of the cells of the resultant coils had not been demonstrated in stained preparations." In Gelasinospora calospora the hyphal branch that goes into the ascogonial coil may correspond to the outer coil of Glomerella, but since there is no branch in Gelasinospora autosteira nor in Gelasinospora cerealis which can be recognized as a second initial, the branch in Gelasinospora calospora can be considered as copulatory rather than vegetative in function.

The type of copulation reported herein for Gelasinospora autosteira has also been found in Glomerella in which a hypha of the conidial culture fused with the tip cell of the inner coil (80).

From the above discussion it is obvious that in the Pyrenomycetes the means of sexual reproduction varies within the families, the genera and even within the same species.

Cookson (23) believed that the central cord of the archicarp differentiated into fertile and sterile elements.

She presumed that they were developed from hyphal outgrowths of an enlarged cell of the archicarp. Andrus (7) confirmed Cookson's work. McGahen and Wheeler (80), as mentioned above found two initials of the perithecium of Glomerella; the inner one develops into the fertile portion and the outer one develops into the sterile portion of the perithecium. In the case of Gelasinospora, it seems that the tip of the coil develops into the fertile portion of the perithecium, and the basal cell of the coil proliferates and forms the sterile portion of the perithecium.

3. Ascus Development and Cytology

Dangeard (26, 27) was the first one to find the croziers proliferated from the ascogenous hyphae. This has been found in a large number of Ascomycetes. Some species (i.e., Gnomonia erythrostoma (14), Ophiobolus graminis (65)) have been reported to develop their asci directly, without crozier formation. In other species both methods have been reported. Ceratostomella (Ophiostoma) furnishes a good example of contradictory observations concerning the presence or absence of croziers. Sartoris (91), Elliot (47), Andrus and Harter (6) reported that the asci were formed without the intervention of croziers; on the contrary, Varitchak (97), Gwynne-Vaughan and Broadhead (57) and Taylorvinje (96) reported the presence of croziers in the same species. In the three species of Gelasinospora studied, there is no question that the croziers are present.

The smear techniques (25) brought great progress to the study of chromosomes in Ascomycetes. However the chromosomes are so small that to count them is very difficult and to study the morphology of each individual chromosome seems to be impossible.

In Neurospora tetrasperma, Colson (22), and Cutter (24) found six chromosomes as the haploid number, whereas McClintock (79) and Fincham (49) found seven chromosomes. Wilcox (100) found six chromosomes in Neurospora sitophila, but McClintock (79) found seven, Lindegren (69) found nine in Neurospora crassa. A very interesting phenomenon is found in Hypomyces. Hirsch reported that the homothallic forms of Hypomyces solani Rke. et Berth. emend. Snyd. et Hans. (62) have six chromosomes in the haploid stage, while the heterothallic Hypomyces solani f. curcurbitae Snyd. et Hans. (63) may have four, three or two chromosomes, depending on the sex of the isolate. In all the cases where the chromosomes are countable, Gelasinospora calospora and Gelasinospora autosteira have the same number of chromosomes, namely six.

Nuclear beaks or spindle horns have been described in Neurospora by Lindegren (69), Cutter (24), Fincham (49) and Colson (22), and in Gelasinospora tetrasperma by Dodge (48). These structures are probably the same as the rods found in the third division (Pl. IV, Figs. G-H; Pl. V, Fig. N) and in the eight-nucleated stage (Pl. V, Fig. O;

Pl. VI, Figs. H-I) in the species under study here. McClintock (79) described the centriole as a rod-like structure in the first and second divisions. It is very possible that the rod found in the three Gelasinospora species is the same as the structure reported by McClintock, but its position as shown in Plate III, Figures N-O, makes its identification as a centriole doubtful.

CHAPTER VII

SUMMARY AND CONCLUSIONS

1. *Gelasinospora calospora* and *Gelasinospora autosteira* are very similar morphologically, but *G. calospora* is a homothallic form and *G. autosteira* is a heterothallic form. A comparative morphological and cytological study was undertaken with the purpose of establishing the relationship between the two species. *G. cerealis* was used here as a contrasting form.
2. The germination of spores and the perithecial development were observed in Van Tieghem rings. Propionocarmine was used for staining smeared preparations of young asci.
3. A heat treatment is necessary to induce spore germination. Spores of *G. calospora* germinate largely by formation of two vesicles one at each end of the spore, although a few germinate by formation of one vesicle only. Spores of *G. autosteira* germinate by forming one vesicle only, while in *G. cerealis* all the spores germinate by two vesicles.
4. The perithecial initials begin as a coiled hyphal branch in all three species. It seems that in *G. calospora* a vegetative hypha from the mother hypha serves as copulatory organ; in *G. autosteira*, a hypha from the opposite

compatible strain is needed for copulation; and in G. cerealis, a trichogyne-like structure is formed to fuse with vegetative hypha.

