STUDIES OF COPROPHILOUS ASCOMYCETES

I. *GELASINOSPORA*[1]

BY ROY F. CAIN[2]

Abstract

Two species of *Gelasinospora* are described for the first time. *G. adjuncta* Cain, a heterothallic species with eight-spored asci, was grown in moist chambers on dung from Germany and Quebec. *G. retispora* Cain, distinguished by means of the large angular pits in the ascospore wall was isolated in culture from seeds of Swiss chard from Holland and from apple twigs in Quebec. *G. tetrasperma* Dowding has been found in moist chambers on dung from Ontario, Quebec, Venezuela, and Germany and isolated from seeds from Quebec. Notes are added regarding the disposition of *Anthostomella destruens* Shear and *Sordaria uvicola* Viala and Marsais.

The Genus *Gelasinospora*

Two species of Pyrenomycetes having ascospores characterized by dark-colored spore walls with pitted sculpturing were described by E. S. Dowding (1) in 1933. For these she erected the new genus *Gelasinospora*. This is closely related to *Neurospora* Shear and Dodge (5) which has longitudinal, wavy ridges on the walls of the ascospores. The species of *Neurospora* produce a conidial stage of the *Monilia* type and spermatia in phialides. In *Gelasinospora tetrasperma* Dowd. and *G. cerealis* Dowd., neither conidia nor spermatia have ever been described to date.

In October 1931, perithecia of *Gelasinospora tetrasperma* were grown on rabbit dung from Thunder Bay District in the laboratory in Toronto. This was recognized as being quite distinct from any known species of *Sordaria* in having the ascospore devoid of any gelatinous sheath or appendages, and in the characteristic marking on the spore wall. These features indicated a closer affinity with members of the Hypocreales. It is now the opinion of the author that most species of *Sordaria* as well as *Neurospora*, *Gelasinospora*, and *Melanospora* should be treated as members of this order in spite of the dark-colored perithecial walls. *Gelasinospora tetrasperma* has since been found on dung from Duchesnay and Lake Mistassini, Que., and from Germany. The type material from near Fort Churchill, Man., has been examined as well as cultures from England referred by Page (2) to *Sordaria fimicola* (four-spored form). It has also been found on seeds from Quebec.

The second species *Gelasinospora cerealis* is known only from the crown of wheat and oats in Manitoba.

In October 1935 a third species of this genus was found on the dung of dog and cow from Germany which was being kept in moist chambers in Toronto.

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Contribution from the Department of Botany, University of Toronto, Toronto, Ont. This study was carried out with the assistance of a grant in aid of research furnished by the University of Toronto.

[2] *Assistant Professor and Curator, Department of Botany, University of Toronto.*

This was isolated in pure culture. In 1938 the same species appeared in moist chambers on dung from Quebec. This species resembles *G. tetrasperma* but has smaller ascospores and eight-spored asci.

A few years later a fourth species was cultured from Swiss chard seed imported from Holland, by Dr. J. W. Groves at Ottawa. This species differs from the others in having much larger pits in the wall of the ascospore. More recently a second collection of the same species was cultured from apple twigs at Ste. Anne de la Pocatiere, Que., by A. Payette, and forwarded to the writer by Dr. Groves.

Key to the Species of *Gelasinospora*

Asci four-spored.....*G. tetrasperma*

Asci eight-spored

Pits on ascospore wall small

Ascospores 26-32 × 23-25μ.....*G. cerealis*

Ascospores smaller 22-27 × 12-15μ.....*G. adjuncta*

Pits on ascospore wall large.....*G. retispora*

Gelasinospora tetrasperma Dowd. Can. J. Research, 9 : 294. 1933.

On malt agar producing a rapid and dense growth of mycelium which is white at first, becoming gray or slightly pinkish. Perithecia superficial or with base slightly immersed, pyriform, 430-600 × 270-300μ, black, bare excepting a fairly dense layer of hyphae at base; neck papilliform, black, bare, 150-200μ long and 100-120μ wide, lined with periphyses; wall moderately thick, slightly coriaceous, composed of dark brown angular cells measuring 8-16μ. Asci four-spored, cylindrical, 150-180 × 16-18μ, truncate and distinctly perforate at the apex, tapering below into a fairly short stipe. A few hyaline, swollen cells surrounding the asci but no paraphyses. Ascospores uniseriate, parallel with ascus, ellipsoid, slightly flattened on one side, 23-27 × 13-16μ, broadly rounded at the ends, dark brown, becoming black and opaque, wall uniformly covered with round or slightly elongated pits measuring up to 1μ in diameter.

Developed in moist chambers on dung of ptarmigan, rabbit, horse, and cow from Quebec, Ontario, Manitoba, England, Germany, and Venezuela. Isolated in culture from seeds of *Beta vulgaris* L. (mangels, beets), and *Festuca rubra* L. (red fescue) from Quebec, by Dr. J. W. Groves, Ottawa.

This species is evidently more common in Northern Canada. It has not been found in Southern Ontario or even at Lake Timagami where extensive collections of dung have been studied.

The pits in the wall of the ascospores cannot be seen in the opaque spores. They are plainly visible, however, in the brown immature spores or when the opaque spores are crushed, especially in lactophenol.

