# A STUDY OF THE LIFE CYCLE AND TAXONOMIC POSITION OF MYCOSPHAERELLA (VENTURIA) RUMICIS.



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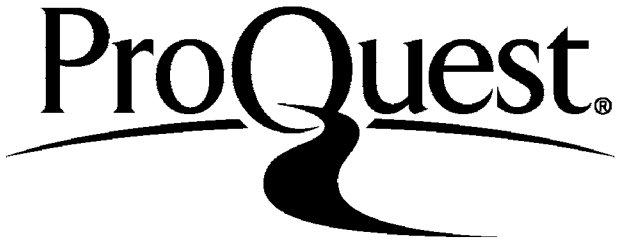

## Abstract

The growth, development and life history of *Mycosphaerella rumicis* (Desm.) Cooke, on its *Rumex* host and in culture, were investigated and described: inoculation experiments were carried out to follow the course of the infection of the host by the fungus. The structure of the ascus and ascospore of the fungus was investigated and described, and compared with other descriptions of bitunicate asci.

Centrum development in *Pleospora herbarum* (Pers.) Rbh., *Venturia inaequalis* (Cooke) Wint., *Venturia pirina* Aderh. and *Mycosphaerella maculiformis* (Pers.) Wint. was described and compared with that in *Mycosphaerella rumicis*.

The classification of the ascostromatic ascomycetes was considered, and in the light of this, the taxonomic position of *Mycosphaerella rumicis* was discussed. It was concluded that the correct name of the fungus is *Venturia rumicis* (Desm.) Wint.

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# I. INTRODUCTION.

## Introduction.

This work was undertaken in an attempt to clear up the confusion in the nomenclature of the pyrenomycete fungus Mycosphaerella rumicis. The fungus is known under two names, Mycosphaerella rumicis (Desm.) Cooke (1866), and Venturia rumicis (Desm.) Winter (1887). It is listed as Mycosphaerella rumicis (Desm.) Cke. in the compilation of British Pyrenomycetes by Mason and Bisby (1940), and is called by this name throughout the present work.

The taxonomy of the Pyrenomycetes has changed since Mycosphaerella rumicis was described by Cooke (1866) and Winter (1887). Primary emphasis in most classifications is now on developmental criteria, and particularly on centrum development, while form and structure of the ascus are also regarded as important taxonomic characters. It follows that an important prerequisite to the correct naming of a pyrenomycete fungus is a knowledge of its development.

A brief review of pyrenomycete classification since the time of Cooke (1866) and Winter (1887), and a short account of the nomenclature of Mycosphaerella rumicis will show the importance of the developmental approach to the correct identification of Mycosphaerella rumicis.

Classification of Pyrenomycetes.

In the early classifications of the fungi, for example by Lindau (I897) and Winter (I887), the Pyrenomycetes were recognised as a distinct group of the Euascomycetes. In Lindau's system the Pyrenomycetes were characterised by asci arising from the base of a spherical to flask shaped fruit body which opens, either by an apical pore, or less frequently by disintegration of the fruit body wall. The system of Winter was basically similar but the arrangement of the asci was not taken into consideration and the Pyrenomycetes in this classification included the Plectascineae sensu Lindau. The subdivisions of the Pyrenomycetes were based on such characters as form and colour of the fruit body, position of the fruit body in relation to its substrate, and form and colour of the ascospore. The general framework of these early classifications has been retained with a few modifications - mainly the recognition of a group of stromatic forms - until relatively recently; it was not until Nannfeldt's work (I932) that a fundamental change of approach was made to the whole system of ascomycete classification.

Nannfeldt's classification attempts a completely new arrangement of the Ascomycetes, based on the development

of the hymenium and on the form of the ascus. He divides the Euascomycetes into the Plectascales, Ascohymeniales and Ascoloculares, and members of the Pyrenomycetes sensu Lindau and Winter, are distributed among all three orders. The Plectascales comprise those Ascomycetes which possess spherical to globose asci developed in a spherical, closed fruit body. The remaining Euascomycetes, which have cylindrical to clavate asci, are contained in the Ascoloculares and in the Ascohymeniales. The Ascoloculares comprise all those forms in which the asci develop in a stroma and are not in a well defined hymenium, and in which there are no free paraphyses in the fruit body cavity. The Ascohymeniales comprise the remaining Euascomycetes; the Pyrenomycetes included in this order are those which possess a walled fruit-body in which the asci arise in a well defined hymenial layer.

Miller (1949) reviews Nannfeldt's proposals and puts forward his own scheme which is basically similar. Miller retains the Pyrenomycetes as a sub-class, but includes in it only - 'all fungi in which the asci are borne in a parallel series in a closed fruit body which ultimately opens by a definite pore or slit'. In this sub-class he recognises two distinct groups :- I) fungi with single walled asci which open by an apical pore and develop in a perithecium,

and 2) fungi with two walled asci which open by the splitting of the outer wall and extrusion of the inner one, and which develop in a stroma; there is no perithecium. A perithecium is defined by Miller as a walled fruit body in which asci develop in a well defined hymenium and which opens by a periphysate ostiole. In the second group, which is equivalent to Nannfeldt's Ascoloculares, Miller recognises two developmental types. In one the asci arise in a basal layer in the locule and grow up among pseudoparaphyses, which are vertically orientated hyphal filaments attached at both top and bottom of the fruit body cavity: this developmental type is used to characterise the order Pseudosphaeriales. In the second type of development, found in the order Dothideales, there are no pseudoparaphyses and the asci grow up in a fascicle from the base of the locule.

Probably the best and clearest arrangement of the Pyrenomycetes (sensu Lindau) proposed so far is that put forward by Luttrell (1951, 1955). Luttrell uses the same criteria as Nannfeldt and Miller, namely fruit body development, or more particularly centrum development, and ascus structure. He uses the term centrum for the ascogenous hyphae and asci together with the locule in which they develop. Luttrell (1951) divides the Euascomycetes into

two series on the basis of ascus structure :- the Unitunicateae, in which the ascus wall is one layered, and the Bitunicateae, where the ascus wall is two layered. Later (Luttrell 1955) he extends and revises these proposals. The main feature of this later work is the removal of the Bitunicateae from the Euascomycetes and the designation of a new sub-class of Ascomycetes - the Loculoascomycetes - to include all ascostromatic Ascomycetes which possess bitunicate asci. This sub-class is synonomous with the Bitunicateae and the Unitunicateae becomes equivalent to the Euascomycetes.

Luttrell bases the subdivisions of the Loculoascomycetes on characters of centrum development. He recognises the two developmental types previously described by Miller (1949). One defines the order Pleosporales and the other the order Dothideales. In the former, vertically orientated pseudoparaphyses, attached at top and bottom of the fruit body cavity, arise before the asci and remain between them at maturity. In the centrum of the Dothideales there are no pseudoparaphyses; the sterile tissue in the locule is pseudoparenchymatous and disintegrates as the asci grow up into it. The result is an unwalled cavity filled with the mature asci.

Much more evidence is required before an adequate

appraisal of these recent proposals of pyrenomycete classification can be made. New information on the development of individual Pyrenomycetes is needed, for it is only by studying the development of a large number of different fungi that types of development can be recognised, and their value in classification assessed.

Nomenclature.

Mycosphaerella was proposed by Johanson (I884) to replace Sphaerella because the latter name was first used by Sommerfeldt (I824) to describe an alga. While there was some opposition to this change (Wakefield I939), Mycosphaerella Johanson has now been adopted. (Ainsworth and Bisby I954).

There has also been some controversy about the validity of the name Venturia for the apple scab fungus and its allies, Müller and Arx (I950), Munk (I953), Korf (I956). Korf (I956) reviews the literature on these fungi and shows that while good authority can be found for placing them in four genera - Venturia, Spilosticta, Endostigme and Phaeosphaerella, a strict application of the Code of Botanical Nomenclature makes Phaeosphaerella Karst. the valid name. However Venturia is the name almost universally used and Korf proposes that to avoid - 'lasting confusion and chaos' - Venturia Sacc. I892 should be conserved, with Venturia inaequalis as lectotype. This proposed conservation of the name Venturia is also supported by Müller and Menon (I955).

For the purposes of the present work the conservation of Venturia as proposed by Korf will be adopted.

In the nineteenth century the presence or absence of appendages on the fruit body was regarded as an important character in the classification of the simple sphaeriacious fungi with two-celled ascospores. If the fruit body possessed appendages the fungus was placed in the genus Venturia, and if no appendages were present, in Sphaerella (Mycosphaerella). Another character used at this time to separate the two genera was presence or absence of paraphyses. Paraphyses are given as characteristic of Venturia (Winter I887; Lindau I897), and their absence as characteristic of Mycosphaerella (Lindau I897).

In recent schemes of classification, the systematic positions of the two genera Venturia and Mycosphaerella are generally clearly separated.

In the classifications proposed by Miller (I949) and Luttrell (I95I, I955) Venturia is included in an order characterised by a centrum in which the sterile tissue is composed of pseudoparaphyses which arise before the asci and remain between them at maturity. Mycosphaerella on the other hand is included in an order characterised by a centrum in which there are no pseudoparaphyses and in which the sterile tissue is pseudoparenchymatous and is non persistent.

Müller and Arx (1950) use somewhat different criteria in their scheme of classification. They include Venturia and Mycosphaerella in the same order. It is only at family level that the two genera are separated. Venturia is included in the Venturiaceae and Mycosphaerella in the Mycosphaerellaceae. Centrum characters are not used to separate these two families, and the division between them is not very clearly defined. The diagnostic character for the Venturiaceae is given as the possession of hyaline, coloured two-celled ascospores, initially pale green, but becoming olive green or olive brown when mature. The two-celled ascospores of the Mycosphaerellaceae remain colourless. Müller and Arx themselves point out that spore colour is not a particularly good taxonomic character, but consider that in this instance its use is justifiable.

The fungus Mycosphaerella rumicis is first recorded and described by Desmazières (I843) as Sphaeria rumicis, and then later by Cooke (I866) as Sphaerella rumicis. The two descriptions are almost identical; neither includes spines or appendages on the fruit body nor mention paraphyses in the fruit body cavity. The only difference in the two descriptions is that Desmazières records the fact that the ascus wall is two-layered.

Venturia rumicis is the name used by Winter (I887) in his description of the fungus in Rabenhorst's 'Kryptogamen-flora'. He records spines on the fruit body and also the presence of paraphyses, and it is for these reasons that he places the fungus in Venturia.

Lindau (I900) and Laibach (I92I) both use the combination Mycosphaerella rumicis in discussing the relationship of the fungus with the hyphomycete Ovularia obliqua: Grove (I933) uses the same combination in describing a form of the fungus which he found on the stem of Rumex pulcher. It is not clear from Grove's account how this new form differs from the species.

On the continent the name Venturia rumicis (Desm.) Wint. has generally been adopted, e.g. Munk (I950), Müller and Arx (I950), while in British mycological literature

Mycosphaerella rumicis (Desm.) Cooke has tended to be used, e.g. Grove (1933), Mason and Bisby (1940), and this name is still used in the official foray lists ( Trans. Brit. mycol. Soc. 39 1956 )

Laibach (1921) records a further complication in the nomenclature of Mycosphaerella rumicis; he reports that Schröter (1908) and Migula (1913) call the fungus Stigmatea rumicis, and he himself favours this name. Their reasons for adopting the name Stigmatea rumicis are based on the habit of the fungus and the fact that they did not find spines on the fruit body. The fungus is found only on the living leaves of its host and this is a feature they consider to be more usual in Stigmatea than in Mycosphaerella and Venturia. In many species of Venturia and Mycosphaerella the conidial stage is found on the living host and the perfect state is not formed until the host tissue is dead.

However Stigmatea rumicis has never been accepted. The dimidiate fruit bodies of Stigmatea are so characteristic, that even in the more recent schemes of classification (e.g. Luttrell 1955), this character separates the genus Stigmatea from genera with the spherical type of fruit body.

Another controversy which has arisen with respect to Mycosphaerella rumicis, is the association with it of the hyphomycete Ovularia obliqua (Cke.) Oud.

The fungus Ovularia obliqua is initially described as Ovularia obliqua by Oudmans (I883) and first reported to be the conidial stage of Mycosphaerella rumicis by Fückel (I869). Lindau (I900) and Langeron (I945) repeat this assertion. However in a detailed account of Ovularia obliqua, Laibach (I92I) reports that the perfect stage of the fungus is a Mycosphaerella-type, but is quite unlike Mycosphaerella rumicis; he calls it Ovosphaerella lapathi. He found this perfect stage on the under surface of Ovularia obliqua lesions on overwintered leaves of the host. Laibach confirmed that he had found the perfect stage of Ovularia obliqua, by obtaining a culture of the fungus from a single ascospore isolated from the perfect stage. He also inoculated leaves of the host with a suspension of asci and ascospores of Ovosphaerella lapathi, and obtained Ovularia obliqua lesions within twelve days. The method used to obtain the ascospore suspension would seem to make it very difficult to ensure that no conidia of Ovularia obliqua were present.

However while the work of Laibach was not repeated, it is shown later (p. 35 ) that similarity of host is almost

certainly the only connection between Ovularia obliqua and Mycosphaerella rumicis.

The foregoing account of pyrenomycete classification, and of the nomenclature of *Mycosphaerella rumicis* show that a detailed investigation of *Mycosphaerella rumicis*, paying particular attention to centrum development, is necessary before the taxonomic position and nomenclature of the fungus can be clarified.

A comprehensive study of *Mycosphaerella rumicis* has been carried out in this present work and is described in section III. In addition the centrum of four fungi related to *Mycosphaerella rumicis* has been investigated, and descriptions of the centrum of these four fungi (*Pleospora herbarum*, *Venturia inaequalis*, *V. pirina* and *Mycosphaerella maculiformis*) are given in section IV.

In section V the results of these investigations are discussed and are used to establish what the author considers to be the correct nomenclature and taxonomic position of *Mycosphaerella rumicis*.

## II. MATERIALS AND METHODS.

A. Sources of Material.

B. Methods.

| Fungus | Host | State of Host |
|---|---|---|
| Mycosphaerella rubicola | Rumex crispus<br>R. obtusifolius<br>R. conglomeratus | On living stems and leaves; throughout the year. |
| M. maculiformis | Quercus sessiliflora<br>Quercus robur<br>Castanea sativa<br>Aesculus hippocastanum | On dead, overwintered leaves; April, May, June. |
| Venturia inaequalis | Malus communis | On dead, overwintered leaves; April. |
| V. pirina | Pyrus communis | " |
| Plaeospora herbarum | Rumex obtusifolius | On dead leaf. |
| Leptosphaeria acuta | Urtica dioica | On dead stems; June. |
| Ovularia obliqua | Rumex crispus<br>R. obtusifolius | On living leaves; throughout the year. |

Mycosphaerella rubicola, Ovularia obliqua and Plaeospora herbarum were grown in culture, and stock cultures for experiment were kept on 2% malt extract agar.

A. Sources of Material.

All the fungi studied in this work were obtained from the grounds of The Royal Holloway College, Surrey. Table I lists the fungi and their respective hosts.

Table I. Fungi and their Hosts.

| Fungus | Host | State of Host |
|---|---|---|
| Mycosphaerella rumicis | Rumex crispus<br>R.obtusifolius<br>R.sanguineus | On living stems and leaves; throughout the year. |
| M. maculiformis | Fagus sylvaticus<br>Quercus robur<br>Castanea sativa<br>Aesculus hippo-castanum | On dead, overwintered leaves; April, May, June. |
| Venturia inaequalis | Malus communis | On dead, overwintered leaves; April. |
| V. pirina | Pyrus communis | " |
| Pleospora herbarum | Rumex obtusifolius | On dead leaf. |
| Leptosphaeria acuta | Urtica dioica | On dead stems; June. |
| Ovularia obliqua | Rumex crispus<br>R.obtusifolius | On living leaves; throughout the year. |

Mycosphaerella rumicis, Ovularia obliqua and Pleospora herbarum were grown in culture, and stock cultures for experiment were kept on 2% malt extract agar.

B. Methods.

I. Media.

Growth of Mycosphaerella rumicis took place on all the media listed in Table 2.

Table 2. Media.

(I) 'Natural' agar media.

Malt extract 0.5 - 5%
Radiomalt extract 0.5 - 5%
Malt extract 0.5% + dockleaf extract
Malt extract 0.5% + portions of dockleaf
Malt extract 2% + sucrose 2%
Potato extract
Potato dextrose
Plain water agar + portions of dockleaf

(2) Synthetic agar media.