5. The development of croziers and the nuclear divisions in the asci follow the typical pattern of Ascomycetes in all three species studied here.

6. A rod-like structure appears in all stages of divisions in the asci of all three species of Gelasinospora. The nature of the rod remains to be studied.

7. The haploid number of chromosomes in Gelasinospora calospora and Gelasinospora autosteira appears to be six and that of Gelasinospora cerealis seven.

8. No major differences can be found in the morphology and cytology of G. calospora and G. autosteira, but both differ significantly from G. cerealis.

9. In accordance with these findings it is concluded that G. autosteira is not sufficiently different from G. calospora to be recognized as a distinct species and should be united with it in support of Moreau's conclusion. However, the two forms differ sufficiently in the following details to be readily recognizable:

    a. One is homothallic, the other heterothallic.

    b. The spores of one generally germinate by a single vesicle, those of the other by two vesicles.

    c. The spores of one have more pointed ends; those of the other have more rounded ends.

10. In view of the above, it is proposed to reduce *Gelasinospora autosteira* to a variety of *Gelasinospora calospora* under the name: *Gelasinospora calospora* (Mouton) Moreau et Moreau var. *autosteira* (Alex. and Sun) n. var.

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APPENDIX

PLATE I.

Fig. A --- Vegetative hyphae of *G. calospora*.

Fig. B --- Chain of thin walled cells with groups of chromatic material. *G. autosteira*.

Fig. C --- Vegetative hypha, showing septa and nuclei. *G. autosteira*.

Fig. D --- Longitudinal section of a perithecium of *G. autosteira*.

Fig. E --- Perithecium of *G. autosteira*.

Fig. F --- Perithecium of *G. calospora*.

Fig. G --- Perithecia of *G. cerealis*.

Figs. H-N --- Ascospore germination

H --- *G. calospora*, four hours after sowing.

I --- The same ascospore, two hours later.

J --- *G. calospora*, one spore germinated from one end only.

K --- *G. autosteira*, four hours after sowing.

L --- The same ascospore, one hour later.

M --- *G. cerealis*, five hours after sowing.

N --- The same ascospore, one hour later.