Gelasinospora adjuncta sp. nov. (Figs. 15-24)

Peritheciis superficialibus, sparsis vel gregariis, piriformibus, 700-1000 × 450-600μ, nigris, superne denudatis, pilis longis, flexuosis, pallide-brunneis, in parte inferiore vestitis; collo papilliformi vel conico, levi, nigro, denudato, 200-400 × 200μ, periphysibus praedito; membrana perithecii mediocriter crassa, interdum coriacea, fere opaca, e cellulis atro-brunneis, angulatis 10-20μ, constituta. Ascis octosporis, cylindraceis, 200-260 × 18-21μ (interdum 400μ 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 × 12-15μ, nigris, opacisque, foraminibus obtectis. Spermatiis ovatis, 2.5 × 2.0μ. Speciebus heterothallicis.

Type: In fimo canino, ex Tamsel, Germania, in laboratorio culta. TRT *23203*.

On agar media, producing a rapid development of copious gray mycelium. Self sterile, hermaphroditic. Perithecia developing from coiled ascogonia, superficial or with base slightly immersed, scattered or loosely aggregated, pyriform, 700-1000 × 450-600μ, black, bare except basal part which is covered with light-brownish hyphae; neck papilliform or tapering, smooth, black, bare, 200-400 × 200μ, lined with slender periphyses; wall moderately thick, somewhat coriaceous, nearly opaque, composed of dark brown angular cells measuring 10-20μ. Asci eight-spored, cylindrical, 200-260 × 18-21μ, elongating to over 400μ, broadly rounded to truncate and distinctly perforate at the apex, tapering below into a stipe which becomes considerably elongated at maturity. Large, hyaline, swollen cells surrounding the asci with a few forming filaments up to 12μ in diameter. Ascospores obliquely uniseriate, ellipsoid, somewhat narrowed toward the ends, 22-27 × 12-15μ, dark brown, becoming black and opaque, wall uniformly covered with round or slightly elongated or irregular pits measuring 1.0-1.5μ in diameter; no hyaline sheath surrounding the ascospores. Spermatia ovate, about 2.5 × 2.0μ, budded from short side tubes from short cells of spermatiophores which are upright, aerial, and branching.

Type: Developed in moist chamber at Toronto on dog dung from Tamsel, Germany, collected by P. Vogel March 1, 1935. Crypt. Herb. Dept. of Botany, University of Toronto *23203* and Herb. RFC *6483*.

Additional material developed in moist chambers at Toronto on cow dung, Tamsel, Germany (RFC *6502*), on horse dung, Duchesnay, Que. (RFC *6950*), and on cow dung, Lac St. Joseph, Que. (RFC *6953*). A specimen of this