Czapek-Dox :-

| | |
|---|---|
| sodium nitrate ($NaNO_3$) | 0.2% |
| potassium phosphate ($K_2HPO_4$) | 0.1% |
| potassium ghloride (KCl) | 0.05% |
| magnesium sulphate ($MgSO_4 7H_2O$) | 0.05% |
| ferrous sulphate ($FeSO_4$) | 0.001% |
| sucrose | 3% |

Westergaard-Mitchell :-

| | |
|---|---|
| potassium nitrate ($KNO_3$) | 0.1% |
| potassium phosphate ($KH_2PO_4$) | 0.1% |
| sodium chloride (NaCl) | 0.01% |
| magnesium sulphate ($MgSO_4 7H_2O$) | 0.05% |
| calcium chloride ($CaCl_2$) | 0.01% |
| yeast extract | 0.01% |
| sucrose | 2% |

(3) Liquid media.

Malt extract 2%
Czapek-Dox as above.

2. Collection of Ascospores.

Ascospores of Mycosphaerella rumicis are actively discharged from the fruit body and can be collected by suspending a 'collecting' slide over ripe fruit bodies.

Diseased host material or cultures of the fungus with ripe fruit bodies, were placed in a sterile moist chamber and a 'collecting' slide held over them. The slide was held in position by a perspex ring. The moist chamber was a petrie-dish, lined with a strip of asbestos which was saturated with water.

When spores were collected for the purpose of culturing the fungus, the collecting slide was sterilised and a thin layer of 3% plain water agar placed on it before it was inverted over the fruit bodies of the fungus. The slide was left in position for twelve hours and then, with the aid of a binocular microscope, the spores were removed with a sterile needle.

When the spores were required for plant inoculation, the slide was cleaned and sterilised before suspending it over the source of ascospores. It was left in position for twentyfour hours and then examined under a binocular microscope, to verify that spores were present. The spores were then transferred to the surface of the plant to be inoculated.

When distance to which spores were discharged was being investigated, the surface of the collecting slide was vaselined to insure that spores reaching the slide were caught. The slide was not supported on a ring as before, but attached with vaseline to the lid of the moist chamber. Height of the slide was varied by sticking two or more slides together, (thickness of slides = 0.5mm.).

3. Cultures.

Ascospores of Mycosphaerella rumicis were used to make cultures of the fungus. A monospore culture was made by picking up a single spore from the agar collecting slide and transferring it to nutrient medium. It was possible to isolate the eight spores from one ascus by raising the collecting slide to such a height that on examining it, groups of eight ascospores were found. In some of these groups each spore was sufficiently isolated from each other to enable the spores to be picked up singly. When equivalent monospore cultures were required, a mature monospore culture was used as the source of ascospores for the inoculum, and the collecting slide was suspended for not more than twelve hours, which insured that the ascospores were approximately the same age.

In mature cultures of Mycosphaerella rumicis, the ascospores are forcibly discharged and land on the lid and sides of the culture vessel. Regular examination of the sides of the tube, in a tube culture, or the lid of a plate culture, enabled one to determine the time at which mature asci were developed in a culture. Another useful criterion for ascus maturity was the appearance of secondary spore colonies in a culture.

When making liquid cultures of the fungus, 100ml. flasks, each containing 10ml. of medium, were inoculated either by ascospores from agar collecting slides, or by a suspension of ascospores and mycelium. The suspension was prepared by shaking up a mature culture of the fungus with sterile water, and centrifuging to remove the larger fragments; 0.5ml. of suspension were added to each flask.

4. Plant Inoculations.

For experimental infections of host plants with Mycosphaerella rumicis, ascospores were used as inoculum. The ascospores were usually collected on a slide and transferred from it to the host, either with a brush, or by rubbing the slide over the surface of the host.

When detached stems or leaves were inoculated, they were first of all washed thoroughly in running tap water, then in sterile distilled water and placed in a moist chamber, with the surface to be inoculated facing upwards. If it were necessary to keep the inoculated surface facing downwards, then the stem or leaf was suspended on glass rods placed on the bottom of the chamber.

When attached leaves were inoculated, the leaves were inoculated as before, and then either/the whole plant was covered with a bell jar, or the inoculated leaf was enclosed in a polythene bag. If the plant were placed under a bell jar, it was sprayed with sterile water, at approximately two and four hours after inoculation, so that the leaves were kept moist.

Occasionally attached leaves were inoculated by using diseased leaves directly as the source of inoculum. Portions

of diseased leaves containing ripe fruit bodies, were placed on the surface of the plant to be infected; they were placed so that the fruit bodies opened on to the surface of the plant, and it was assumed that infection was accomplished by ascospores discharged from these fruit bodies. The plant was then sprayed with water and covered either with polythene or a bell jar. The portions of diseased leaf were removed after twentyfour hours.

5. Investigations of Structure and Development.

Several methods were used to investigate the development of Mycosphaerella rumicis in its Rumex host. The initial stages of infection were studied, either by taking epidermal strips of inoculated leaves, or by clearing such leaves in chloral hydrate. The lower epidermis was more easily stripped than the upper one, and therefore when infections were being investigated by the epidermal strip method it was the lower surface which was inoculated. When clearing a leaf in chloral hydrate, McBryde's (I936) technique was used. The host-parasite relationship in the later stages of infection was studied by sectioning diseased tissue; hand or freezing microtome sections of fresh material were used for this.

Development and structure of the fruit bodies of Mycosphaerella rumicis, and of the four other fungi studied in the present work, were investigated by making sections of the fruit bodies. Sectioning was carried out on fresh and fixed material, either by hand or with a freezing microtome Microtome sections were also made of wax-embedded material.

Cotton blue in lactophenol proved to be the most satisfactory stain to use, both for the study of host-parasite relatioships, and for the investigations of the development of the fungi.

## III GROWTH, DEVELOPMENT AND LIFE HISTORY OF MYCOSPHAERELLA RUMICIS.

Introduction

A. The relationship of Ovularia obliqua and Mycosphaerella rumicis.

B. Infection and growth of Mycosphaerella rumicis in host.

C. Growth and development of Mycosphaerella rumicis in culture.

D. Development and structure of fruit body of Mycosphaerella rumicis, including development and structure of fruit body centrum.

E. Structure of ascus and ascospore discharge.

Introduction.

Mycosphaerella rumicis causes a leaf-spotting disease of Rumex species. It also attacks the stems and petioles of its host, where elongated lesions are formed. Very frequently the spots have a red border, which makes them very distinctive and easily noticed. Red bordered spots are not unique to Mycosphaerella rumicis; they are often formed by other Rumex leaf pathogens, and can sometimes be induced by wounding a leaf. These red areas of the host are caused by the sap of its cells turning red. Examination of a spot shows a brown area of dead leaf tissue containing a number of black fruit bodies of the fungus. The oldest and largest fruit bodies are in the centre of the spot and the others are arranged more or less concentrically in the dead host tissue, figure 8. At the edge of a spot, young fruit bodies may be seen developing in leaf tissue which is still green; in fact when the diseased leaf is beginning to turn yellow, the area at the edge of infected spots often remains much greener than the rest of the leaf. Sometimes there are no fruit bodies in the centre of a spot and then the fruit bodies are arranged around a central area of dead tissue. A 'shot hole' type of spot is sometimes found when the central dead tissue has disintegrated.

Diseased leaves are found all the year round, the fungus seems to thrive especially in cool wet conditions, and the disease is least prevalent in hot dry weather.

The fungus has been recorded on most species of Rumex, but not on Rumex acetosa or R. acetosella. It is reported to be widespread in Europe (Lindau 1897), and has been widely recorded in Great Britain.

Mycosphaerella rumicis is readily obtainable in culture and has continued to produce a perfect stage through continued sub culturing. Despite all attempts to induce asexual reproduction, no conidial stage of the fungus has ever been found either on the host or in culture.

A. The relationship of Ovularia obliqua and Mycosphaerella rumicis.

Before proceeding to the detailed account of Mycosphaerella rumicis it will be convenient to consider and establish the position of Ovularia obliqua.

Ovularia obliqua has at times been considered to be the perfect stage of Mycosphaerella rumicis (see p. 17); it causes a leaf-spotting disease of Rumex species, and like Mycosphaerella rumicis has not been found on Rumex acetosa and R. acetosella. The spots, which may or may not be red bordered, are at first like those caused by Mycosphaerella rumicis, but gradually the diseased area widens, and often, especially in moist conditions, the spot may cover a large area of leaf. The dead leaf tissue in the centre of a large spot often disintegrates. An examination of the under surface of a spot shows the conidiophores and conidia appearing as a white powder over the decayed leaf tissue. The conidiophores arise from aggregations of hyphae, which develop in the sub stomatal chambers of the host, grow up through the stomata and project from the leaf surface as brush-like clumps (figure IA). The young conidiophore is a thick straight hypha, which tapers slightly at its tip. The conidia are borne singly, though occasionally in moist

conditions a short chain of spores may be observed. They develop at the tip of the conidiophore, but soon become lateral due to the continued growth of the conidiophore, which itself becomes bent. Figure IC shows development of a conidium. The conidium is unicellular, elongate-oval in shape and white to pink in colour. It germinates very rapidly on a moist slide; more than one germ tube may arise from one spore, the first formed germ tube frequently developing from the pointed end of the spore. As Laibach (I92I) observed, a germ tube may develop directly into a conidiophore, figure IB.

All attempts to obtain a perfect stage of Ovularia obliqua were unsuccessful. It proved very difficult to collect overwintered Rumex leaves in their natural surroundings, for the fallen leaves disintegrate very rapidly. Some overwintered leaves were collected and examined in the Spring, but no perfect stage was found in any of the Ovularia lesions. Similarly when Ovularia obliqua infected leaves were collected in the Autumn and overwintered, either in the laboratory, or out of doors, or in a refrigerator for three months and then in the laboratory, no perfect stage of the fungus was formed in the Ovularia obliqua infected lesions.

In further attempts to obtain a perfect stage, the fungus was grown in agar culture media, and the cultures were subjected to various conditions. The composition, concentration and pH of the medium were varied and so also was the circum-ambient temperature. Ovularia obliqua grew and reproduced its conidial stage on all the media used. Small, spherical, dark brown sclerotia were subsequently developed on most media, but no perfect stage was seen in any of the cultures.

A perfect stage of Ovularia obliqua was not obtained during the present investigation; the only connection which has been demonstrated between the fungus and Mycosphaerella rumicis is identity of host. The two fungi parasitise species of Rumex, but while Ovularia obliqua exists and reproduces, on the host and in culture, in its conidial stage, Mycosphaerella rumicis is known only in its perfect stage, which develops on the host and in culture. No evidence whatever was found for associating the conidial stage of Ovularia obliqua and the perfect stage of Mycosphaerella rumicis.

Ovularia obliqua is therefore excluded from the present investigation of Mycosphaerella rumicis.

B. Infection and growth of Mycosphaerella rumicis in host.

Infection takes place if detached Rumex leaves are placed in moist chambers and inoculated, either on the upper or on the lower surface, with an ascospore suspension of Mycosphaerella rumicis.

Table 3 shows the results of an experiment carried out at room temperature (average daily reading I4°C). The ascospores used for inoculation were obtained from infected leaves of twenty different Rumex plants. The leaves which were inoculated were from twenty other plants.

Table 3. Infection of Rumex leaves by Mycosphaerella rumicis.

| Position of inoculum | No. of leaves inoculated | No. of leaves infected | Time, in days, for infection to appear |
|---|---|---|---|
| Upper epidermis | IO | 9 | Minimum I2<br>Maximum I3 |
| Lower epidermis | IO | 9 | Minimum I2<br>Maximum I3 |

These results show that the ascospores can accomplish infection, and that infection takes place equally readily

via the upper or lower epidermis. They also show that at least in the conditions of this experiment, different sources of host and pathogen have no noticeable effect on speed of infection.

When pieces of Rumex stem were inoculated with ascospores of Mycosphaerella rumicis, obtained from infected leaves, infection of the stems took place. This result shows that the same fungus can infect both stem and leaf, and casts doubt on the validity of Grove's stem form of the species (c.f.p. 15 ).

The ascospore is the sole agent by which Mycosphaerella rumicis spreads from host to host. The ascospore germinates very rapidly; on a moist slide one may germinate within eight hours of its liberation from the ascus. Such rapid germination is a character often associated with asexual spores rather than with ascospores.

As the results given in table 3 show, infection of Rumex leaves by Mycosphaerella rumicis is a fairly rapid process, and this together with the fact that a large number of ascospores are produced throughout the year, enables the fungus to survive very successfully, and that without the production of accessory spores.

Growth of fungus in host.

The ascospore, which consists of two cells of unequal size, see page 54, germinates on the surface of the host. Usually only the larger cell produces a germ tube, but two germ tubes, one from each cell, may develop, and penetration of the host by two such germ tubes has been observed. The germ tube is short, seldom extending far beyond the spore before penetrating the surface of the host (see figures 4 and 5); long germ tubes have never been seen to penetrate the host.

The germ tube pierces the cuticle and a sub cuticular mycelium is developed. This mycelium radiates out from the point of entry, the hyphae forming an almost continuous sheet, figures 4 and 5. Although the individual epidermal cells are not penetrated by the fungus, the epidermis does not prove an effective barrier, for hyphae from the sub cuticular mycelium grow down between the cells and form a sub epidermal mycelium from which hyphae grow out and invade the other tissues of the host.

While the germ tube of the fungus does not appear to penetrate the epidermal cell walls, it seems to alter them in some way. When epidermal strip, or cleared leaf preparations were stained with cotton blue, small greenish-

blue areas were seen in the epidermal wall, just below where the cuticle was pierced by the germ tube of the fungus. Figure 2 B, a drawing of cleared leaf preparations, shows some such stained areas in the epidermal walls, and in the epidermal strip preparations, drawn in figure 3, similar stained areas are shown. These blue stained areas are somewhat similar to the ones found by Corner (1935), when he was investigating penetration of epidermal cells by haustoria of powdery mildews. Corner found, even in some resistant hosts, where the cuticle but not the wall of the epidermal cell was pierced by the haustorium, that an alteration in the epidermal cell wall could be detected by staining with cotton blue. By carrying out mic-rochemical tests, Corner was able to demonstrate that the cellulose of the cell wall was altered in the areas which stained blue with cotton blue. The nature of the change caused by the germ tubes of Mycosphaerella rumicis has not been demonstrated.

In many of the epidermal strip preparations it was found that the anticlinal walls of some epidermal cells also stained blue with cotton blue. As shown in figures 3 and 4, the sub cuticular hyphae grow above the junction of the epidermal cells and it is here that the staining of the anticlinal walls is noticed. The alterations in these

walls possibly results from the growth of the fungus between the epidermal cells. Figure 2 $A_2$ shows the growth of the pathogen between two epidermal cells, a thickening of the cell walls has occurred; a similar thickening of a periclinal wall is shown in figure 2 $A_2$.

When the hyphae have passed through the epidermal layer, they grow extensively immediately below the epidermis. Figure 6 A shows an epidermis invaded by the fungus, sub cuticular, sub epidermal and intercellular hyphae are shown. In figure 5 a stage of infection is shown where there is considerable development of a sub epidermal mycelium, which has extended over a wider field than has the sub cuticular mycelium. When the epidermal cells become surrounded by hyphae, they often become filled with tannin before they break down. The sub cuticular and sub epidermal hyphae then come together and form a stromatic layer below the cuticle.

The other tissues of the host are similarly invaded. At first the hyphae grow between the host cells, causing little disorganisation; figures 6 and 7 illustrate the intercellular growth of the pathogen. In 6 B invasion of mesophyll by hyphae which have grown out from the sub epidermal mycelium, is shown, and in figure 6 C, where palisade tissue of

a leaf is viewed from above, the intercellular growth of the hyphae is clearly seen. Figures 6 D and 7 are of transverse sections of infected Rumex stems, and they show the intercellular invasion by the pathogen of parenchyma and collenchyma tissues of the cortex.

The host cells eventually become disorganised, and the hyphae tend to come together and form a loose stroma, which is most apparent in regions where fruit bodies are developing.

The mycelium in the host is composed of relatively broad hyphae which branch frequently. It does not appear to be restricted in any way at the edge of a leaf spot, for the hyphae are always in advance of the dead leaf tissue. It is in this region of advancing hyphae that the leaf tissue is often greener than in the rest of the leaf. The red areas round some spots do not appear to impede the advance of the fungus. When grown on dead sterilised leaves and stems of Rumex, the fungus was able to cover the whole tissue, provided the substrate was not allowed to dry up.