PLATE I

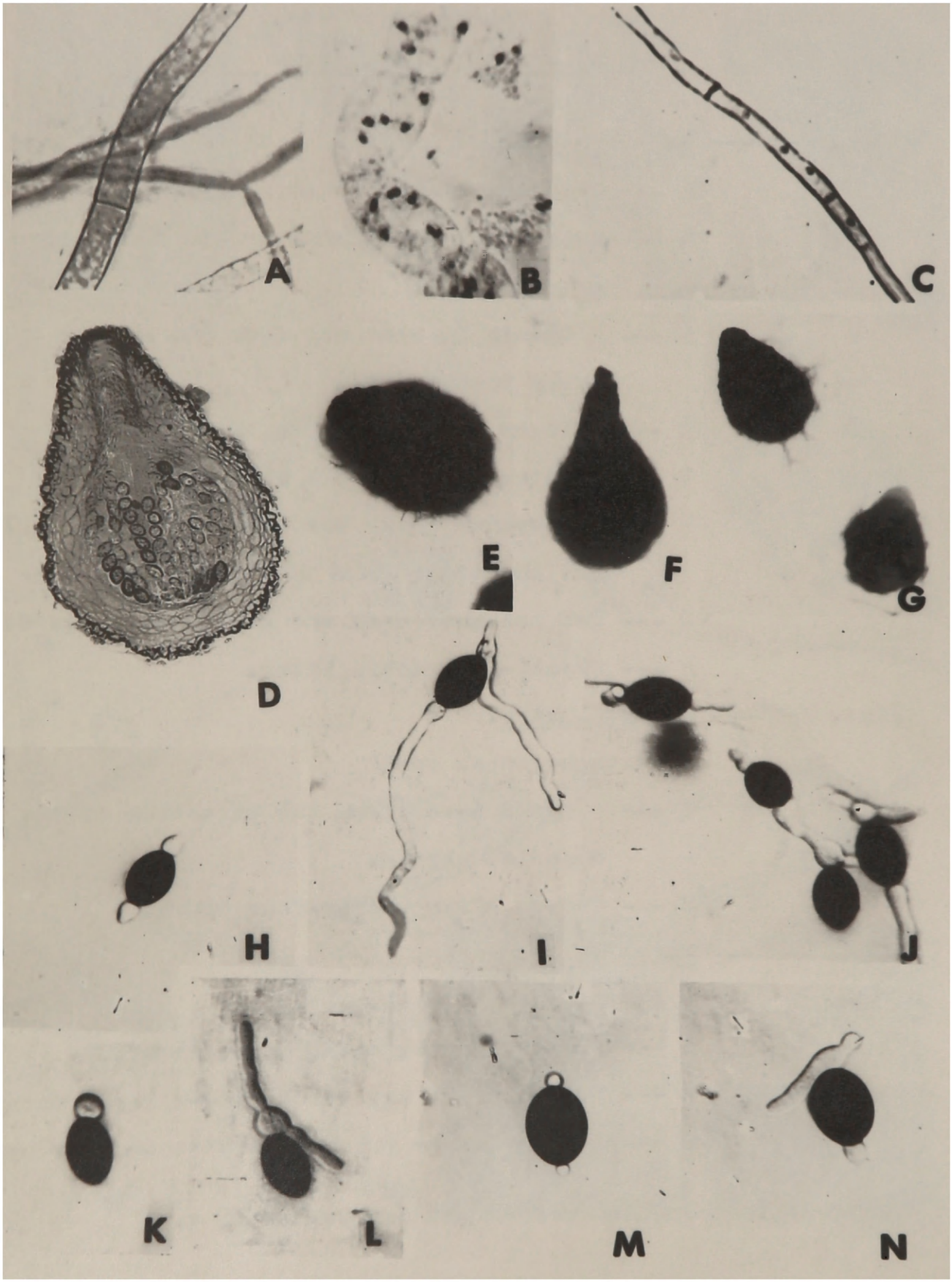

PLATE II

Perithecial Initials

Figs. A-H --- *G. calospora*.

A --- Ascogonial coil which develops by itself.

B --- Ascogonial coil forms at the terminus of the hypha.

C --- A branch is sent out from the mother hypha to the coil.

D --- Half an hour after Fig. C.

E --- Ascogonial coil as a side branch.

F --- One branch grows out from the basal cell and the other from the mother hypha.

G --- Two branches meet and grow toward the coil.

H --- Twenty-four hours later.

Figs. I-K --- *G. autosteira*.

I --- Ascogonial coil

J --- A hypha comes from the direction of the opposite strain.

K --- Growth after twenty-four hours.

Figs. L-O --- *G. cerealis*.

L --- Ascogonial coil.

M --- The same coil, eight hours later.

N --- Beginning of the trichogyne-like structure.

O --- Fusion of the trichogyne with another hypha.

Each division on the lower right represents 10 u.