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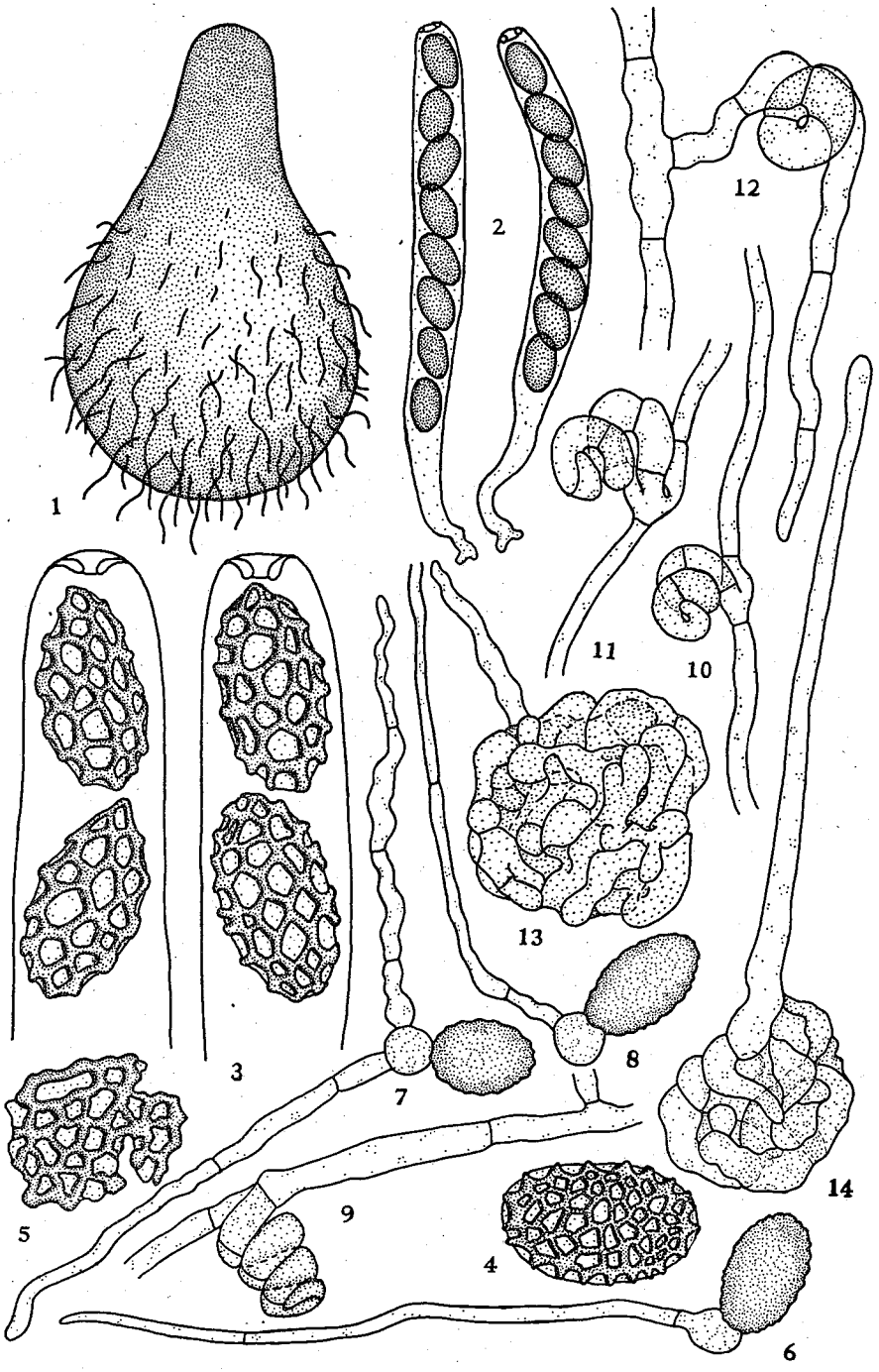
FIGS. 1-14. *Gelasinospora retispora. Fig. 1. Perithecium.* ×*93. Fig. 2. Two asci.* × *300. Fig. 3. Upper end of two asci with ascospores.* × *900. Fig. 4. Ascospore.* × *900. Fig. 5. Portion of wall from crushed ascospore showing the thickened ridges and large angular pores.* × *900. Figs. 6-8. Germinating ascospores.* × *560. Figs. 9-11. Coiled ascogonia and hyphae.* × *900. Fig. 12. Coiled ascogonium with developing trichogyne filament.* × *900. Figs. 13, 14. Early stages in development of the perithecium.* × *900.*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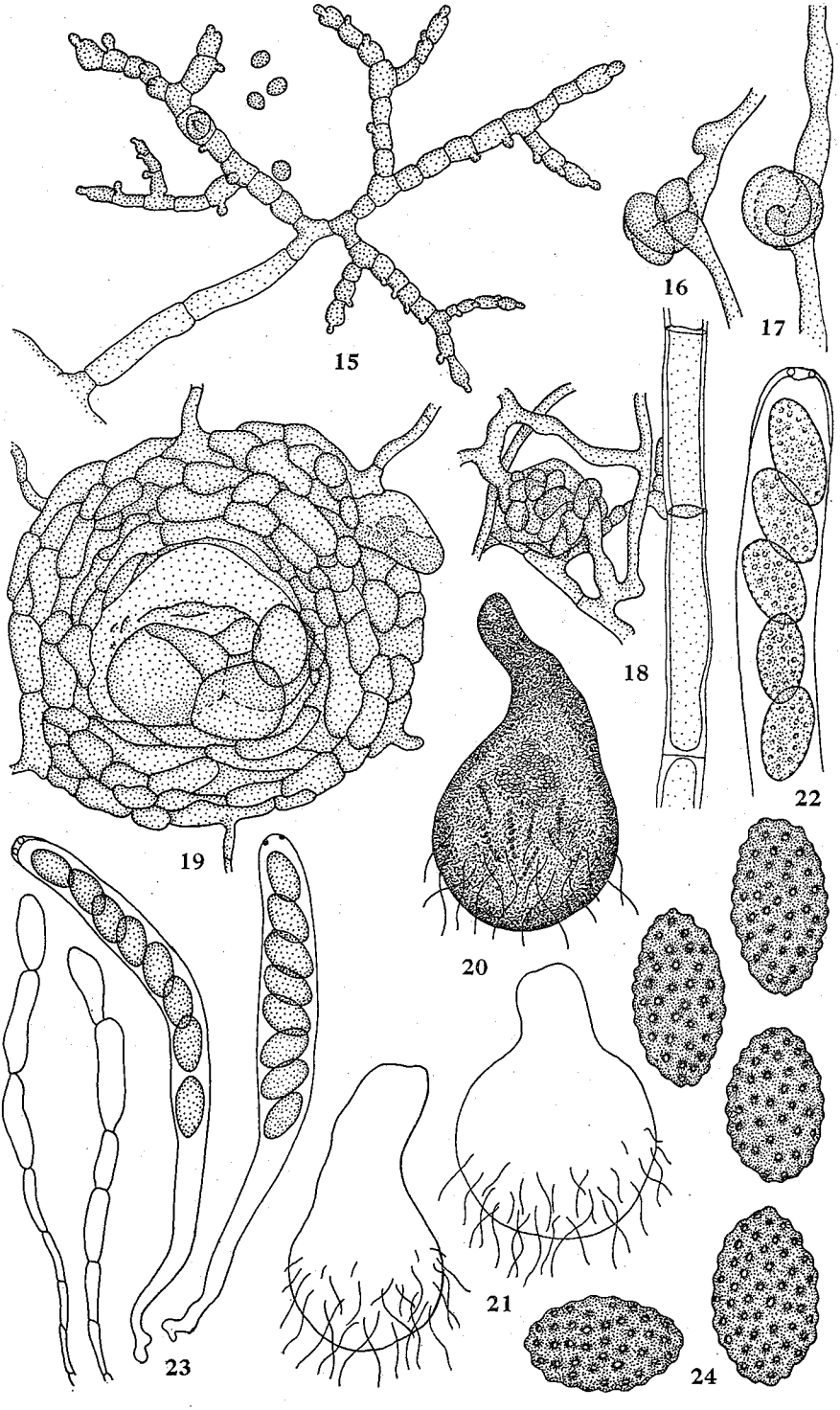
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species was found in the Herbarium of the United States Department of Agriculture, Bureau of Plant Industry, at Washington labelled "*Anthostomella destruens* Shear, (in culture from) *Vaccinium* sp. (blueberry), Wareham, Mass. transfer from culture of Sawyer's, comm. C. L. Shear."