The fruit bodies of the fungus are always, at least in part, and often wholly, submerged in host tissue until mature. They begin to develop between the cells of the host, figures 6 D and 7, but as they enlarge the host tissue round about them becomes broken down and disintegrates.

When young, the fruit bodies are spherical and closed, but as maturity is approached the upper part grows out as a papilla-like structure, which, if the fruit body is still immersed in host tissue, penetrates and projects through the surface of the host. The papilla appears lighter coloured than the rest of the fruit body, which is dark brown to black in colour. When the fruit body is mature the tissue at its apex breaks down and it becomes open to the exterior by a pore of irregular shape, through which the ascospores are actively discharged.

The fruit bodies generally open on to the upper surface of the leaf. It was found, by inoculating upper and lower surfaces of leaves, that the position of the fruit body in the leaf is partly an effect of gravity.

The fruit bodies are often separate, but frequently two or more coalesce to give a compound structure. These large compound fruit bodies may have one large , or two to three smaller ostioles when mature. In the diagram of a leaf spot in figure 8 A, a compound fruit body opening by one large pore, and another one opening by two smaller pores are shown. A section of a compound fruit body which has three apical papillae is shown in figure I4.

Spine-like appendages may occur on the upper part of the fruit body, but are not always present. They are most frequently found on fruit bodies which are not deeply immersed in host tissue. In completely immersed fruit bodies, it is only as maturity is reached, that the upper part becomes free from host tissue, and this probably accounts for the frequent absence of spines on such fruit bodies. A spine is composed of a short non-septate hypha, which grows out from the surface of the fruit body, becomes thick walled, pointed and dark brown in colour, figure 9. Occasionally spines with one cross wall have been seen.

C. Growth and development of Mycosphaerella rumicis in culture.

The extreme rapidity with which the ascospores germinate, and the fact that they are actively discharged from the fruit body, make ascospores the obvious starting point for obtaining the fungus in culture.

Mycosphaerella rumicis is homothallic. From a single ascospore sown on a suitable agar medium, mature fruit bodies producing viable ascospores are obtained.

The fungus will grow on all the common agar culture media, the richer media stimulating aerial growth, but aerial growth is not extensive on any medium.

Viewed macroscopically, a monospore colony on malt extract agar at room temperature, becomes visible about five days after inoculation of the agar. At first it is a pale olive green colour, but soon becomes dark olive green, and eventually, as fruit bodies begin to be formed, turns almost black. On media where aerial growth is more evident, the colony remains the dark olive green colour. The fungus grows slowly on agar media. The spread of the fungus over the medium is hastened by the formation of new colonies, which have developed from spores discharged from fruit bodies formed in the initial colony.

The ascospore germinates on agar, as on a moist slide, by producing a germ tube from each cell of the spore (figure IOE). The largest cell is usually the first of the two to emit a germ tube. The germ tubes soon penetrate the surface of the agar and give rise to mycelium which ramifies through the medium. The hyphae branch frequently, and often several branches arise from the same point. Figures IO B, C and D show this typical type of branching. At first the hyphae are thin walled and colourless or with a slightly greenish tint; their segments may be of fairly even width, but are often constricted from each other to give an almost moniliform appearance to the hyphae. Later, when fruit body formation begins, the vegetative hyphae anastomose and compact together to form a stroma, and they become dark coloured and thick walled. The segments of these hyphae are often almost spherical. The stroma is loosely compacted at first, but as the fruit bodies mature it becomes hard and the fruit bodies become embedded in this hard stroma. Fruit body formation takes place first in the centre of the colony, hence it is here that the hard stroma is initially developed, but the stromatic region gradually becomes extended as fruit bodies continue to be formed when the fungus spreads through the medium. New fruit bodies can

develop on the stroma so that the centre of the colony becomes raised.

With a hand lens, fruit bodies can be distinguished as small black bodies in the stroma. They are most easily seen at the edge of a colony, where the stroma is less developed. The mature fruit bodies formed in agar culture are very similar to those formed on the host. The only difference is, that while fruit bodies on the host frequently lack spine-like appendages, such appendages are invariably present on fruit bodies in culture. For example, spines were developed on the fruit bodies formed in thirty monospore cultures, each derived from different sources, ten of which were fruit bodies which lacked spines. Presence or absence of spines on the fruit body can therefore have little value as a taxonomic character.

When the fungus is grown on liquid malt extract, or liquid Czapek-Dox medium, it forms colonies, and later a mat of mycelium firmly attached to the bottom of the culture vessel. The fungus remains immersed in the liquid and becomes exposed only when the culture begins to dry out. No fruit bodies were formed in these liquid cultures.

The slow growth of Mycosphaerella rumicis makes difficult

any measurement of rate of growth in culture, but a measurement of speed of fruit body development can be made relatively easily, by recording the time taken from inoculation of cultures to the production of ascospores in the cultures.

Experiments on the growth and development of Mycosphaerella rumicis in culture are described in an appendix, page 107 . They show that the fungus is a non-exacting one; it can grow and reproduce on a synthetic medium which lacks all growth factors. It can utilise sucrose and glucose as sources of carbon. It can use nitrate nitrogen and can also grow on a medium where the sole source of nitrogen is asparagin. The fungus can tolerate a wide range of pH, but the optimum for both growth and reproduction is on the acid side, about pH 4. The optimum temperature for growth and reproduction is between 14°C and 20°C. Reproduction is favoured by a low concentration of phosphate in the medium.

D. Development and structure of the fruit body of Mycosphaerella rumicis, including development and structure of the fruit body centrum.

The initial stages of fruit body formation are more easily studied in culture than in the host, and figures II A - F illustrate the early stages of fruit body development in culture. The fruit body initial is first recognised as a small hyphal knot, figures II A, C; hyphae grow out from and round this knot and the initial soon becomes a dense and spherical aggregation of hyphae, figures II D, E, F. The initial shown in figure II D is more or less spherical and the walls of the outer hyphae have become slightly thickened. In figures II E, F, later stages in development are shown, the outer hyphae are thick walled and are also dark coloured. Hyphal anastomoses (figure IO A) are frequent in regions of fruit body formation.

To study further stages in the development of the fruit body, sections have to be made, and figures I2 - I4 show a series of sections of fruit bodies of Mycosphaerella rumicis at different stages of development.

At first, when the fruit body is at the small spherical stage, shown in figure II F, a section of it reveals little

internal differentiation. Figure I2 A is of such a section and shows an outer layer of thick walled hyphae and a mass of deeply staining hyphae in the centre.

Sections of older fruit bodies (see figures I2 B - I4) show differentiation of this central tissue, and it can be seen that in the development of the centrum, asci grow up in a layer into sterile tissue which is composed of vertically orientated pseudoparaphyses. Figures I2 B - D show the initial stages in the differentiation of the centrum. In the fruit body shown in figure I2 B asci are not yet developed in the centrum but pseudoparaphyses are differentiated and are vertically orientated, thus showing that the vertical orientation of the pseudoparaphyses is not dependent on the upgrowth of the asci. Figure I2 C shows a fruit body at a later stage of development; here a layer of young asci are growing up among the pseudoparaphyses. The fruit bodies shown in figures I2 D and I3 A are rather more developed than the one shown in figure I2 C, but the asci are still not ripe and the fruit body cavity remains enclosed. In these two fruit bodies the pseudoparaphyses are very evident, especially at the apex of the locule, where their apical attachment can clearly be seen. The basal ends of the pseudoparaphyses are somewhat obscured by the developing

asci so that attachment at their basal end is not so easy to demonstrate. However, in both figures I2 D and I3 A, a few pseudoparaphyses with attached basal ends can be seen. Figure I2 E is of a transverse section of a fruit body which is at about the same stage of development as the one drawn in figure I3 A, and it gives a good picture of the arrangement of the pseudoparaphyses between the asci, and also shows their vertical orientation.

While in the vertical sections of developing fruit bodies, the pseudoparaphyses are usually most prominent at the apex of the locule, no evidence was found to show that the pseudoparaphyses arise apically and grow downwards, as suggested by Miller (1949) and Luttrell (1951). The first formed pseudoparaphyses appear to be permanently attached at both ends, figure I2 B, but it is difficult to visualise how for example, a centrum as in figure I2 B, could develop to give a centrum as in figure I2 D, without further development of pseudoparaphyses from apex or base of the locule. If such development takes place, naturally occurring free ends to the pseudoparaphyses ought to be found in vertical sections of the fruit bodies. Free ends to pseudoparaphyses are however difficult to see, and when seen, difficult, if not impossible to interpret. From the many

preparations of Mycosphaerella rumicis which were examined in the present work, it was found impossible to determine the exact way in which the pseudoparaphyses arise and develop. It seems unlikely that examination of more fruit body sections will solve the problem; the difficulty of interpretation appears to be insurmountable.

As the fruit body matures it develops a papilla-like projection at its apex, the tissue in the centre of which breaks down to form an irregular ostiole which exposes the fruit body cavity to the atmosphere. Figure I3 B shows a section of a mature fruit body; the tissue at the top of the cavity has broken down and the centrum is open to the exterior. Asci are ripe and some elongated 'extension tubes' (see p. 56 ) are evident. The pseudoparaphyses are rather obscured by the asci; some are free at their apical end, a condition which could be the result of, either the formation of the fruit body ostiole,or merely because the pseudoparaphyses were cut in making the section. It is in sections like this that pseudoparaphyses can easily be confused with true paraphyses. Figure I4 is also of a vertical section of a mature fruit body; the fruit body is a compound one with three apical papillae,and is a good illustration of the stromatic nature of the fruit body of Mycosphaerella rumicis.

The asci in a fruit body do not all ripen at the same time, see for example figures I2 - I4; when the ascus is mature, and under suitable conditions, it extends through the ostiole of the fruit body and the ascospores are forcibly discharged. The structure of the ascus and ascospore liberation are fully described later (p. 55 ).

There are eight ascospores in an ascus. The ascospores are two celled, the larger cell always points towards the apex of the ascus. The broadest part of the spore is in the middle of this larger cell which is rounded at its apex; the smaller cell tapers to a point. The spore is slightly constricted at the septum. Initially it is colourless, but when mature has a slight olive green tint. If, because of unsuitable conditions, the spores are not liberated when first mature, they become darker coloured, often brownish, but they still remain hyaline.

Ascospore measurements based on I00 spores were as follows :-

Length . . .range I6.6 - 22.5 µ average I9.5 µ
Breadth . . range 6.6 - 9.9 µ average 8.I µ
(at widest part)

E. Structure of the ascus and ascospore discharge.

All accounts of spore discharge from the bitunicate ascus agree on the main points of the process. The first step is the splitting of the outer layer of the ascus wall, and this is followed immediately by the rapid extension of the inner layer. When the inner layer has fully extended, the ascospores are forcibly discharged from the apex of the ascus. Ingold (1953) gives a general account of spore discharge from the bitunicate ascus. He describes the process in Sporormia intermedia and refers to good accounts of the process in Pleospora scirpicola, by Pringsheim (1858) and in Leptosphaeria acuta, by Hodgetts (1917). In Sporormia intermedia the inner wall extends until it bursts and the eight ascospores are discharged simultaneously. In Pleospora scirpicola and in Leptoshaeria acuta the eight ascospores are disharged successively from the tip of the extended ascus; in the former, the process takes place in water, but in Leptosphaeria acuta no active release of spores from the ascus occurs when the fruit bodies are immersed in water, the process occurring only in humid conditions.

The structure of the bitunicate ascus can be appreciated fully only after studying spore discharge from the ascus, hence the account of ascospore discharge in Mycosphaerella rumicis is given before the structure of the ascus is described.

I. Discharge of ascospores.

Humid conditions are essential for the process of ascospore discharge in Mycosphaerella rumicis, to take place. Discharge occurs and can be watched in comparatively normal conditions, when fruit bodies of the fungus (either on the host or in culture), are placed in a glass ring moist chamber and viewed under a microscope. A more drastic way of obtaining ascospore discharge is to mount squashed fruit bodies in water on a slide. This second method enables more detail of the discharge process to be seen, and the process as seen in a water mount is now described.

The first indication that spore discharge is about to occur is a slight overall enlargement of the ascus, with the ascospores seeming to become suspended in the middle of the ascus and not touching the wall, figure I5 A. Suddenly the outer layer of the ascus wall splits at the apex and the inner layer, surrounding the spores, extends smoothly but rapidly to form a long 'extension tube', about twice the original length of the ascus, figure I5 B; the spores now lie in a row in the extension tube. The outer layer of the ascus wall remains as a rather wrinkled sleeve at the base of the extended inner layer, figure I5 B. The ascus may remain in this elongated condition for two or

three minutes before its spores are liberated, but more frequently spore liberation begins as soon as the ascus is fully extended. At the tip of the extension tube there is a thin area which protrudes slightly as a small papilla when the ascus is fully extended. When spore release is iminent the apical spore moves up so that its blunt end fits into the papilla, figure I5 C. Pressure within the ascus gradually forces this spore through the papilla which opens to form a pore. The spore is forced through until its broadest part is through and then it is violently ejected. The next spore immediately fills the apex of the ascus and since it fails to pass through at once, it can be assumed that the wall at the tip of the ascus is elastic and the apical pore closes or is at least partly reduced in size immediately a spore is released. The next and following spores are released successively in a similar manner: the process continuing until all the eight spores are liberated. Ejection of one spore and the replacement of it in the tip of the ascus by the next spore follows so rapidly that it is possible only to see the result. When a spore is ejected the ascus contracts slightly, but elongates again before the next spore is released: this retraction and elongation make the process appear jerky. The ascospores are always

ejected singly; all eight may discharged one immediately after the other, or there may be a time lag between the release of each spore; again, some may be released in quick succession followed by a delay before the rest are discharged. Immediately the eighth spore is released, the extension tube of the ascus collapses and a considerable swelling of the inner layer of the ascus wall is now observed, figure I5 D. The apical pore is now clearly visible. Occasionally asci are seen in which the outer layer of the wall has split, other than at the apex, but no release of spores from such asci has been witnessed. This abnormal splitting is probably caused by the rough treatment to which the asci are subjected when squashing the fruit body. Figure I8 J shows such an ascus, the outer layer of the wall has split irregularly and part of it remains as an apical cap.

When viewing ascospore discharge from an unmutilated fruit body mounted in a moist chamber, the only part of the ascus which can be seen is the extension tube. Figures I6 A - D show views of extension tubes obtained by this method. The extension tube slides out rapidly and either discharges its spores straight away, or may remain projecting for some minutes before doing so. The spores may sometimes be seen in the extended ascus, figure I6 $A_2$. When spore

liberation takes place the apical spore is forced part way through the tip of the ascus, figure I6 B, and then is liberated with a jerk; the ascus contracts as a spore is liberated and elongates again before the discharge of the next spore. The retraction and subsequent elongation of the ascus are not quite complementary, with the result that the extension tube becomes slightly shorter as the eight spores are released. This is indicated in figure I7 which summarises the successive release of two ascospores from an extension tube. The discharge of the eight ascospores from one ascus may be continuous, or it may be spasmodic: immediately the eighth spore is released the extension tube collapses completely, frequently falling back into the fruit body cavity; it may collapse sideways and lie on the edge of the mouth of the fruit body. Some asci were observed to collapse before all their eight ascospores were discharged; this premature collapse often occurred when the humidity of the moist chamber had been allowed to fall. The ascus just sinks slowly sideways - there is not a rapid and complete collapse such as occurs at the termination of the normal discharge process. If moist conditions are restored these prematurely collapsed asci are able to extend again, but no spore release from such re-extended asci has been seen. Figure I6 C shows a series

of drawings of one ascus as it slowly collapsed when the coverslip was removed from the moist chamber, which surrounded the fruit body. Figure I6 D shows a prematurely collapsed ascus and its subsequent re-extended position.

More than one ascus can discharge ascospores from a fruit body at the same time; figure I6 B shows two such asci. As many as five extension tubes have been seen protruding from one fruit body simultaneously.

The ascospores are shot a considerable distance from the fruit body, and height to which the spores were discharged was determined by suspending vaselined slides at various distances from infected leaves. (See p. 25 ). The slides were examined after being suspended for twenty-four hours. Twentyfive leaves were used and a complete range of distances were used for each leaf. The results are given in table 4,which is on the next page. It can be seen from the results that the ascospores are discharged a sufficient distance from the leaf to enable them, either to land directly on another leaf, or to get carried off and dispersed in air currents.