PLATE II

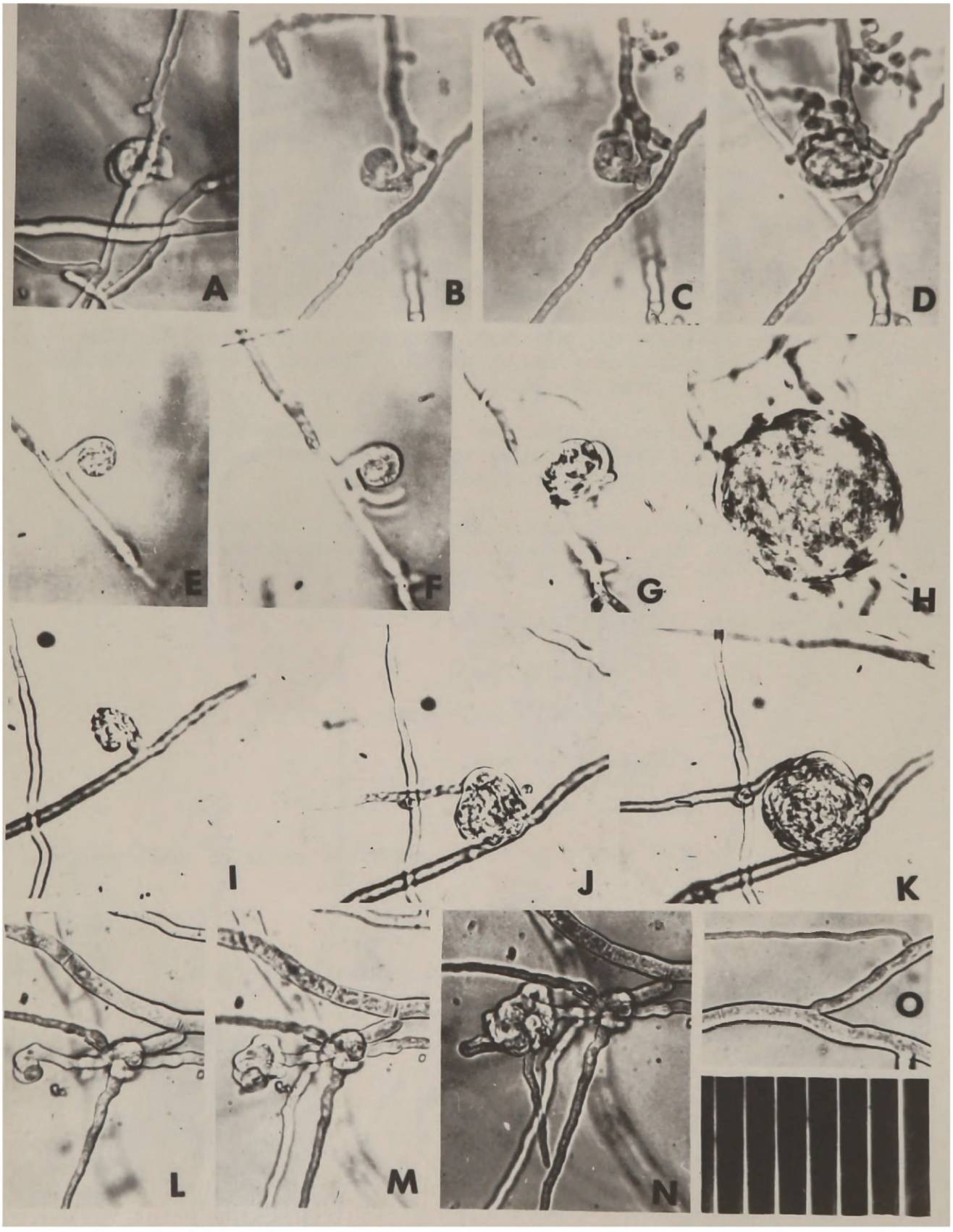

PLATE III

Cytology of Asci in G. calospora

Fig. A --- Formation of the hook; four nucleated crozier with septa; young ascus with fused nucleus; and four nucleated crozier with septa; from bottom to top respectively.

Fig. B --- Crozier on the top showing conjugate division; young ascus with crozier attached.

Fig. C --- Crozier on the top showing elongation of the penultimate cell; on the bottom showing crozier with four nuclei.

Fig. D --- Crozier on the top showing elongation of the penultimate cell; on the bottom showing crozier with four nuclei and septa.

Fig. E --- Crozier on the right showing fusion of nuclei; on the left showing crozier proliferated from the basal cell of the crozier.

Figs. F-O --- First metaphase.

F-G --- No rod.

H-I --- One rod, three nucleoli.

J-K --- One rod.

L-M --- Two rods.

N-O --- Two rods, showing spindle and centrosome.

Each division on the lower right represents 10 u.