Distribution: Massachusetts, Quebec, and Germany.

Spermatiophores have not been observed on dung substrata. They are sometimes produced in abundance on some media especially those with little or no sugar. On many culture media such as malt agar, no spermatiophores or spermatia are produced and yet perithecia mature in abundance. Under these conditions it is apparent that fertilization is accomplished by means of a fusion between ordinary hyphae and the ascogonium or branches from the ascogonium. The spermatiophores are upright about 100μ in length, aerial, with about four to five main branches each of which is again branched once or twice. The cells are very short, being about 3-6μ long and 4μ wide in contrast with the very long cells in the ordinary hyphae. The cells of the spermatiophore have denser contents. A short side tube is produced from each cell and the spermatia are formed one at a time by budding from the open end, just as in the ordinary phialide. It is probable that the short side tubes represent minute phialides.

The ascogonium develops as a stout side branch with dense contents on an ordinary hypha. It twists into a compact, close coil with ultimately about three turns. As it twists, septa are formed so that four or five cells are produced. Hyphae develop from the basal cell and adjoining filaments and surround the ascogonium. These are at first filamentous and septate but very soon form a pseudoplectenchymatous tissue to produce the wall of the perithecium. The cells in the inner layer remain thin-walled and colorless, those in the outer layer become thick-walled and brown. Each layer is several cells in thickness.

These perithecial initials reach a diameter of 50 to 100μ (occasionally up to 250μ) without fertilization. In monospore cultures the perithecia develop no further. A series of monospore cultures were paired in all possible combinations and it was found that they could be divided into two groups, A and B, such that, when any culture of Group A was crossed with any culture in Group B, mature perithecia with ascospores were formed. Monospore cultures are hermaphroditic but self sterile. However, mature perithecia are frequently developed in cultures of fertile combinations without the formation of spermatia. It is probable that this is accomplished by hyphal fusion. It

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FIGS. 15-24. *Gelasinospora adjuncta. Fig. 15. Branching spermatiophore showing the short cells with small projections from the tips of which spermatia are budded. Four free spermatia.* × *900. Figs. 16, 17. Coiled ascogonia.* × *900. Fig. 18. Coiled ascogonium being enveloped by hyphae.* × *680. Fig. 19. Young perithecium with unfertilized ascogonium from 12 weeks old monospore culture.* × *900. Fig. 20. Perithecium.* × *46. Fig. 21. Outline of two perithecia.* × *46. Fig. 22. Upper end of ascus showing pore at the apex and five ascospores.* × *560. Fig. 23. Asci and filamentous paraphyses-like structures.* × *300. Fig. 24. Five ascospores showing small pits in the wall.* × *900.*

is likely that a trichogyne filament obtains the required nucleus and transfers it to the ascogonium. But at this stage there are numerous long hyphae produced as branches from the outer cells of the wall and these could not be distinguished from a trichogyne filament.

Anthostomella destruens Shear

This species was described by Shear (3) in 1907 and transferred to *Melanospora* (5) in 1927. The type was isolated in culture from diseased cranberry from New Jersey. This specimen cannot now be found in the Herbarium of the Bureau of Plant Industry in Washington. The author has examined four other collections from this Herbarium. One of these is *Gelasinospora adjuncta* and consists of a dried culture from *Vaccinium* sp. (blueberry), Wareham, Mass., transfer from culture of Sawyer's, comm. C. L. Shear. The other three are *Sordaria fimicola* (Rob.) Ces. & De Not. These are labelled as follows:

(1) Ex *Vitis*. Isolated and det. C. L. Shear. Cultured on *Rubus* stems.

(2) On cranberry, E. Wareham, Mass., No. *329*, dried test tube specimen. May 19, 1928.

(3) *Sordaria uvicola* Viala & Marsais (*Melanospora destruens* Shear – det. C. L. Shear) on *Vitis* sp., type culture from Italy. March 22, 1930.

It is thus apparent that Shear considered specimens of *Sordaria fimicola* as being *Anthostomella destruens*. The photographs published by Shear and Dodge (5) in Plate IF and Plate IIID represent *Sordaria fimicola*. The illustration published by Shear (4) in Plate IV, Figs. 8-11, may refer to *Gelasinospora adjuncta*. This, in all probability, was drawn from the type since in this publication Shear stated that the fungus had been found only once. In the description of the type, the spore size given is nearer *Sordaria fimicola* than *Gelasinospora adjuncta*. There is nothing else in the description that would distinguish between these two species. The gelatinous sheath around the ascospores of *Sordaria fimicola*, and the pits in the wall of the ascospore of *Gelasinospora adjuncta* easily separate the two species, but neither of these characters is mentioned. When mature opaque spores of the latter species are examined in water, it is easy to overlook the pits. The shape of the ascospores is slightly, but distinctly, different in the two species.

If the type of *Melanospora destruens* is found, it may be necessary to reduce *Gelasinospora adjuncta* to synonymy. On the other hand, *M. destruens* may become a synonym of *Sordaria fimicola*.