Table 4. Heights to which ascospores of Mycosphaerella rumicis are discharged.

| Distance of slide from leaf, in cms. | No. of slides on which spores landed, out of 25. |
|---|---|
| I.2 | 25 |
| I.3 | 22 |
| I.4 | II |
| I.5 | 2 |
| I.6 | 0 |

Ascospore discharge exhibits no periodicity to light, it can take place under conditions of continuous dark or of continuous light.

The account of spore discharge in Mycosphaerella rumicis agrees in most details with the accounts of discharge in Leptosphaeria acuta and Pleospora scirpicola, by Hodgetts(I9I7) and Pringsheim (I858). In both Mycosphaerella rumicis and Pleospora scirpicola spore discharge occurs when asci are mounted in water; Hodgetts found that with Leptosphaeria acuta, extension of the inner layer took place in water mounts, but the spores were not released. He considered that under these conditions the inner layer became over extended, and as a result ascospore discharge could not occur. Water mounts of asci of Leptosphaeria acuta were examined for comparison with Mycosphaerella rumicis, and it was found that the inner layer of the wall of the ascus extended very rapidly and remained in that extended condition; in many of these extended asci, after a few minutes, the ascospores suddenly shot down to the base of the ascus, indicating a release of pressure at the base. This phenomenon was never found in Mycosphaerella rumicis.

The simultaneous discharge of ascospores from several asci in one fruit body, which was seen in Mycosphaerella rumicis was not recorded by Hodgetts (I9I7) and Pringsheim (I858). It seems likely that in Mycosphaerella rumicis this simultaneous discharge is possible because the fruit body

has no appreciable neck and opens by a rather wide irregular ostiole. As Ingold (1953) points out, in fruit bodies with well developed necks, the neck often has a narrow canal, which permits the emergence of only one ascus at a time.

2. Structure of the ascus.

Many accounts of the bitunicate ascus indicate that the wall at the apex of the ascus is thickened. For example, Higgin (I920, I929) describes the ascus wall of Mycosphaerella bolleana and of Mycosphaerella personata to be 'apically thickened'; Wehmeyer (I946) considers an ascus with a 'much thickened wall at its tip' to be very characteristic of the genus Mycosphaerella; Müller and Arx (I950) and Menon (I956) state that in the Venturiaceae, the ascus wall may be'apically slightly thickened'.

The ascus of Mycosphaerella rumicis has the following structure. The wall is two layered and of even thickness, except at the apex, where there is a small thinner area which is not always clearly visible. It is only the inner layer of the wall which thins out at the apex, but since the outer layer develops an apical split at spore discharge, it presumably has a point of weakness at the ascus tip. The outer layer appears to be fairly rigid and more or less inextensible, while the inner layer is capable of considerable extension and also of swelling.

When asci of Mycosphaerella rumicis are mounted in water, or other liquids such as lactophenol, glycerine and saturated sucrose solution, the inner wall may swell.

This does not occur in all asci, and has never been observed in very young ones. It is thought that asci which show this swelling have been damaged in some way in removing them from the fruit body and mounting them. Figure I8 shows drawings of several asci which were mounted in water. It can be seen that asci of apparently similar age may behave differently, for example figures I8 B, E. The swelling of the inner layer of the wall is shown clearly in figures I8 D, E; it is not confined to the upper part of the ascus, though it is perhaps most noticeable in this region. Prematurely elongated asci are often seen in liquid mounts and they may also exhibit a similar swelling in the inner layer of their wall, figures I8 H, I. It was seen in the investigation of the process of spore discharge in Mycosphaerella rumicis that the inner layer of the ascus wall is capable of swelling, but does so under normal conditions only at the liberation of the eighth spore, when the pressure in the ascus is released, figure I5 D. It seems possible that the swelling in the unelongated and prematurely elongated asci, found in liquid mounts, is the result of a similar release of tension in the ascus, but in this instance brought about abnormally. Factors which might contribute to such a release of tension could include, plasmolyses, the external pressure supplied by the coverslip

and damage to the ascus as a result of its abnormal treatment. Figure I8 K shows that structural damage to the ascus allows the inner layer of the wall to swell, and this is also demonstrated by figure I2 E, which is of a transverse section of a fruit body in which all the asci have been cut.

In all the asci of Mycosphaerella rumicis, in which a thickening was observed, the phenomenon could be accounted for by a swelling of of the inner layer of the wall; thus the ascus wall can not be described as apically thickened - it has a thin region at the apex of the ascus, but is otherwise equally thickened.

It is possible that other bitunicate asci, which have been described as apically thickened, may possibly have the same structure as the ascus of Mycosphaerella rumicis, - the thickened apex being the result of a swelling of the inner layer of the wall. However, even if the bitunicate ascus can not strictly be called apically thickened, the swelling of the inner layer of the wall is so characteristic, that in the absence of mature and discharging asci, it may be used as a diagnostic character for the bitunicate ascus. See for example figures I5, 20, 30, 3I.

## IV. DEVELOPMENT AND STRUCTURE OF THE CENTRUM OF FOUR RELATED LOCULOASCOMYCETES.

Introduction

A. Pleospora herbarum.

B. Venturia inaequalis, V. pirina.

C. Mycosphaerella maculiformis.

Introduction.

Luttrell (I95I, I955) recognises and describes different developmental types of centrum in the ascostromatic ascomycetes. He gives examples of fungi possessing the different types of centrum, and for these examples uses fungi he himself has studied and also those described by other workers. Some of the latter accounts do not provide complete information of the nature of the centrum, at least partly no doubt, because the fungi were described before the possible significance of centrum structure and development was appreciated.

To illustrate the Pleospora-type of centrum, Luttrell uses as his principal examples, Melanomma pulvis-pyrius, described by Chesters (I938), Pseudotricha viridicoma, described by Wehmeyer (I94I), and three fungi described by himself, Miocopron smilacus, Ellisodothidia inquinans and Glomium stellatum (Luttrell I944, I948, I953). All these papers cited, include good descriptions of the development of the centrum. Luttrell considers that the descriptions of Pleospora herbarum by Cavara and Mollica (I907) and Arnaud (I925) furnish good circumstantial evidence that this fungus also possesses a Pleospora-type of centrum.

Some recent accounts of development in Pleospora (Wehmeyer I954, I955) (Moreau and Moreau I956) are somewhat conflicting, but these will be considered later. (p. 90 ).

Luttrell (I95I) includes Venturia inaequalis as an example of a fungus possessing the Pleospora-type of centrum. His evidence for this is based on the work of Killian (I9I7) and Frey (I924), which though not conclusive gives a strong indication that Venturia inaequalis has a Pleospora-type of centrum. This view is supported by the recent work of Müller and Arx (I950), Arx (I952) and Menon (I956), which although not particularly concerned with centrum structure, describes pseudoparaphyses in the fruit body of Venturia inaequalis and of other species of the genus.

In his account of the Dothidea-type of centrum development, Luttrell (I95I) uses Dothidea collecta, which he himself has studied, (Luttrell I95Ia) as his principal example. The descriptions of Mycosphaerella personata and Mycosphaerella tulipifera by Higgin (I929, I936) and those of Mycosphaerella cerasella and Mycosphaerella arachidicola by Jenkins (I930, I938) are among several cited by Luttrell to show that the Dothidea-type of centrum is found in the genus Mycosphaerella. Although these accounts are not primarily concerned with centrum

development, the descriptions and drawings are very clear and give ample evidence that the centrum is of the Dothidea-type in Mycosphaerella; asci are shown to grow up into pseudoparenchymatous tissue, which disintegrates almost before they reach it, leaving the mature centrum as a locule containing a fascicle of asci.

The investigations described in section III of the present work have shown that in the development of the centrum of Mycosphaerella rumicis, bitunicate asci develop in a locule in a stroma and grow up in a layer from the base of this locule. The sterile tissue in the locule is composed of pseudoparaphyses which remain between the asci at maturity. This type of development suggests that Mycosphaerella rumicis is not a typical Mycosphaerella, but is more closely allied to Venturia. It was considered desirable therefore to re-investigate the development of the fruit body of an established species of Venturia, with special reference to its centrum development, and similarly to study another species of Mycosphaerella.

Accordingly the following three fungi were investigated:- Venturia inaequalis, V. pirina and Mycosphaerella maculiformis.

*Pleospora herbarum* was also studied because variations in centrum development in the genus *Pleospora* have been described. (Wehmeyer 1954, 1955: Moreau and Moreau 1956)

A. Pleospora herbarum (Pers.) Rbh.

When grown on malt extract agar the fruit body of Pleospora herbarum develops as a black, more or less spherical stroma, partially immersed in the medium: as the fruit body enlarges it becomes somewhat elongated and develops a short neck at the apex. When maturity is reached, the tissue at the centre of the neck breaks down, thus opening the fruit body cavity to the exterior. Compound fruit bodies may be formed, either by two or more of the simple fruit bodies becoming confluent, or by more than one locule developing in a single ascostroma. In the latter instance, as Wehmeyer (1954) shows for Pleospora trichostoma, the orientation of separate locules is not necessarily similar.

Centrum development and structure of Pleospora herbarum were investigated by examining sections of fruit bodies of various ages, figures 20 - 26. As these figures show, Pleospora herbarum was found to exhibit Luttrell's Pleospora-type of centrum development. The asci, which are bitunicate (figure 19), develop in a basal layer and grow up between vertically orientated pseudoparaphyses. The pseudoparaphyses are attached at the apex and the base of the fruit body cavity; they arise before the asci develop,

and remain between the asci at maturity. Figure 20 is a drawing of a vertical section of an almost ripe fruit body and shows the structure of the mature centrum, which at this stage consists of a locule containing a basal layer of asci interspersed with pseudoparaphyses; the locule is surrounded by a stroma, the outer layers of which are compacted and have become hard and dark coloured. Figure 21 is of a transverse section of a fruit body at a similar stage of development to the one in the previous figure, and here the pseudoparaphyses and asci are seen in cross section. Figure 22 shows a vertical section of a young fruit body in which the asci have not yet developed, and in figure 23 the fruit body is slightly older, and asci are shown growing up among the mass of pseudoparaphyses. These last two figures show that the pseudoparaphyses arise before the asci, and that their vertical orientation is not dependent on the upgrowth of the asci. In these figures the pseudoparaphyses are shown to be attached at both ends, and in all preparations examined, the pseudoparaphyses appeared to be attached in this way. No pseudoparaphyses which unequivocally showed naturally occurring free ends were observed. There was no evidence of radially ingrowing sterile filaments, (as described

by Wehmeyer, I954, I955), in the centrum of any of the fruit bodies examined. While in some of the thicker sections the appearance of radial ingrowth was sometimes given, this effect is regarded as an artefact attributable to the cutting and, or the displacement of the pseudoparaphyses in preparing the sections. The derangement of the pseudoparaphyses tends to be most marked in thick sections, where clearly there are more pseudoparaphyses to become mutilated than there are in thinner sections. Figures 24, 25, and 26 show three sections of fruit bodies of Pleospora herbarum in which the appearance of radially ingrowing hyphae, and hyphae with free ends is given.

Despite the difficulties of interpretation, the investigation of Pleospora herbarum shows that in the development of its centrum a basal layer of asci grows up into sterile tissue, which is composed of persistent pseudoparaphyses. Thus Pleospora herbarum exhibits Luttrell's Pleospora-type of centrum development.

B. Venturia inaequalis (Cke.) Wint., V. pirina Aderh.

These two fungi form their fruit bodies on the dead, overwintering leaves of their respective hosts, apple and pear. The fruit bodies develop immersed in the dead leaf tissue and as they mature, grow towards and finally project through the leaf surface. The projecting part of the fruit body may develop spine-like appendages. Figure 28 shows spines on a fruit body of Venturia inaequalis, and similar appendages were found on some fruit bodies of V. pirina. When the asci are ripe the fruit body cavity becomes open to the exterior by tissue in the centre of the projecting apex breaking down to form an irregular pore, through which the asci project when spore liberation takes place. The ascospores are forcibly ejected from the ascus; they are two celled, and hyaline with an olive green tint; they become dark olive brown in old fruit bodies in which ascospore liberation has been delayed.

The development and structure of the centrum were investigated by means of longitudinal sections of fruit bodies of different ages, figures 28 - 31 show some of these sections. It was found that Venturia inæqualis and V. pirina possess a Pleospora-type of centrum, - asci grow up among persistent sterile tissue which is composed

of vertically orientated pseudoparaphyses. Sections of mature fruit bodies, figures 28, 29, show asci in a basal layer with pseudoparaphyses between them. In the sections shown in these figures, as in most sections of ripe fruit bodies, the pseudoparaphyses are somewhat obscured by the asci and ascospores; some of the pseudoparaphyses are free at their apical end, a phenomenon, which is possibly the result of the break down of the apical tissue of the fruit body in the formation of the apical ostiole, but may also be caused by the cutting of the pseudoparaphyses in preparing the sections. In sections of younger fruit bodies, figures 30, 31, the pseudoparaphyses are more clearly seen, in figure 30, for example, the pseudoparaphyses are shown attached at both ends and with a layer of asci growing up among them.

This investigation of centrum development in Venturia inaequalis and V. pirina fully supports Luttrell's view that the genus Venturia possesses a Pleospora-type of centrum, and that it is correctly placed in the Pleosporales.

C. Mycosphaerella maculiformis (Pers.) Wint.

There are two species of Mycosphaerella which are recorded as common on the overwintered leaves of broad leaved trees; they are Mycosphaerella maculiformis and Mycosphaerella punctiformis (Pers.) Wint. (see Winter I887, Munk I957). The arrangement of the fruit bodies and the size of the ascospores are the characters used to separate the two species. The fruit bodies of Mycosphaerella maculiformis are aggregated, the aggregations appearing as small blackish patches on the leaf: the ascospores measure 9 - I4 µ by 2 - 4 µ. The fruit bodies of Mycosphaerella punctiformis are scattered and the ascospores are smaller than those of Mycosphaerella maculiformis, they measure 7 - 8 µ by 2 - 4 µ. These differences are not very great, and as Winter (I887) suggests a thorough investigation of the two species is required.

The species of Mycosphaerella which is described in this section, was found on overwintered leaves of beech, oak and chestnut. The fruit bodies were in groups and the ascospores measured II - I3 µ by 2 - 3 µ; hence this fungus was tentatively accepted as Mycosphaerella maculiformis.

The fruit bodies of Mycosphaerella maculiformis develop immersed in dead leaf tissue, but project from the leaf surface when mature. When the asci are ripe the tissue at the apex of the fruit body breaks down and the fruit body is opened to the exterior by an irregular roundish pore. The fruit bodies are generally separate, but two or more may be joined together at their edges.

The development and structure of the centrum were investigated by means of longitudinal sections of fruit bodies of different ages. It was found that the centrum of Mycosphaerella maculiformis possesses all the features of the Dothidea-type of development.

Figure 32 shows sections of two fruit bodies which demonstrate the structure of the mature centrum. In these fruit bodies, the centrum consists of a cavity filled with a fascicle of bitunicate asci and there is no sterile tissue between the asci. The cavity is surrounded by stromatic tissue the outer layers of which are hard and dark coloured. The bitunicate nature of the asci is demonstrated in figure 32 A, where some extended asci are shown. The fasciculate arrangement of the asci shown in figure 32, was found in all sections of mature fruit bodies.

Centrum development is illustrated by figures 33 and 34, which show sections of young fruit bodies. In figure 33, which incidently, indicates the position of the fruit body in the leaf, it is seen that the sterile tissue of the centrum is composed of thin walled parenchyma-like tissue; there are no pseudoparaphyses. The deeply stained area in this section is the region of the developing asci. As the asci grow upwards into the thin walled tissue of the centrum, the latter disintegrates. Figure 33 shows young asci just beginning to grow up and the sterile tissue above them is in the process of disintegration.

The ascospores of Mycosphaerella maculiformis are two celled, figure 27d, and are hyaline with a very slight greenish tint. The colour of the ascospores is very similar to that of newly matured ascospores of Mycosphaerella rumicis, Venturia inaequalis and V. pirina. In Mycosphaerella maculiformis however, no subsequent darkening of the ascospores, such as is found in these other three fungi, has been observed.

The study of centrum development in Mycosphaerella maculiformis has shown that a fascicle of asci grows up into non persistent sterile tissue, so that the mature centrum consists of a bundle of asci in a locule in a stroma.