PLATE III

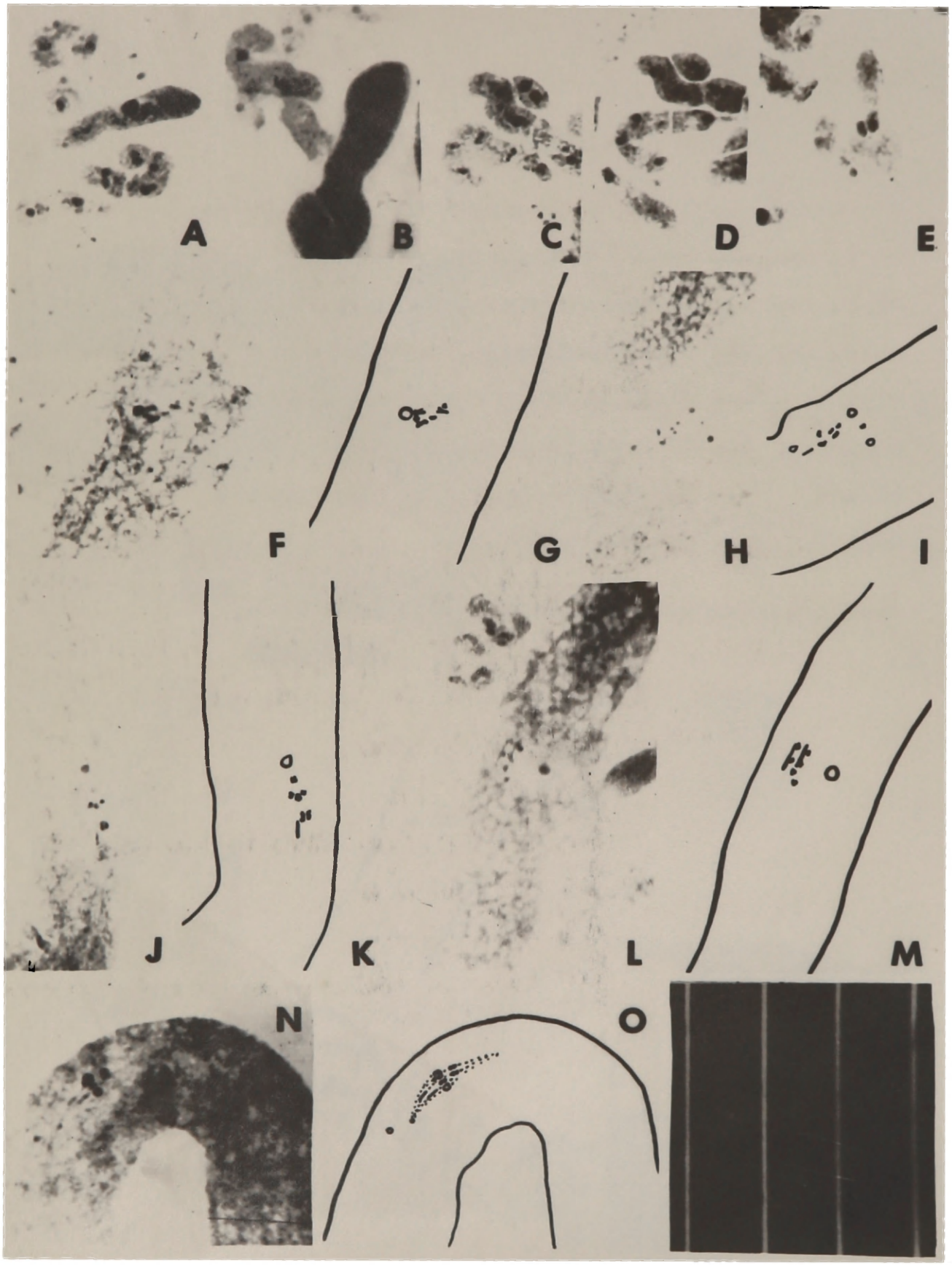

PLATE IV

G. calospora (Continued)

Figs. A-B --- First anaphase, showing two rods.

Figs. C-D --- Second metaphase, no rod.

Figs. E-F --- Second anaphase, no rod.

Figs. G-H --- Third metaphase, rods on end of spindles.

Fig. I --- Eight nucleated stage, with rods.

Fig. J --- Mitosis in ascospores.

Fig. K --- Young ascospore with two nuclei.

Fig. L --- Mature ascospores.

Each division on lower right represents 10 u.