Sordaria uvicola Viala and Marsais

It was described in 1927 (6) from a culture isolated from diseased grapes. Dried cultures of the type of this species grown on agar, carrot plug, and stems (labelled clover but undoubtedly *Rubus*) located at Washington, have

been examined by the author. These are all *Sordaria fimicola* (Rob.) Ces. & De Not. The perithecia agree in every respect as do the asci which are eight-spored, cylindrical, and with a thickened ring surrounding a pore at the apex. The ascospores are the normal size for *S. fimicola*, but are slightly variable as is usually the case when grown in culture. The lower end is minutely pointed with a round germ pore. They are surrounded by a hyaline gelatinous sheath which, however, does not cover the germ pore.

The description of *S. uvicola* includes, along with the ascomycete, various species of Fungi Imperfecti such as *Alternaria* and a pycnidial form. *S. fimicola* produces coiled ascogonia and the fertilization is not as illustrated by Viala and Marsais. *S. uvicola* was isolated from diseased grapes from Chisinau in Bessarabia. The species is not tenable since it includes a mixture, the ascomycete component of which is *Sordaria fimicola*.

Gelasinospora retispora sp. nov. (Figs. 1-14)

Peritheciis superficialibus, separatis vel gregariis, piriformibus, 700-1000 × 400-600μ (interdum 1200μ longis), nigris, levibus, superne denudatis, pilis longis, flexuosis, hyalinis, septatis, in parte inferiore vestitis; collo conico interdumve longiore, nigro, denudato, 200-300μ longo, periphysibus praedito; membrana perithecii mediocriter crassa, interdum coriacea, opaca, e cellulis atro-brunneis, angulatis, 10-15μ, constituta. Ascis octosporis, cylindraceis, 250-300 × 20-24μ, apice truncatis distincte perforatisque basi in stipitem longum 60-100μ attenuatis. Sine paraphysibus sed cellulis magnis, hyalinis, vesiculiformibus praeditus. Ascosporis oblique monostichis, ellipsoideis, 28-33 × 14-17μ, initio hyalinis dein olivaceo-brunneis usque olivaceo-nigris opacisque, foraminibus angulatis, 2-5μ in diam. et jugis 1-2μ latis praeditis. Spermatiis et conidiis non observatis. Speciebus homothallicis.

Type: Ex seminibus *Beta vulgaris* L. var. *ciclae* L., Enkhuizen, Holland. TRT *23204*.

On agar media producing a rapid growth of white fluffy mycelium. Perithecia developing from coiled ascogonia, superficial, scattered or loosely aggregated, sometimes crowded in small groups on surface of agar, pyriform, 700-1000 × 400-600μ, sometimes reaching 1200μ in length due to more elongated neck, dull black, smooth, bare except the lower part which is covered with white hyphal-like hairs; neck conical, or sometimes more elongate, black, bare, about 200-300μ long, lined with slender periphyses; wall moderately thick, somewhat coriaceous, opaque, with dark-brown, obscure angular cells measuring 10-15μ in diameter; hairs hyaline, flexuous, irregularly and remotely septate, rarely branching or anastomosing, with slightly thickened walls. Asci eight-spored, cylindrical, 250-300 × 20-24μ, truncate at apex with large distinct perforation surrounded by a thickened ring, with a stipe 60-100μ long which gradually tapers to the base, surrounded by hyaline filaments which in the upper part are tubular and in the lower part

with cells swollen to 15μ in diameter or in older perithecia up to 30μ in diameter. Ascospores obliquely uniseriate, ellipsoid, 28-33 × 14-17μ, becoming olivaceous-brown and then olivaceous-black and opaque; spore wall ornamented with low ridges 1-2μ wide which form a reticulate pattern surrounding angular pores which measure 2-5μ in diameter; no gelatinous sheath. No spermatial or conidial stage observed. Homothallic.

The fresh spores germinate in water only after heating (44° C.). They produce a single apical vesicle from which a hyphal filament is produced. On malt agar growth is very rapid and a white fluffy colony completely covering the tube is produced. The hyphae are hyaline or faintly brownish, measuring 3-8μ in diameter. There is considerable aerial growth.

Type: Isolated in culture from seeds of *Beta vulgaris* L. var. *cicla* L. (Swiss chard), Enkhuizen, Holland. Crypt. Herb. Dept. of Botany, University of Toronto *23204*. Additional specimens from same isolation in Herb. Div. of Botany and Plant Pathology, Dept. of Agric. Ottawa, and in Herb. RFC *12105*).

A second collection was isolated from apple twigs, Ste. Anne de la Pocatiere, Que., by A. Payette (No. *595*).

Distribution: Holland and Quebec.

This species differs considerably from the others so far described in the genus *Gelasinospora* in the character of the pits in the ascospore wall. Here they are much larger and angular with the intervening area reduced to narrow ridges. This is considered to be only a specific character and not of sufficient importance for the establishment of a separate genus. Furthermore, there appear to be no other distinguishing features other than the size of perithecia, asci, and ascospores. The admission of this species in the genus *Gelasinospora* requires a slight modification of the original concept.

The ascogonium develops as a side branch from a hyphal thread (Figs. 10-11). It becomes closely coiled and septate with the terminal end extending out as a long trichogyne (Figs. 12-14). Branches from the basal cell (Fig. 11), grow out and enclose the ascogonium to form the perithecial wall (Figs. 13-14).

Acknowledgments

I am deeply indebted to the following for their contributions of material for study: J. Rousseau, J. Savage, the late H. Sydow, P. Vogel, and the late Prof. H. H. Whetzel. Grateful acknowledgment is made to Mr. J. Stevenson for sending specimens from the Herbarium of the U.S. Department of Agriculture, to Dr. J. W. Groves for his contribution of cultures isolated from seeds, to Dr. A. Payette for a culture of *Gelasinospora retispora*, to Mrs. Kaye (née Miss Daphne F.Vick) for inking the drawings, and to Mr. L. Garay for assistance in preparation of the manuscript. I wish to thank Prof. H. S. Jackson for his interest and advice.