Thus Mycosphaerella maculiformis affords another, and previously undescribed, example of the Dothidea-type of of centrum in Mycosphaerella, and it also provides additional support for Luttrell's view (1951, 1955) that the Dothidea-type of centrum is characteristic of Mycosphaerella.

The five fungi, Pleospora herbarum, Venturia inaequalis, Venturia pirina, Mycosphaerella maculiformis and Mycosphaerella rumicis, all have bitunicate asci. A detailed investigation of the structure of the ascus and of the process of ascospore discharge was made only in Mycosphaerella rumicis (p. 55 ), but in the other four fungi the ascospores are also actively discharged from the fruit body, and in water mounts of their asci, extended asci, such as found in Mycosphaerella rumicis (figure I7), were seen. The large asci of Pleospora herbarum, figure I9, show very clearly the two layered wall, especially when the ascus is extended, figure I9 B. No liberation from such extended asci was ever seen, and it seemed that in water mounts of asci, ascospore liberation in Pleospora herbarum was unable to proceed further.

The five fungi, under present consideration differ from one another in the size and septation of their ascospores. Figure 27 shows the range of size, from the small, one septate spore of Mycosphaerella maculiformis, (27d) to the large and muriformly septate spore of Pleospora herbarum,(27a). It is interesting to find however that their ascospores have all the same shape; they have a blunted apical end with the other end somewhat pointed

and have the broadest part of the spore toward the apical end. The two species of Venturia (figure 27 b, c), clearly show this: in both, the ascospores are composed of two unequal cells, in Venturia inaequalis (27c), the smaller of the two cells is uppermost, while in Venturia pirina the smaller of the two cells is distal, but the broadest part of both spores is in the same position. Ingold (1954) discusses the advantage of this somewhat oviform shape, in ascospore liberation; he considers that it is the most efficient shape of spore, for spore discharge from an inoperculate ascus.

## V. DISCUSSION.

A natural classification can be built up only from a detailed knowledge of the structure, ontogeny and behaviour of the organisms concerned. Where knowledge is meagre only a few characters may be available for purposes of classification and the system, as in the Fungi Imperfecti, is in the main artificial and not taxonomic. In the words of Nannfeldt (1958), ' The natural system is an ideal that can only be approached, never reached ', and the practical aim of all taxonomists is to construct a workable classification which appears best to set forth the relationships of the organisms being classified.

In the classification of the Pyrenomycetes an apparently natural, as opposed to an artificial, system has been evolved during the last twenty years as more information about members of the group has accumulated. The erection of the sub-class Loculoascomycetes by Luttrell (1955) seems a good attempt at a natural grouping. It is the logical consequence of the earlier work of Nannfeldt (1932), Miller (1949) and Luttrell (1951), and the delimitation of these ascostromatic Ascomycetes with bitunicate asci is now generally accepted.

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Natural groupings within the Loculoascomycetes are much more difficult to define and there is as yet no general

agreement as to how this group of fungi should be split up. Luttrell (1955) uses characters of centrum development to make the primary divisions, while Müller and Arx (1950, 1954) place more emphasis on characters of external morphology.

In the Loculoascomycetes Luttrell (1951, 1955) recognises two developmental types of centrum, the Pleospora-type and the Dothidea-type. In the former the asci grow up in a layer from the base of the locule into persistent sterile tissue which is composed of vertically orientated pseudoparaphyses; and in the Dothidea-type the asci arise from a more localised region in the base of the locule and grow up in a bunch into sterile tissue which is pseudoparenchymatous and non-persistent; there are no pseudoparaphyses.

Luttrell uses these two types of centrum development as the main criteria for separating the orders Pleosporales and Dothideales. The Pleosporales have a spherical centrum in which development is of the Pleospora-type. The families Dothideaceae and Capnodiaceae of the Dothideales have the Dothidea-type of centrum, but in the other two families (Pseudosphaeriaceae and Dothioraceae) in the order, the Dothidea-type of centrum is somewhat modified. In these two families the sterile tissue in the centrum is pseudoparenchymatous, as it is in the Dothidea-type s.s., but

differs in that this pseudoparenchymatous tissue does not entirely disappear at maturity and the asci do not grow up in a fascicle. In the Pseudosphaeriaceae the asci arise singly or in small groups, while in the Dothioraceae they arise in a continuous layer. In the two families only a few developmental studies have so far been made, but the information obtained seems to indicate a close affinity between the families and the other Dothideales. For the present therefore it seems best to follow Luttrell and include them in the order.

The use of the fasciculate arrangement of the asci as a character in taxonomy is questioned by Munk (1954). He considers the fascicle to be a 'negative character', a phenomenon resulting from the methods of examining the centrum coupled with the rudimentary nature of the sterile tissue; since there is no interascicular tissue to support the asci, they assume a bunched arrangement when the fruit bodies are mounted. In a series of sections of Mycosphaerella maculiformis (see section IV) the fascicle of asci was very evident and when sections of wax-embedded fruit bodies of this fungus were examined before the wax was removed, the fasciculate arrangement of the asci was still distinct. In this fungus at any rate, the fascicle appears to be real

enough and it seems likely that this is also true in the examples referred to by Luttrell (I95I, I955).

The examples used by Luttrell (I95I, I955) to illustrate the Pleospora-type of centrum development all show that the centrum consists of a locule filled with vertically orientated pseudoparaphyses, with the asci among them. There is no general agreement on how the pseudoparaphyses arise and variations in pseudoparaphyses development have been recorded. Since possession of pseudoparaphyses is the main difference between the Pleospora-type of centrum and the Dothidea-type, these variations should perhaps be investigated, for it may be that the Pleospora-type of centrum, as defined by Luttrell, might arise in more than one way, and if this is so it might be necessary to define the Pleospora-type of centrum more exactly before it is used as a criterion in classification.

Luttrell (I95I) and Miller (I949) consider that pseudoparaphyses arise from the apex of the young ascostroma as elongating hyphae with free ends, which grow downwards. These hyphae soon push to the base of the locule where they become attached at their free ends. Further growth is by intercalary elongation. According to Chesters (I938), in Melanomma pulvis-pyrius the pseudoparaphyses are formed by

the lateral separation and subsequent elongation of hyphae in the centre of the young ascostroma. Their further growth is also by intercalary elongation. Thus in the centrum development of Melanomma pulvis-pyrius pseudoparaphyses never have free ends.

The divergent accounts of fruit body development in Pleospora emphasise the difficulty of interpreting the course of centrum development, particularly the development and growth of the pseudoparaphyses. Luttrell (I95I, I955) uses the excellent drawings and descriptions by Cavara and Mollica (I907) and Arnaud (I925) as evidence that Pleospora herbarum possesses the Pleospora-type of centrum, but in these accounts the exact origin of the pseudoparaphyses is not clearly explained. Wehmeyer (I955) describes the development of pseudoparaphyses in Pleospora armeriae, a fungus he includes in a Pleospora herbarum complex, and which is separated from Pleospora herbarum solely on ascospore size. In Pleospora armeriae, Wehmeyer finds that the pseudoparaphyses arise as a somewhat irregular radial ingrowth of elongating hyphae, which soon become parallel to each other in a vertical direction to form a palisade of pseudoparaphyses into which the asci grow up. The pseudoparaphyses increase in length by intercalary elongation. Wehmeyer considers that some, if not all the pseudoparaphyses remain free at one end.

In the centrum of Pleospora trichostoma, Wehmeyer (I954) describes radially ingrowing hyphae which branch and twine about each other and which are not attached at both ends. Only as the asci grow up do these hyphae become vertically orientated and they become - 'compressed into almost non-existent filaments by the enlarging asci, or even compacted into thick walled pseudoparenchyma'. In the latter case the mature centrum would be like that found in the Pseudosphaeriaceae of the Dothideales, a similarity which suggests that comparisons of mature fruit bodies may be misleading. Moreau and Moreau (I956) in an account of Pleospora herbarum, conclude that centrum development in the fungus is similar to that described by Wehmeyer for Pleospora trichostoma; however they describe the development of the pseudoparaphyses as typical hyphal filaments which grow down from the apex of the fruit body cavity, a development similar to that described by Miller (I949) and Luttrell (I95I). If the figures and descriptions given by Wehmeyer (I954, I955) and by Moreau and Moreau (I956) are compared, the difficulty of interpretation is apparent. For example, the figures for Pleospora herbarum (Moreau and Moreau, I956) and those of Pleospora trichostoma (Wehmeyer, I954) could equally well be used to illustrate Wehmeyer's description and

interpretation of centrum development in Pleospora armeriae (Wehmeyer, 1955).

It seems probable that a number of the variations recorded in the development of the Pleospora-type of centrum are largely due to differences in interpretation of sections. A solution to this problem might be reached if the same worker investigated several fungi in which differences had been recorded. In this way recorded differences, which were solely the result of the difficulty of interpretation, might be avoided, and actual differences and similarities in development might be more easily recognised.

The investigations of development in Mycosphaerella rumicis, Venturia inaequalis, Venturia pirina and Pleospora herbarum, described in sections III and IV of the present work, illustrate this point. While no success was met in determining the exact origin of the pseudoparaphyses in any of these four fungi, their centrum development was essentially similar. In none of them was there an indication that the pseudoparaphyses arose apically and grew downwards, as described by Miller (1949) and Luttrell (1951); the pseudoparaphyses appeared more prominent at the apex of the locule, for at the base they were soon obscured by the developing asci. There was also no evidence that the pseudoparaphyses originated as radially ingrowing hyphae with permanently free ends, as

described by Wehmeyer (1954, 1955). The pseudoparaphyses appeared to be always attached at both ends. However the number of pseudoparaphyses in the centrum seems to increase as the fruit body develops (e.g. figures 12 B - E), and since it seems unlikely that an increase could be the result of the longitudinal splitting of the original pseudoparaphyses, it is difficult to visualise how it could arise without the development of some hyphae with free ends. The development of pseudoparaphyses was studied most extensively in Pleospora herbarum, since its large fruit body made it the easiest one of the four to work with, but even in thick longitudinal sections of the fruit bodies, free ends to the pseudoparaphyses were not seen. The appearance of free ends may be simulated: the pseudoparaphyses are often cut when the fruit body is sectioned, which is almost enevitable with a spherical centrum, while in other preparations the pseudoparaphyses may become folded or otherwise displaced when the sections are mounted. Such possibilities have to be considered when interpreting the various preparations.

Centrum development in all four fungi furnishes additional support for the Pleospora-type of centrum as described by Luttrell (1951, 1955). In the centra of these fungi there was no indication that the Pleospora-type

may develop in more than one way; in fact more support is given to the view that the variations described in the Pleospora-type of development could be the result of differences in interpretation. The difficulty of interpretation makes it practically impossible to distinguish any other than clear cut, and therefore probably fundamental, differences in centrum development. Even though the exact origin of the pseudoparaphyses has not been ascertained, the present investigations have certainly shown that centra with pseudoparaphyses can be recognised as a distinct type, and the evidence all suggests that Luttrell's Pleospora-type of centrum is a sound taxonomic criterion. Until more evidence is forthcoming, particularly from comparative developmental studies of fungi at present included in the Pleosporales, the Pleospora-type of centrum appears to be a good basis on which to erect the order.

In the Pleosporales, Luttrell (1955) adopts, with certain reservations, the Venturiaceae as designated by Müller and Arx (1950). Müller and Arx, unlike Luttrell, do not use characters of centrum development to define the orders of the ascostromatic Ascomycetes; instead they use characters of external morphology of the fruit body, and in their classification, the Venturiaceae is in an order, the Pseudo-

sphaeriales, characterised by the possession of a spherical fruit body which opens by a roundish pore. Characters of the ascospores are used in the subdivision of the Pseudosphaeriales; in the family Venturiaceae the ascospores are two celled and olive brown or greyish green when mature, while in the Mycosphaerellaceae the two celled ascospores are colourless, even at maturity. Both these families include fungi with a pseudoparaphysate centrum and fungi in which the centrum has no pseudoparaphyses. In the designation of the Venturiaceae, Müller and Arx describe the sterile tissue of the fruit body locule as composed of: 'hyaline parenchymatous cells, or vertically orientated pseudoparaphyses'. While Luttrell adopts Venturiaceae, based as it is on spore characters, he includes in it only members of the Pleosporales. The Venturiaceae as emended by Luttrell includes therefore only those fungi which possess both olive tinted ascospores and the Pleospora-type of centrum. The difference between the Venturiaceae of Müller and Arx and the Venturiaceae in Luttrell's classification follows from the use of different characters for the primary divisions of the Loculoascomycetes. The Pseudosphaeriales of Müller and Arx, based on external morphology of the fruit body, is a much larger group of fungi than is the Pleosporales of Luttrell, which is based

on a type of development. With the possession of a similar type of development, members of the Pleosporales may be assumed to have some natural affinity, but such an assumption is less admissible for the Pseudosphaeriales, since similar external form of the fruit body may not necessarily indicate homology. There is more reason therefore for regarding the Pleosporales as monophyletic than for making the same assumption with the Pseudosphaeriales.

Spore colour is one of the characters used to make the subdivisions of both these orders. It seems possible that such a character, which may result from a single gene mutation and may be expected to evolve more than once, is likely to be a more suitable character when applied to the Pleosporales, than when used to subdivide the possibly more heterogenous Pseudosphaeriales.

The position of Venturia and Mycosphaerella in the schemes of classification is interesting: the two genera have certain features in common besides the bitunicate ascus and ascostromatic fruit body; these include a simple unilocular fruit body and two celled ascospores. In Luttrell's (1955) classification the two genera fall into separate orders, Venturia in the Pleosporales and Mycosphaerella in the Dothideales, whereas in the system of Müller and Arx (1950) they are both included in one order, the Pseudosphaeriales.

It is only in Luttrell's system that the distinct and apparently fundamental difference in the development of the centrum in Venturia and Mycosphaerella is recognised; a difference which is clearly shown by the present investigations of the two genera.

For identification the use of developmental criteria has obvious disadvantages; it is time consuming and, certainly with herbarium material, not always possible. However, the mature state frequently reflects development, and a complete developmental study is not always necessary. For example, sections of the mature fruit bodies of Venturia inaequalis and Venturia pirina (figures 28 - 29) give sufficient information to show that the centrum development in these fungi is of the Pleospora-type, while those of Mycosphaerella maculiformis (figure 32) show that its centrum development is of the Dothidea-type.

It may be argued that spore characters are much easier to use as criteria for identification than are developmental ones. This is often true, and for identification the use of any reliable character is defendable. The spore colour character, used by Müller and Arx to characterise Venturiaceae, was found to be a positive character for the two species of Venturia which were studied in the present work. However, in

some newly ripened fruit bodies the colour of apparently mature ascospores was found to be not significantly different from that of ascospores of Mycosphaerella maculiformis, and it would be difficult with such specimens to separate Venturia from Mycosphaerella on this character alone. However in herbarium material spore colour is a reliable character; it was found in the species of Venturia, under present consideration, that a darkening of unreleased ascospores always took place after a fruit body was mature, while in Mycosphaerella maculiformis the ascospores never became similarly darkened.

While the two systems of classification under consideration are generally adequate for the identification of Venturia and Mycosphaerella, the affinities of the genera appear to be shown much more clearly by the system of Luttrell than by that of Müller and Arx. It seems likely that a system of classification, which ignores such an apparently fundamental difference in development, as has been shown to exist in the centrum of the Loculoascomycetes, will have little value in indicating natural relationships; the evidence of the present work all suggests that in this respect Luttrell's system is better than that of Müller and Arx, not only for Venturia and Mycosphaerella, but also

for the Loculoascomycetes as a whole. The system of Müller and Arx seems to lack the clarity of Luttrell's system; one feels that their system may be a good one for identification, when in the hands of experts like themselves, who appreciate the sometimes rather subtle differences they use to separate families and genera. It may be, when more developmental studies of fungi have been made, that some of the clarity of Luttrell's system will be lost. Luttrell's scheme is based on published data and in some instances, as he himself is the first to admit, the examples are few and the information rather meagre, for it is only relatively recently that emphasis has been placed on developmental criteria for the classification of the Pyrenomycetes. The investigations in the present work furnish additional evidence in support of Luttrell's system of classification, a system, which though still tentative, forms an excellent basis for the further work which must be done before it can be said with any assurance, this is or this is not a better classification.

The main purpose of this present work was the determination of the taxonomic position of Mycosphaerella rumicis. The confusion arose because the first descriptions of the fungus by Desmazières (1843) and Cooke (1866) omitted presence of spines on the fruit body, and at this time presence of spines was a character used to separate Venturia from Mycosphaerella (Sphaerella). Winter (1887) was the first to record the presence of spines on the fruit body of the fungus and thus to show that the fungus was to be referred to Venturia rather than Mycosphaerella.