PLATE IV

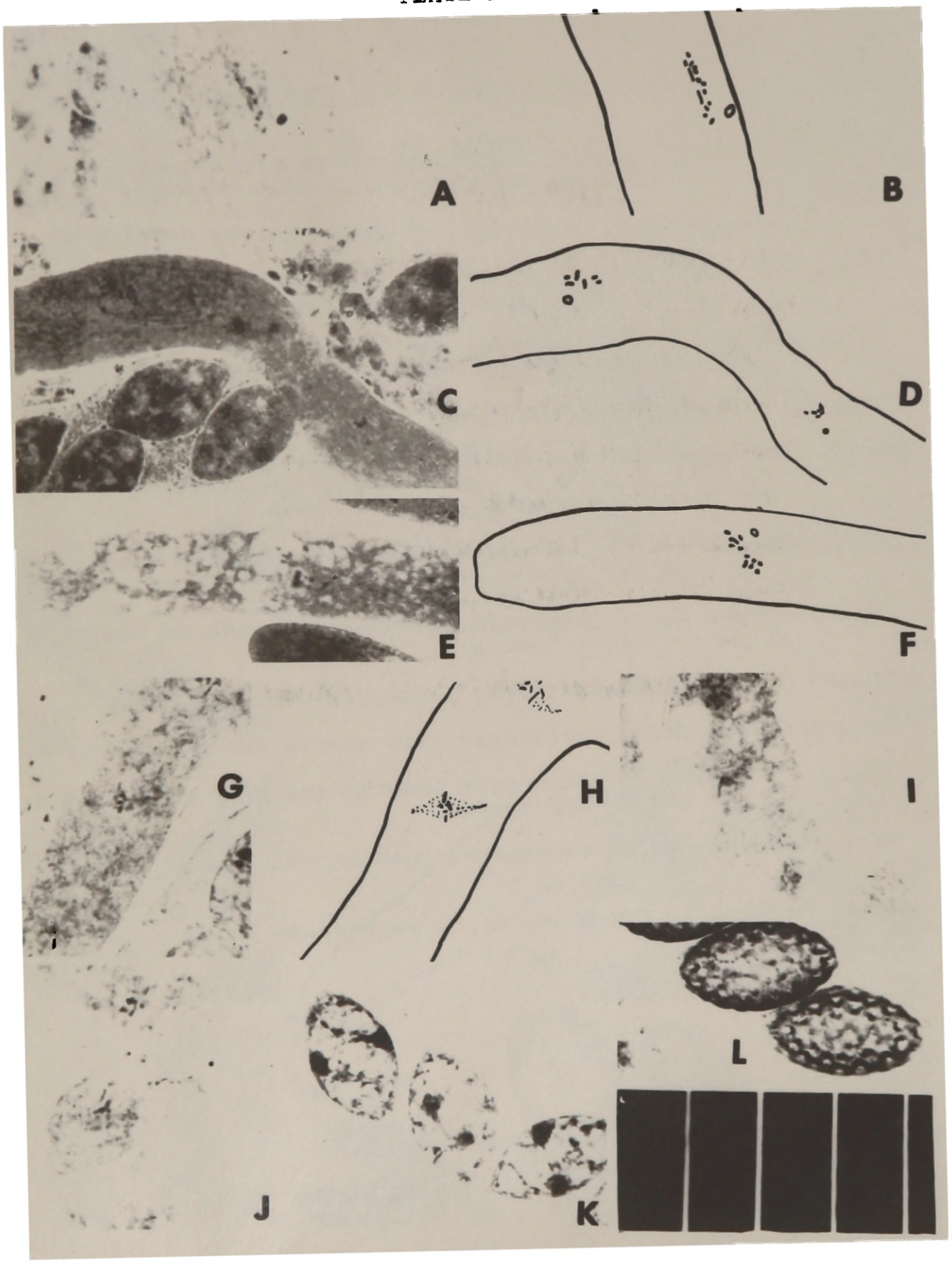

PLATE V

Cytology of Asci in *G. autosteira*

Figs. A-B --- Conjugate division in crozier, showing six univalent chromosomes in each nucleus.

Figs. C-D --- Young asci still attached to the croziers.

Figs. E-F --- Diakinesis, showing six bivalent chromosomes, one nucleolus, a rod and a centrosome.

Figs. G-H --- First anaphase, showing six pairs of separating chromosomes, a rod and two nucleoli.

Fig. I --- First telophase.

Figs. J-K --- Second metaphase, the lower nucleus showing six pairs of chromosomes, a nucleolus, a rod, and a centrosome.

Figs. L-M --- Portion of the ascus showing second anaphase of one nucleus with twelve chromosomes and a rod.

Fig. N --- Third metaphase, showing the rods on the ends of the spindle.

Fig. O --- Eight nucleated stage, showing the rod.

Fig. P --- Young ascospores with one to two nuclei.

Fig. Q --- Young ascospores showing one germ pore on the end of the spore.

Fig. R --- Mature ascospores showing the pits.

Each division on the lower right represents 10 u.