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* *In a paper by Claude and Mireille Moreau* [*Rev. Mycol. 14* (*suppl. col. No. 2*) *: 53. 1949.*] *a specimen from Congo is described as Gelasinospora calospora* (*Mouton*) *Cl. et Mir. Moreau, that is, according to the description, very similar to G. adjuncta. This publication did not come to my attention until the present paper had gone to press.*

(NOTE: *Plates I-IV follow.*)

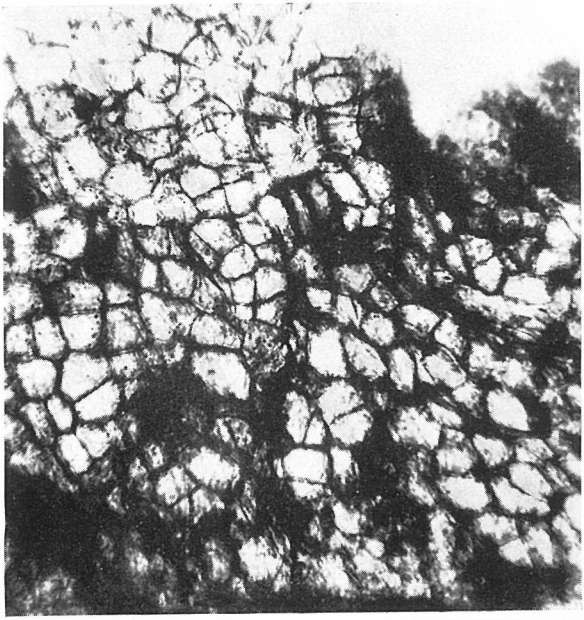
Plates I-IV

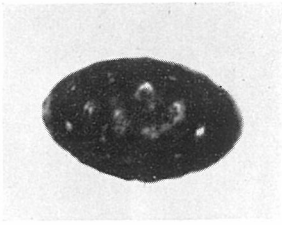
PLATE I. *Gelasinospora adjuncta. Microphotographs of perithecial wall, asci with ascospores, and two separate ascospores showing pits in wall.*

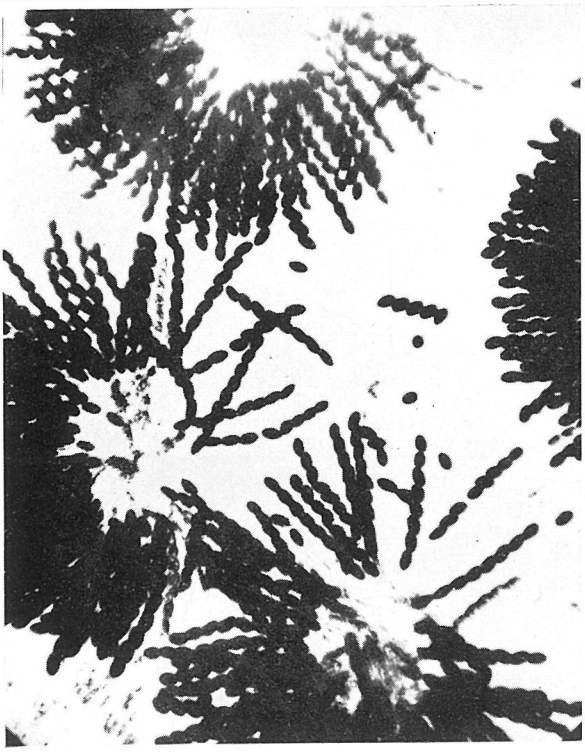
PLATE II. *Gelasinospora adjuncta. Microphotographs of crushed perithecium and ascospores. The mature ascospores appear black and the lighter colored ones with the pits showing are in an immature condition.*