The present work on the fungus has shown that the presence of spines on the fruit body is an unreliable taxonomic character; for while spines were always present on fruit bodies produced in culture, they were lacking on many of the fruit bodies developed on the host. This frequent absence of spines on the fruit bodies of Mycosphaerella rumicis, when growing on its host, is possibly one of the main reasons why Winter's naming of the fungus was not universally adopted.

The centrum of Mycosphaerella rumicis has been shown to be of the Pleospora-type and not of the Dothidea-type and the fungus is therefore correctly placed in the Pleosporales sensu Luttrell; its olive tinted two celled ascospores refer

it to the Venturiaceae and its simple spherical fruit body to Venturia. Mycosphaerella rumicis is also correctly placed in the Pseudosphaeriales of Müller and Arx, by virtue of its spherical ascostromatic fruit body. In the Pseudosphaeriales, the colour of its two celled ascospores is the factor which determines whether the fungus should be assigned to Mycosphaerella of the Mycosphaerellaceae, or to Venturia in the Venturiaceae. In Mycosphaerella rumicis, as in the other species of Mycosphaerella and Venturia which were studied in the present work, spore colour may prove misleading. The mature ascospores of Mycosphaerella rumicis are initially very pale green in colour, they may be discharged and germinate in this condition, but if their discharge is delayed they assume a dark olive colour, which is characteristic of Venturia. Dark coloured ascospores were not found so frequently in the fruit bodies of Mycosphaerella rumicis as in those of Venturia inaequalis and Venturia pirina, but this may possibly be explained by the different habitat and life cycle of the fungi. Fruit body production in Mycosphaerella rumicis occurs on the living host, and it is a fairly rapid process; in suitable conditions the ascospores may be discharged within twenty days of infection, and they are capable of germinating very quickly after discharge.

On the other hand, the fruit bodies of the two species of Venturia develop in the dead overwintering leaves of the host which are infected in the Summer or Autumn; they reach maturity in the Spring, when release of ascospores may often be delayed by unsuitable conditions for their discharge. No attempt was made to germinate the ascospores of these two species of Venturia.

As the mature ascospores of Mycosphaerella rumicis become dark coloured with age, the fungus may be correctly assigned to the Venturiaceae of Müller and Arx, and to Venturia.

Thus in the different classifications, with their different criteria for separating Venturia and Mycosphaerella, Mycosphaerella rumicis is shown to be a species of Venturia. The fungus should therefore be called Venturia rumicis (Desm.) Winter.

## VI. SUMMARY

1. *Mycosphaerella rumicis* was shown to infect its *Rumex* host by ascospores, the germ tubes of which pierce the host cutucle: the invading mycelium grows intercellularly.

2. The fungus is homothallic; it will grow and reproduce its perfect stage on the living host and in culture; it is non-exacting in its growth factor requirements. The perfect stage is an ascostroma: no asexual reproductive stage has been found; there was no evidence that the asexual reproductive stage is *Ovularia obliqua*.

3. A developmental study showed that centrum development in *Mycosphaerella rumicis* is of the *Pleospora*-type.

4. The *Pleospora*-type of centrum was found also in *Pleospora herbarum*, *Venturia inaequalis* and *Venturia pirina*.

5. *Mycosphaerella maculiformis* was found to have a *Dothidea*-type of centrum.

6. It was concluded that *Mycosphaerella rumicis* is a species of *Venturia*, and should be called *Venturia rumicis* (Desm.) Wint.

# VII. APPENDIX

Some factors affecting the growth and development of *Mycosphaerella rumicis* in culture.

1. Inoculum
2. Temperature
3. Medium

I. Inoculum.

When investigating the growth and development of Mycosphaerella rumicis in culture, freshly discharged ascospores were used as the inoculum. Initially, monospore inocula were always used, but it was found later (see table 5) that within limits size of inoculum did not affect development, and so 20 - 50 spore inocula were used in the subsequent work.

Table 5 shows the results of an experiment in which the number of spores in the inoculum was varied. There were ten cultures of each inoculum size and the cultures were kept at room temperature (average daily reading = I2.8°C)

Table 5 Effect of size of inoculum on the development of Mycosphaerella rumicis.

| No. of spores in inoculum | Time between inoculation and discharge of ascospores; recorded in days. | |
|---|---|---|
| | range | average |
| I | 4I - 45 | 43 |
| 2 | 4I - 42 | 4I.5 |
| 4 | 42 | 42 |
| 6 | 4I - 42 | 4I.5 |
| Io | 40 | 40 |
| 20 - 50 | 33 | 33 |

In culture work a large inoculum has two main advantages over a monospore one, it is more easy to obtain and with it growth is more certain. The latter point was illustrated by comparing the growth of the fungus in 40 monospore agar slope cultures, with growth in 40 >10 spore cultures. The spores of the inocula were all from the same source and the cultures were kept in similar conditions. Growth occurred in all the >10 spore cultures, but only half of the monospore cultures grew.

2. Temperature.

No critical work was carried out on the effect of temperature on the growth and development of Mycosphaerella rumicis, but by growing the fungus at various temperatures it was found that Mycosphaerella rumicis is a relatively 'low temperature fungus'. Table 6 summarises the results of three experiments in which similar cultures of the fungus on malt extract agar were placed in different temperature conditions. The temperature was constant only at the two higher temperatures, and at the other three varied as indicated.

Table 6 Effect of temperature on development of Mycosphaerella rumicis.

| Temperature (in °C) | No. of cultures | No. of cultures which grew | Time between inoculation and discharge of ascospores average (in days) |
|---|---|---|---|
| 4 - 6 | 36 | 36 | 77 |
| 8 - II | 3I | 3I | 5I |
| I4 - I7 | 3I | 3I | 34 |
| 22 | I6 | I6 | no spores formed |
| 25 | 20 | 0 | - |

It is seen that the temperature range at which mature fruit bodies develop is smaller than the range permitting growth of the fungus. It is therefore most important when investigating the development of the fungus to insure that the temperature conditions are not above the maximum for reproduction.

At 22°C, where growth of Mycosphaerella rumicis takes place but is somewhat restricted, mature fruit bodies are not formed, and if monospore inocula are used to initiate the culture no growth occurs. In liquid cultures, subjected to a similar range of temperature conditions, growth of the fungus does not take place at 22°C or temperatures above. It would seem therefore that 22°C is at, or is near, the upper limiting temperature for the growth of Mycosphaerella rumicis.

3. **Medium.**

*Mycosphaerella rumicis* grows and produces fertile fruit bodies on all the common natural agar media. It also grows on synthetic Czapek-Dox medium, but even with addition of yeast extract, no fertile fruit bodies are produced.

To investigate this lack of reproduction of the fungus, various other changes were made in the composition of the synthetic medium. Table 7, on the next page, lists the various modifications made to the medium, and summarises the results obtained when *Mycosphaerella rumicis* was cultured on the modified medium.

Table 7. Effect of alterations in the composition of Czapek-Dox medium, on Mycosphaerella rumicis.

| Nature of change | Growth occurred + | Ascospores formed + |
|---|---|---|
| Carbon | | |
| 1. Sucrose reduced from 3% - 0.2% | + | - |
| 2. Sucrose replaced by glucose | | |
| a) Glucose 3% | + | - |
| b) Glucose 0.2% | + | - |
| Nitrogen | | |
| 3. Sodium nitrate replaced by asparagin 2% | | |
| a) with glucose 3% | + | - |
| b) with glucose 0.2% | + | - |
| c) with sucrose 3% | + | - |
| d) with sucrose 0.2% | + | - |
| Potassium | | |
| 4. Potassium chloride reduced from 0.05% to 0% | + | - |
| 5. Potassium phosphate replaced by sodium phosphate($Na_2HPO_4$) 0.1% | + | - |
| Phosphate | | |
| 6. Potassium phosphate reduced from 0.1% to 0% | + | + |

It was found that when the phosphate concentration of the medium was reduced reproduction of the fungus took place.

Table 8 shows the result of an experiment in which the fungus was grown on Czapek-Dox medium of various phosphate concentrations. Six tube-cultures were made at each concentration of phosphate, ascospores were used as inoculum. Growth occurred in all cultures, and it appeared, from visual examination of the cultures, that the amount of growth increased with increased concentration of phosphate. Since all cultures in which mature asci were developed reached maturity at approximately the same time, the concentration of phosphate apparently did not affect the speed of development of the fungus. In table 8 the number of secondary colonies developed from discharged ascospores, in each culture is recorded.

Table 8. Effect of different concentrations of phosphate in Czapek-Dox medium, on the reproduction of Mycosphaerella rumicis.

| % $KH_2PO_4$ | No. of spore colonies developed in each culture | | | | | | Total no. of spore colonies |
|---|---|---|---|---|---|---|---|
| | I. | 2. | 3. | 4. | 5. | 6. | |
| 0 | I9 | 7 | I4 | I0 | 0 | 0 | 50 |
| 0.00I | >I00 | >I00 | >I00 | >I00 | >I00 | >I00 | >600 |
| 0.005 | 28 | 2o | 6 | 20 | 5 | 20 | 99 |
| 0.0I5 | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| 0.025 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.035 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.045 | 0 | 0 | I | 0 | 0 | 0 | I |
| 0.055 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.055 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.065 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.II0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

The experiment shows that in culture the reproduction of Mycosphaerella rumicis is greatly influenced by the concentration of phosphate in the medium. It is seen that the fungus can grow and reproduce on a medium with a very low concentration of phosphate, for its growth and reproduction occurred on a medium to which no phosphate had been added. In this medium the phophate requirements of the fungus must be provided for, either by impurities from the glassware, and or from the constituents of the medium. As the results in table 8 show, reproduction of the fungus is stimulated by a very small addition of phosphate to the basic medium. A further small increase in the phosphate concentration depresses reproduction, and reproduction is inhibited by a phosphate concentration which is considerably lower than is the phosphate concentration in normal Czapek-Dox medium.

Similar results were obtained when the fungus was grown on the following agar media :-

Potato extract + sodium phosphate ($Na_2HPO_4$)

Malt extract + sodium phosphate ( " )

Malt extract as above, but buffered, with citric acid, at pH 5.

The results of these experiments are shown in table 9.

**Table 9. Effect of addition of phosphate to natural media, on the reproduction of Mycosphaerella rumicis.**

| % phosphate | Growth | | Ascospores formed | |
|---|---|---|---|---|
| | + | | + | |
| **Potato extract** | | | | |
| 0.005 | + | | + | |
| 0.015 | + | | + | |
| 0.025 | + | | + | |
| 0.035 | + | | + | |
| 0.045 | + | | + | |
| 0.055 | + | | - | |
| 0.065 | + | | - | |
| 0.075 | + | | - | |
| **Malt extract** | | | | |
| a) buffered at pH 5 | | | | |
| b) unbuffered | a | b | a | b |
| 0.005 | + | + | + | + |
| 0.012 | + | + | + | + |
| 0.023 | + | + | + | + |
| 0.046 | + | + | + | + |
| 0.092 | + | + | + | + |
| 0.184 | + | + | - | - |
| 0.369 | + | - | - | - |
| 0.738 | + | - | - | - |

On the unbuffered malt extract medium, growth of the fungus at 0.I8% phosphate was rather restricted, and there was no growth at all at the higher concentrations of phosphate; but in the buffered medium, growth was good in all the cultures. The inhibition of growth in the unbuffered medium is probably the result of a high pH of the medium, caused by the high concentrations of phosphate.

On a natural medium, Mycosphaerella rumicis appears to be able to tolerate a higher concentration of phosphate in the medium, for reproduction, than when the fungus is on synthetic Czapek-Dox medium. The complex nature of the natural media makes it difficult to determine any direct reason for this difference. It was found however, that fruiting of Mycosphaerella rumicis took place when the fungus was grown on synthetic Westergaard-Mitchell medium, and the phosphate concentration of this medium is 0.0I%, which is the same as in Czapek-Dox medium.

To investigate the different behaviour of Mycosphaerella rumicis on the two synthetic media, the fungus was grown on combinations of the media. It was grown on :- Westergaard-Mitchell medium, minus calcium chloride, and on Czapek-Dox medium, plus calcium chloride 0.0I - 0.04%.

The results obtained were inconclusive, the fungus grew

on all the media, but mature fruit bodies were developed in only four cultures. The cultures in which reproduction took place were four out of ten cultures on Czapek-Dox medium, plus calcium chloride 0.04%. In Westergaard-Mitchell medium the concentration of calcium chloride is 0.01%.

The preceding experiments, while differing in details, all show that reproduction of Mycosphaerella rumicis is influenced by the phosphate concentration of the medium on which the fungus is growing. As shown in table 8, the difference in the phosphate concentration which stimulates and which inhibits reproduction is very small, and it appears unlikely that the effect is one of pH. The results of the experiments which are summarised in table 9 also seem to indicate this; it is shown that reproduction of Mycosphaerella rumicis was inhibited by the same concentration of phosphate, in a buffered and in an unbuffered mediium. It was found, in another experiment, that growth but not reproduction of Mycosphaerella rumicis occurred on Czapek-Dox medium buffered at a range of pH, pH 3.5 to pH 7.0. However the buffer used was Mc Ilvaine's phosphate - citrate buffer (see Clark 1927), but if the effect of phosphate on reproduction is purely one of pH, then the use of/a phosphate buffer might even be expected to cancel the effect of the phosphate in the medium.

When investigating the effect of pH of the medium, on the reproduction of Mycosphaerella rumicis in culture, it is clear that a phosphate buffer should not be used to adjust the pH of the medium; the use of such a buffer can give a misleading result. For example, when using potato extract as a basic medium and Mc Ilvaine's buffer solutions to adjust pH, it was found that reproduction of Mycosphaerella rumicis would not take place above pH 3.4, but reproduction of the fungus took place on the unbuffered potato extract medium, the pH of which was between pH 5 and pH 6.
It is perhaps possible that in some instances, effects of pH on reproduction which have been recorded for other fungi, may be the effects of changes in phosphate concentration, rather than a direct effect of pH.

It was shown that Mycosphaerella rumicis will grow on media of widely differing pH. Tube-cultures of the fungus were made on media of pH range - pH 2.4 to pH 8.4, with malt extract agar as the basic medium and Mc Ilvaine's buffer solutions to adjust pH. Growth of the fungus occurred at pH 2.8 to pH 8.0. In liquid cultures, using Czapek-Dox medium as base and the same buffer solutions to adjust pH, a pH range of pH 3.4 to pH 7.0 was used, growth of the fungus took place at pH 3.4 to pH 6.2. No measurement of growth

was made in these experiments, and as phosphate buffer solution were used it was not possible to correlate pH and reproduction.

An attempt was made to relate reproduction and pH of the medium, by growing Mycosphaerella rumicis on media buffered with a non phosphate buffer solution. Sorenson's sodium citrate, sodium hydroxide or hydrochloric acid, buffer solutions (see Hale 1958) were used, with malt extract as the basic medium. The pH range investigated was pH 2.97 to pH 6.97. The cultures were made in medicine flats instead of tubes, which enabled colony diameter to be measured at the end of the experiment. The results of this experiment are shown in table 10. There were four cultures at each pH; the cultures were kept at room temperature which varied and was not recorded.

Table IO. Effect of pH of the medium, on the development of Mycosphaerella rumicis.

| pH | Colony diameter after 40 days (average, in cms) | Time between inoculation and ascospore discharge (average, in days) |
|---|---|---|
| 2.97 | I.9 | 40 |
| 3.95 | 2.4 | 26 |
| 5.02 | 2.4 | no spores formed |
| 5.95 | 2.2 | no spores formed |
| 6.97 | I.2 | no spores formed |

It can be seen from table IO, that in the conditions of this experiment, mature fruit bodies of Mycosphaerella rumicis were formed only on the two most acid media, at pH 2.97 and pH 3.95. The fungus grew at all points on the pH range, but growth was limited at the two extremes of the range.

It appears therefore that growth and reproduction of Mycosphaerella rumicis are both favoured by a low pH, but that the pH is more critical for reproduction than it is for growth.

When the natural substrate of the fungus is considered, it is not surprising that in culture, growth and reproduction are favoured by an acid medium; the pH of Rumex leaf tissue ground up with water is in the range pH 4.5 to pH 4.8.