PLATE V

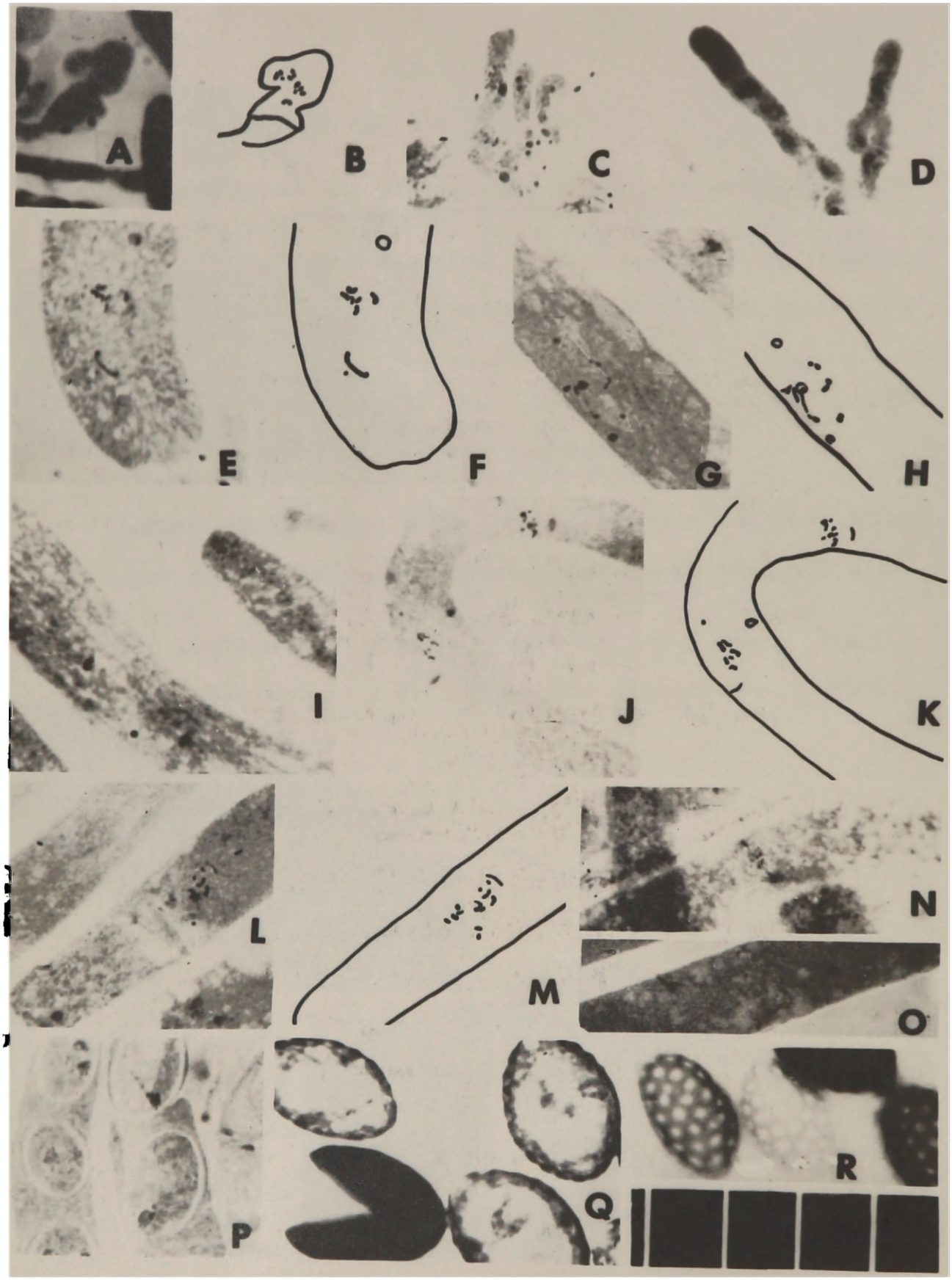

PLATE VI

Cytology of Asci in *G. cerealis*

Figs. A-B --- Conjugate division in the croziers, showing seven univalent chromosomes.

Fig. C --- Young asci with fused nuclei.

Figs. D-E --- First metaphase, showing seven bivalent chromosomes and the nucleolus chromosome attached to the nucleolus.

Figs. F-G --- Ascus on the left is in second metaphase, showing the rod; ascus on the right is in first metaphase, showing seven bivalent chromosomes and the nucleolus chromosome.

Figs. H-I --- Eight nucleate stage showing the rods.

Fig. J --- Young ascospore showing two nuclei and two germ pores.

Fig. K --- Young ascospore showing two nuclei.

Fig. L --- Mature ascospores showing the pits.

PLATE VI