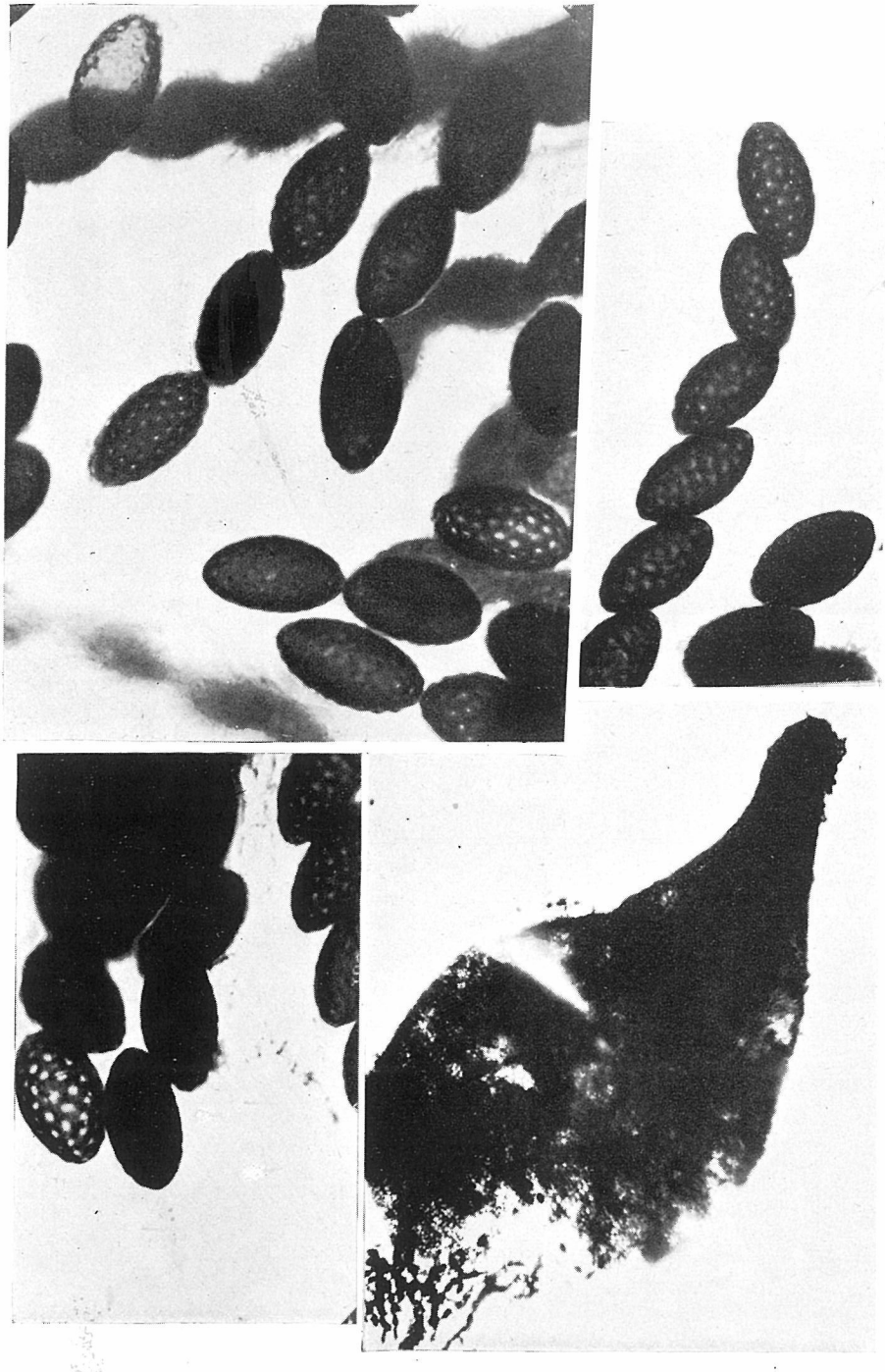
PLATE III. *Gelasinospora retispora. Microphotographs of crushed perithecium, perithecial wall, and ascospores that vary from immature to mature condition.*

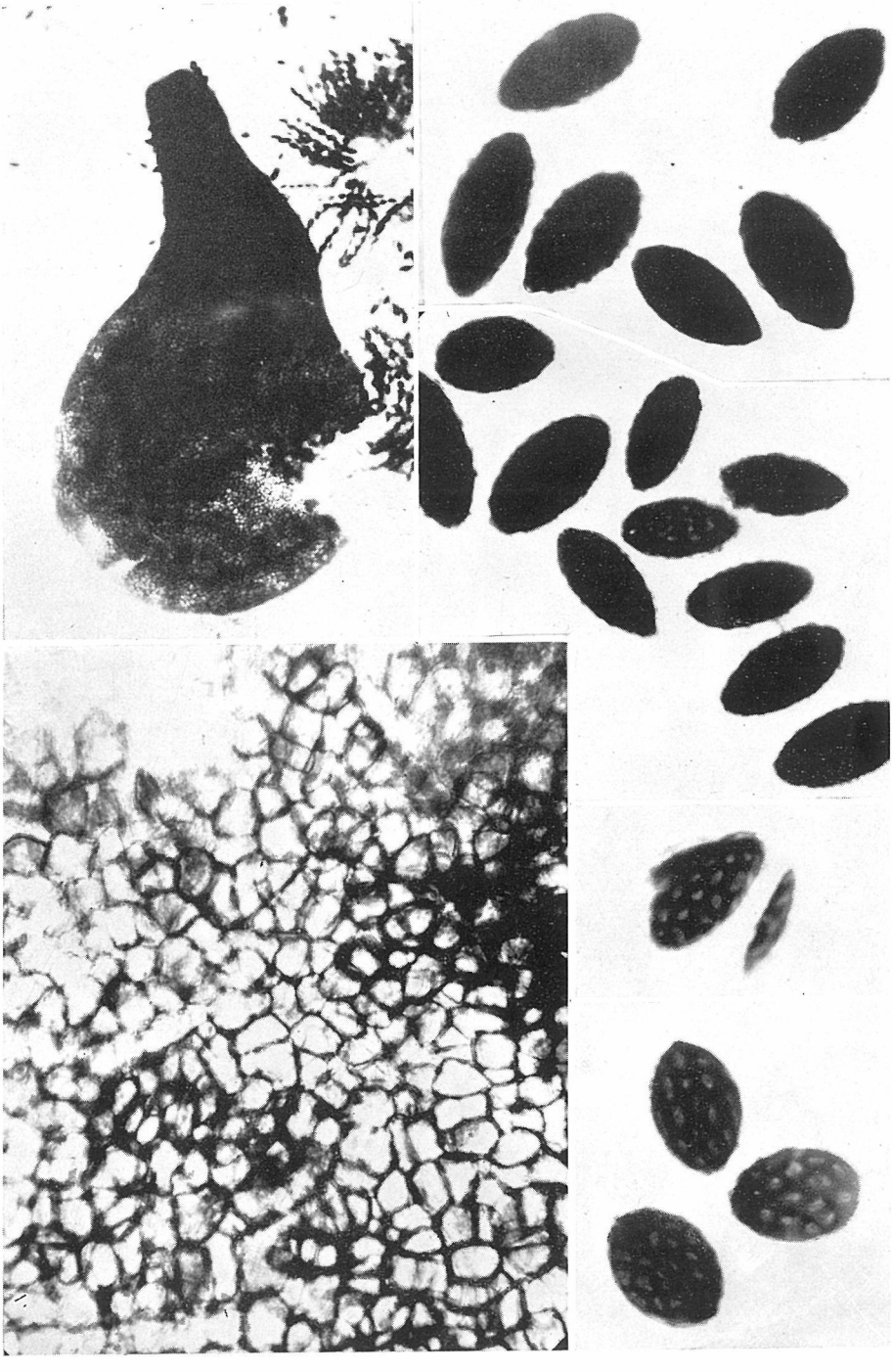
PLATE IV. *Gelasinospora retispora. Microphotographs of ascospores varying in maturity from nearly colorless to black and opaque. Note the large angular pores. Near the upper left hand corner is an ascus that is filled with a single ascospore with the characteristic markings on the wall. Some of the ascospores in this area have abnormally elongated pits in the walls.*