The experiments which have just been described show that Mycosphaerella rumicis will grow and reproduce on a synthetic medium, and that it is non-exacting in its growth factor requirements. They also show that like a great many fungi, the conditions which influence its reproduction are more critical than are those which influence growth.

Both growth and reproduction are favoured by low temperature conditions, but as can be seen from table 6, growth occurs at a higher temperature than the highest limiting temperature for reproduction.

The medium has a definite influence on reproduction, but as no measurement of growth was made in most experiments, no clear effect of the medium on growth has been demonstrated. Two factors of the medium which have been shown to have considerable influence on reproduction, are the pH of the medium and its phosphate concentration. Their effect may be linked, but the results indicate that the inhibitory effect of phosphate is at least not a direct pH effect. It was also found that the actual amount of phosphate in the medium is not directly related to its inhibitory effect on reproduction. For example, in natural media, reproduction of Mycospaherella rumicis occurred where there was

a larger concentration of phosphate than that which inhibited reproduction in synthetic Czapek-Dox medium. The relative proportions of the individual constituents of the medium are probably very important. The experiment in which calcium chloride was added to Czapek-Dox medium was inconclusive, but would appear to be worth expanding; with critical experiments it might be possible to obtain more exact information on the role of phosphate in the stimulation and inhibition of reproduction in Mycosphaerella rumicis. The experiment suggests that both the phosphate and calcium concentration of the medium may influence reproduction. It may be that the stimulatory effect of calcium on reproduction, shown by the four cultures in which reproduction occurred, is the indirect result of the inter relationship of the phosphate and calcium in the medium. However a great deal of work will have to be done before the effects of the various constituents of the medium can be understood.

Growth and reproduction of Mycosphaerella rumicis have been shown to be favoured by a medium of low pH. The experiments with pH, show that it is undesirable to use a phosphate buffer when investigating the effect of pH on growth and reproduction. If possible, for such investigations a non phosphate buffer should always be used.

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## IX. FIGURES.

Figure 1.

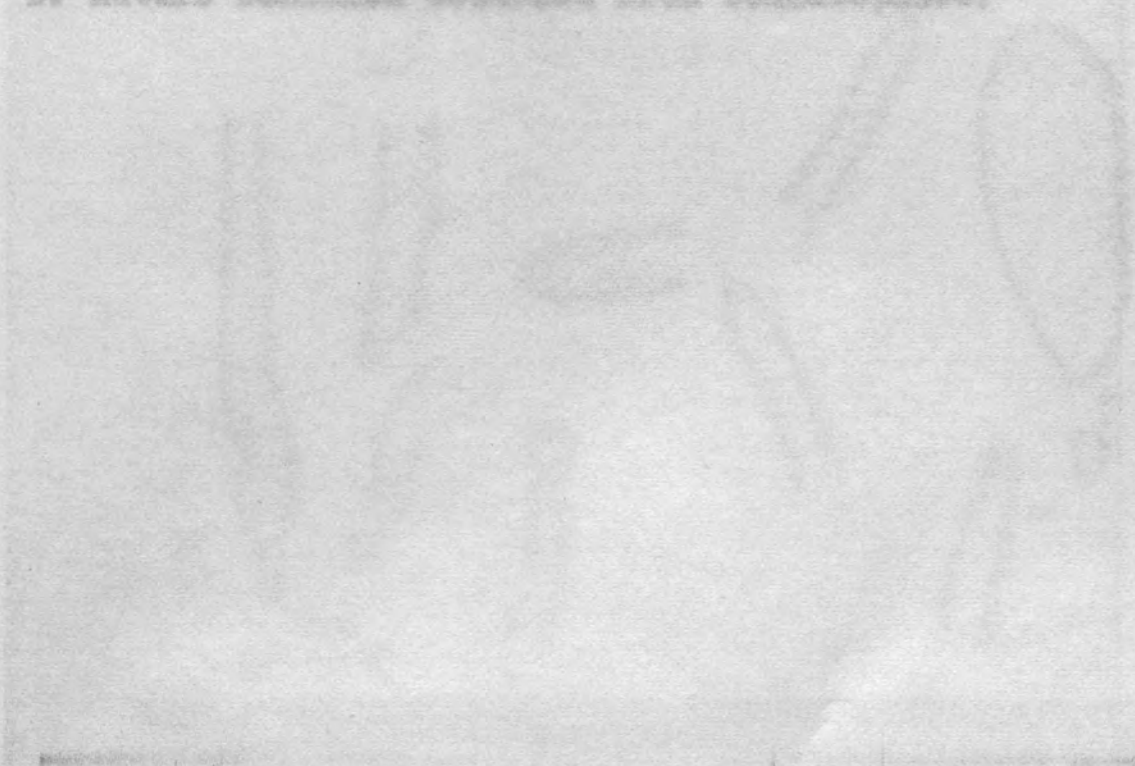
Ovularia obliqua.

A. Conidiophores projecting from a stoma of Rumex leaf, shown in outline only.

B. Conidium which has germinated to give a conidiophore while still attached to the parent conidiophore.

C. Tips of conidiophores to show stages in the development of a conidium.

D. Mature conidium detached from conidiophore.



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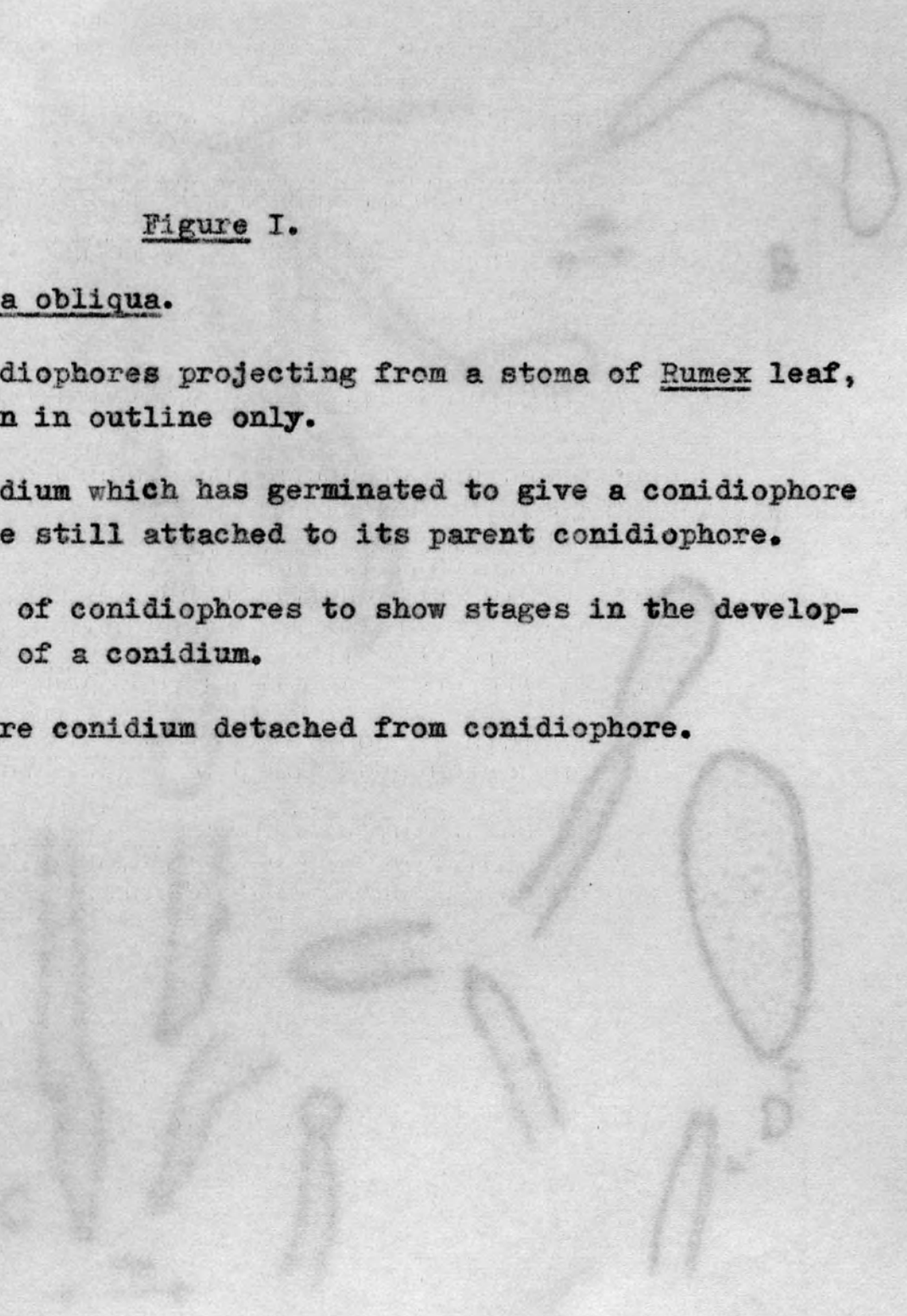
C. Tips of conidiophores to show stages in the development of a conidium.

D. Mature conidium detached from conidiophore.



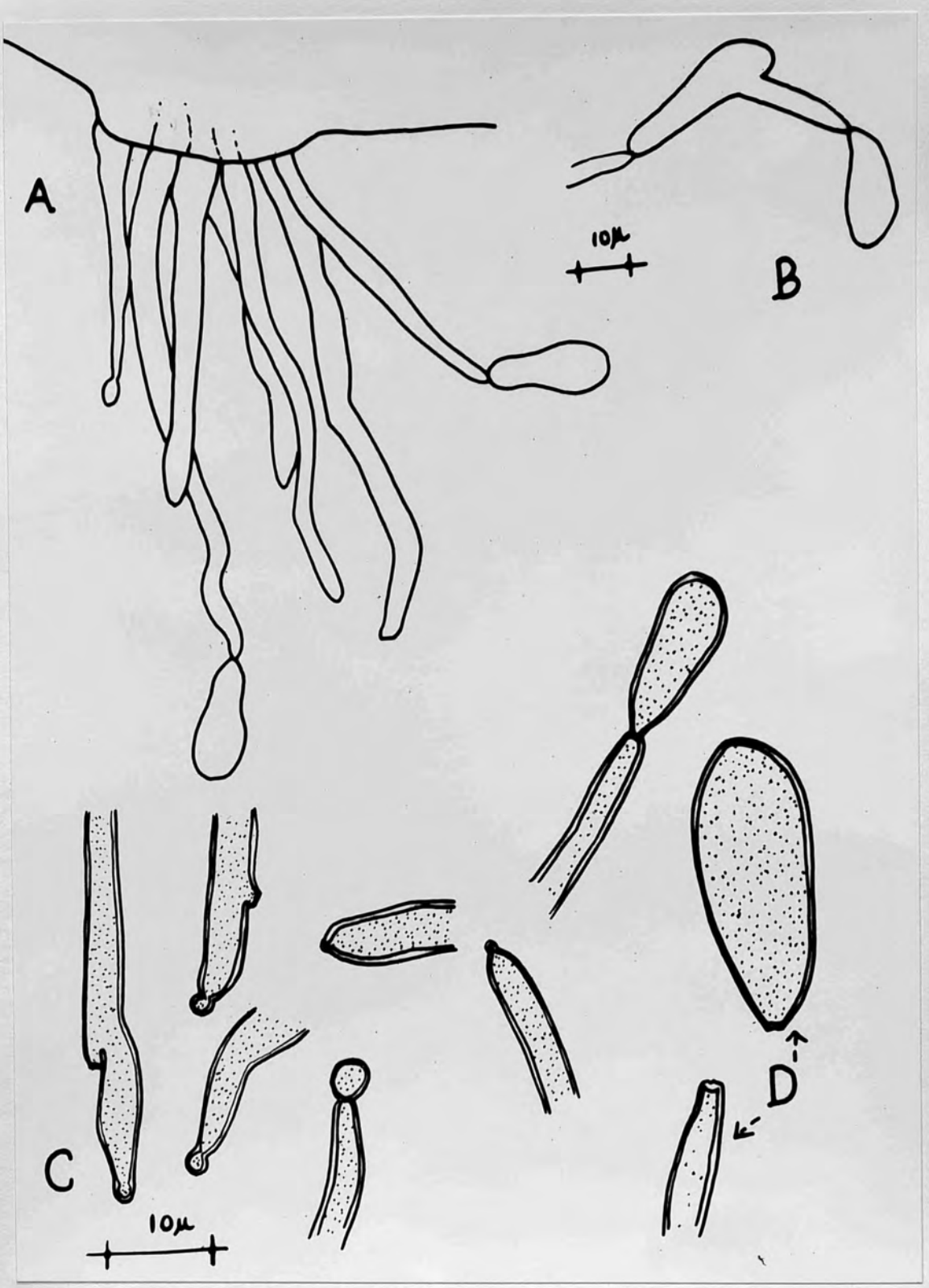



fig.1

Figure 2.

Mycosphaerella rumicis. Preparations of infected Rumex host to show stages in infection.

$^{A}$I - 2 Transverse sections of epidermis of leaf.

I. Penetration of host has occurred between two epidermal cells, 'alteration' of the adjacent anticlinal walls is shown.

2. Shows 'alteration' in periclinal wall, caused by germ tube of an ascospore which is no longer in position.

$^{B}$I - 5 Cleared leaf preparations showing 'altered' areas in periclinal walls, caused by ascospore germ tubes.

I. Small 'altered' area on either side of germ tube, at point of entry to host.

2. Two 'altered' areas, one either side of a junction of two epidermal cells.

3. One 'altered' area at point of penetration.

4. One 'altered' area, with a lighter blue 'halo' (dotted).

5. Similar to 2, but ascospore not in position.

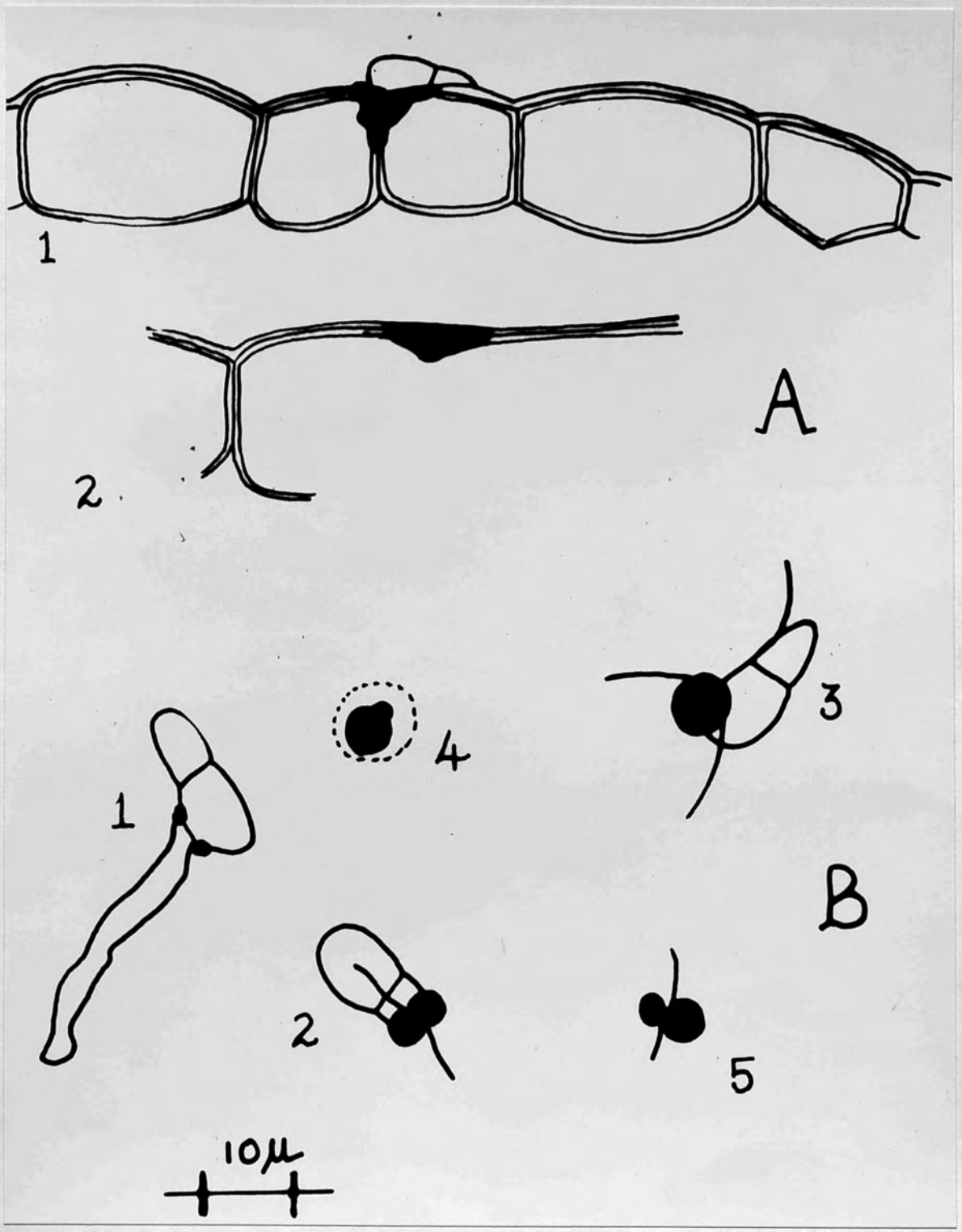



fig.2

Figure 3.