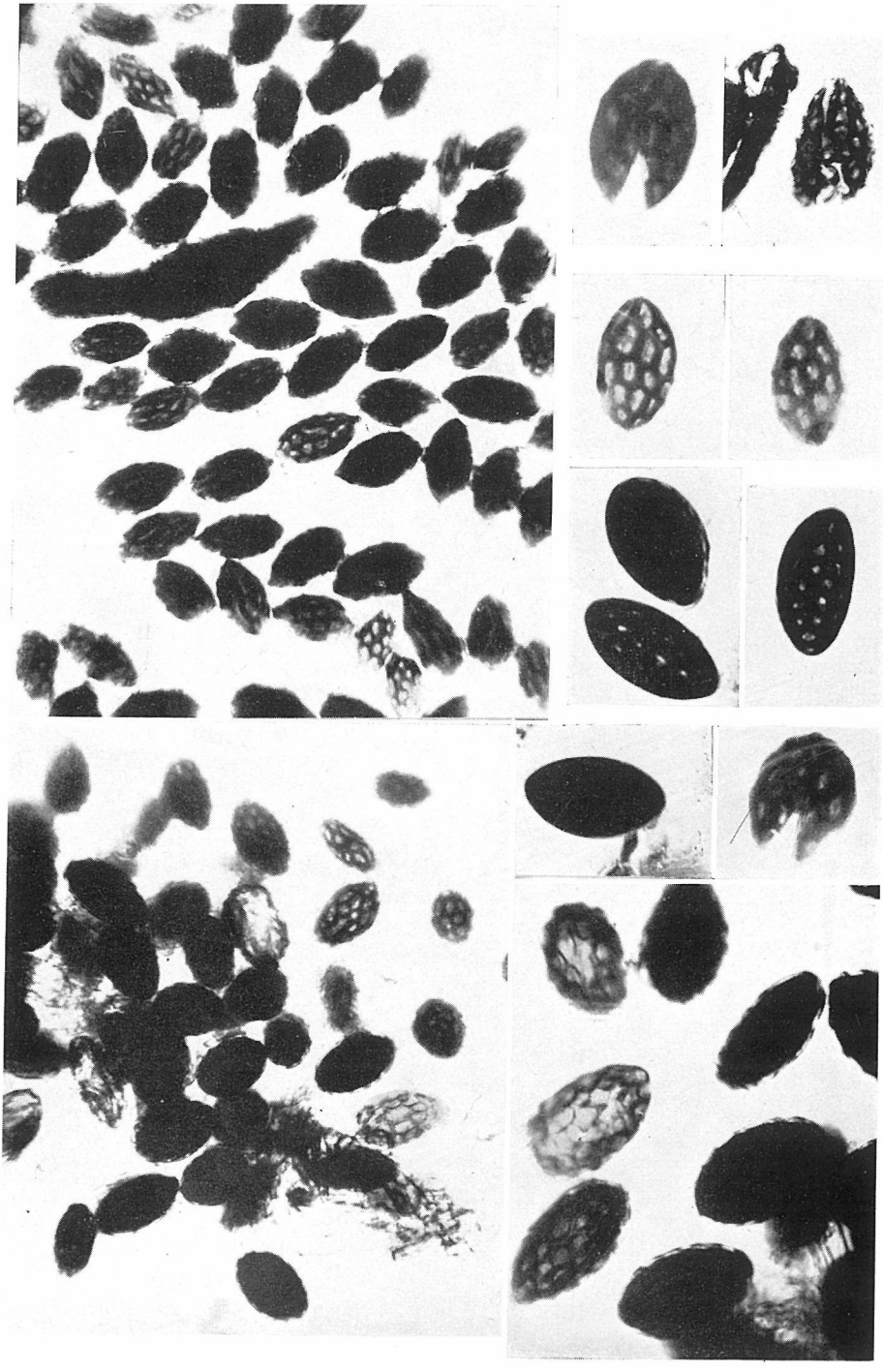












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New Techniques for Mounting Fungi.	1949.	1s. 6d.
Proceedings of the First Commonwealth Conference on Tropical and Subtropical Soils.	1948.	25s.

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