Mycosphaerella rumicis. Epidermal strips of infected Rumex leaves to show stages in infection.

A. Ascospore on surface of leaf; germ tube has penetrated and 'alteration' of periclinal wall of epidermal cell is shown.

B. Ascospore germ tube has extended beyond spore before penetrating host, 'altered' area in periclinal wall of epidermal cell is shown at point of entry.

C. Ascospore has germinated over the junction of two epidermal cells, invading hyphae growing above the junction of the cells. 'Altered' areas in anticlinal walls are shown.

D,E. As in C, but growth of invading hyphae more extensive, and becoming lateral.

F. A wide sub cuticular hypha developed, the germ tube did not enter over the junction of two epidermal cells, but the invading mycelium follows the contours of the epidermal cells.

('Altered' areas in the periclinal walls of the epidermal cells are shown in black; in the anticlinal walls they are indicated by drawing the middle lamella as a thick black line. Where the fungus is sub cuticular and growing above the junction of the epidermal cells, the epidermal walls are shown as a single continuous('altered') or dotted('unaltered' line instead of the usual three lines.)

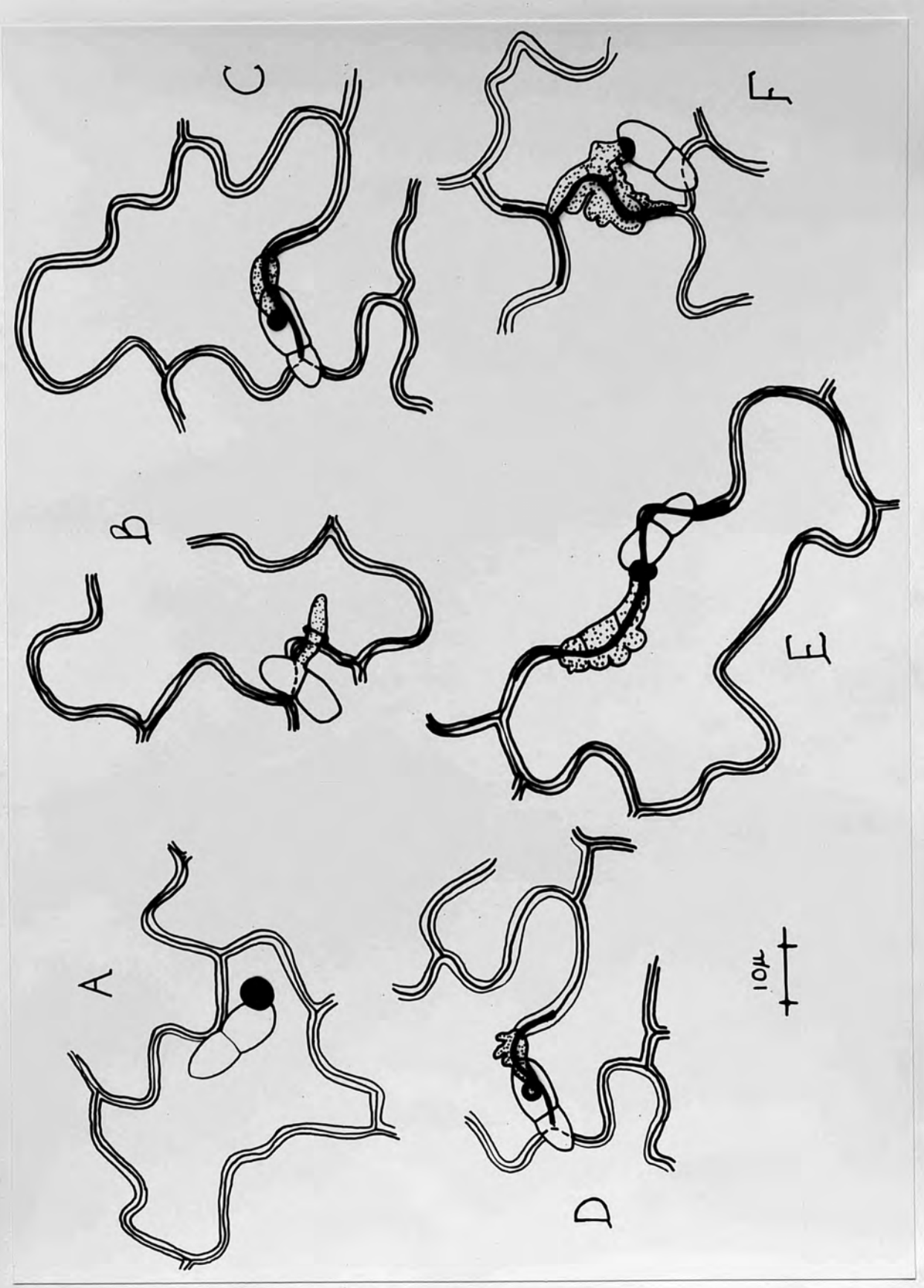



fig.3

Figure 4.

Mycosphaerella rumicis. Epidermal strips of infected Rumex leaves to show stages in infection.

A. Shows extensive development of sub cuticular hyphae, radiating out from the point of entry in a sheet like layer, and following the contours of the epidermal cells.

B. Similar to A, but shows also the beginning of sub epidermal hyphae (shaded). These sub epidermal hyphae arise below the junction of two epidermal cells, where the anticlinal walls appear 'altered'.

(The strips were stained in cotton blue, and 'altered' areas are indicated as in figure 3.)

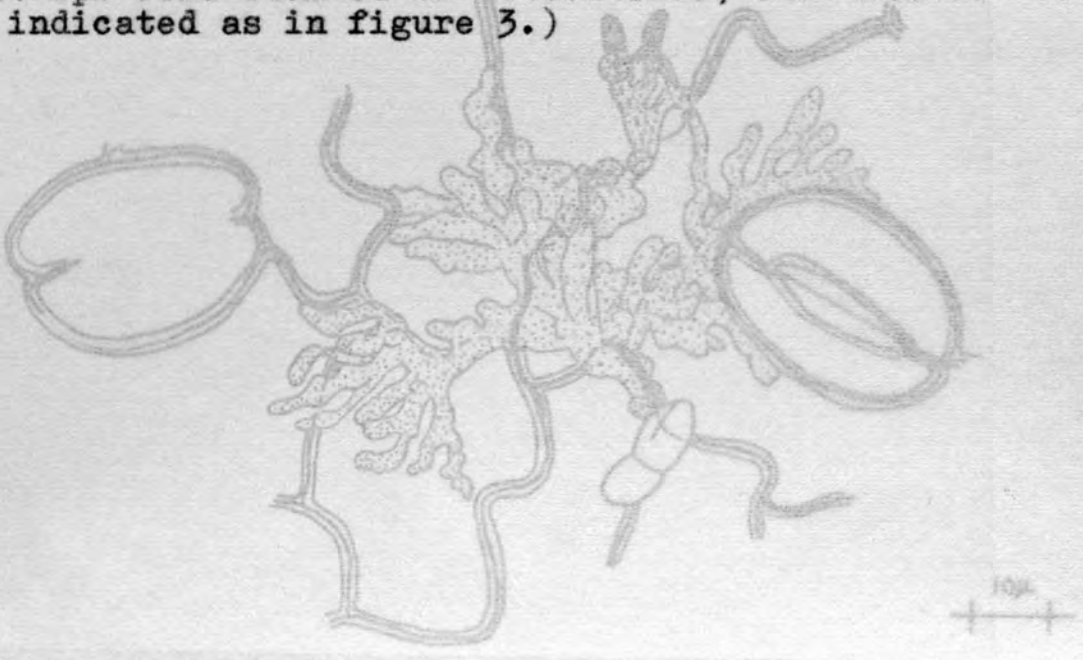



fig. 4

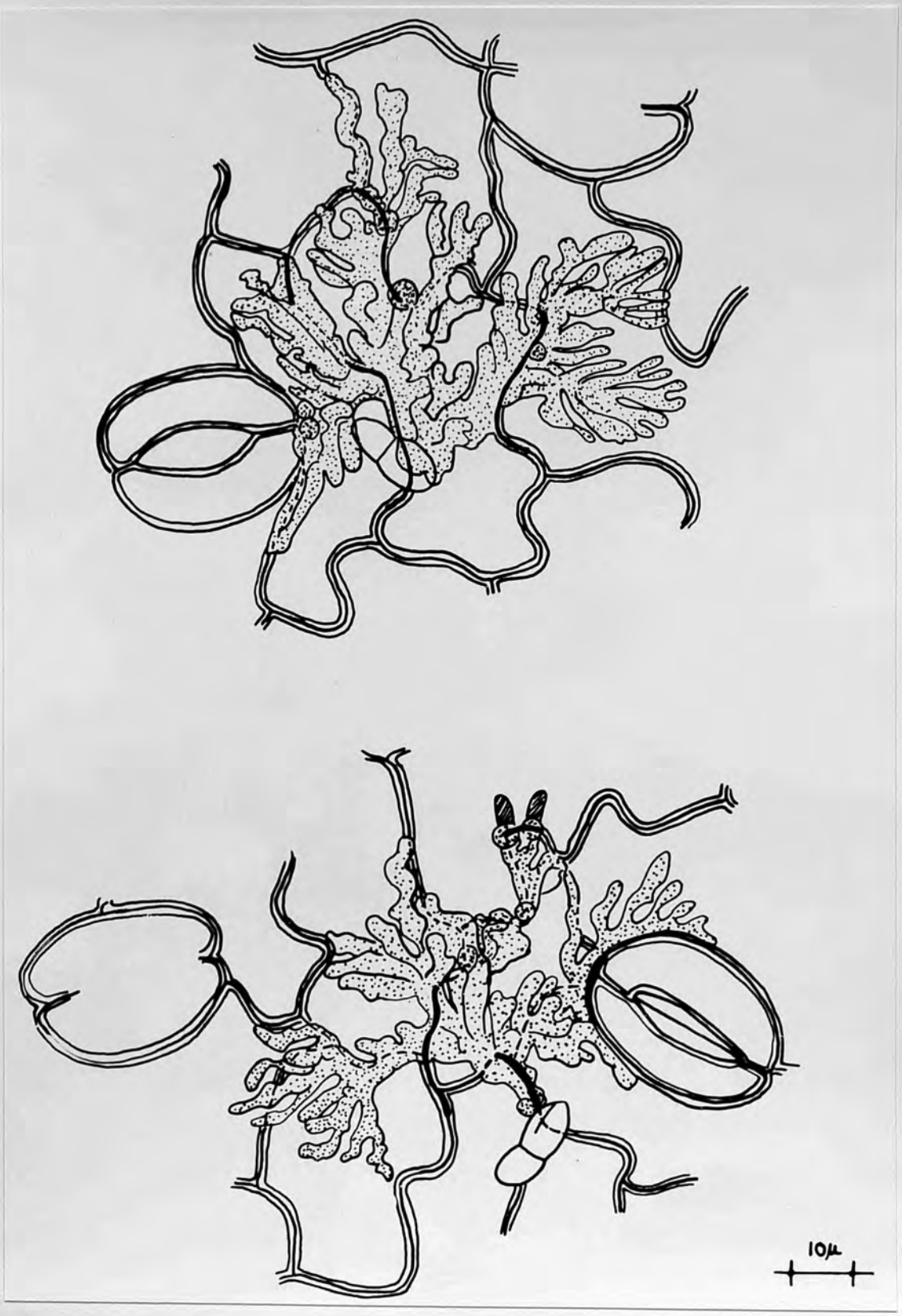



fig. 4

Figure 5.

Mycosphaerella rumicis. Epidermal strip of infected Rumex leaf. Shows extensive development of both sub cuticular (dotted) and sub epidermal hyphae (shaded). The sub epidermal mycelium extends further than does the sub cuticular mycelium, but its hyphae tend to remain separate and do not form the sheet like mass of the sub cuticular hyphae.

fig 5

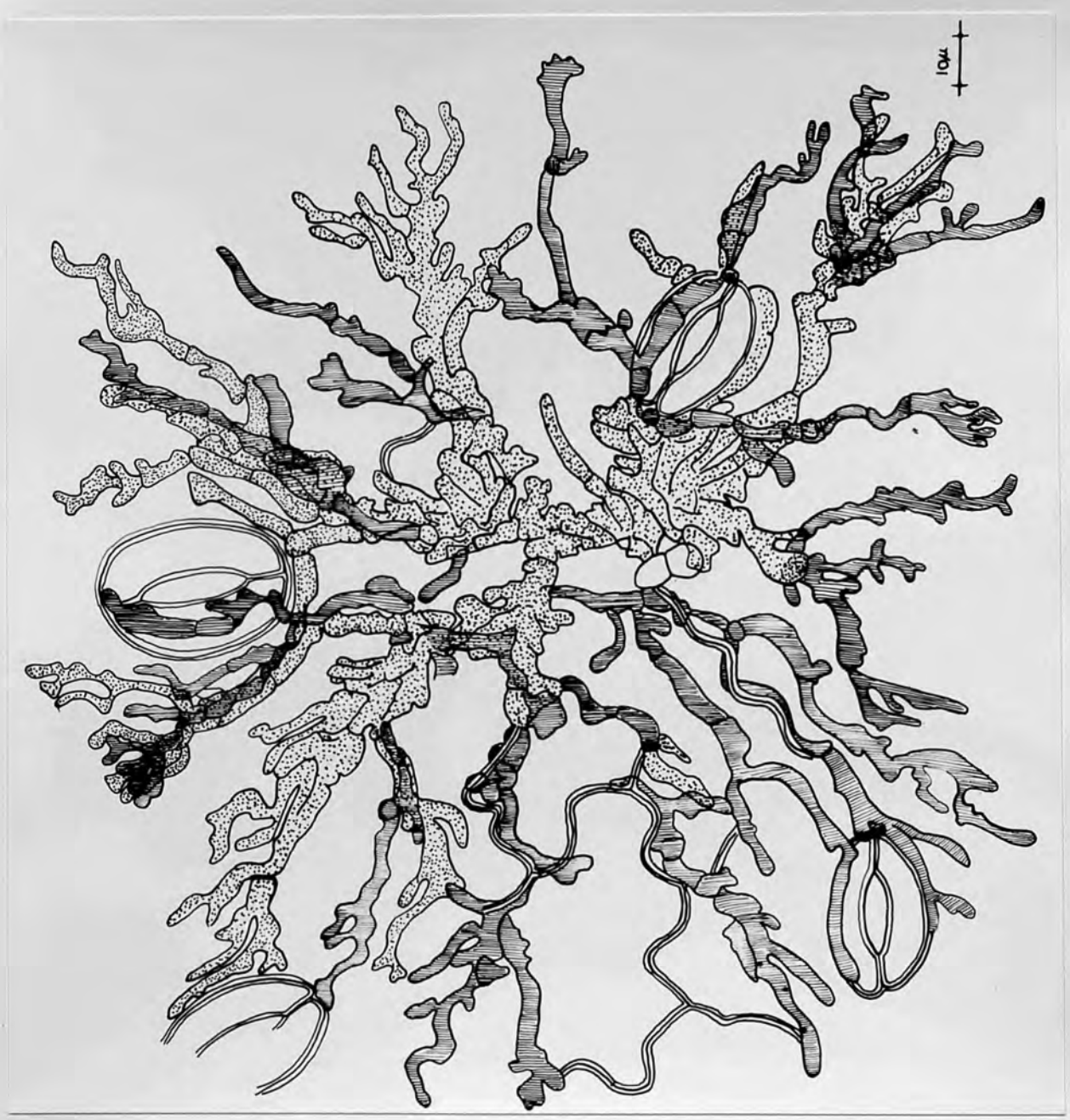



fig. 5

Figure 6.

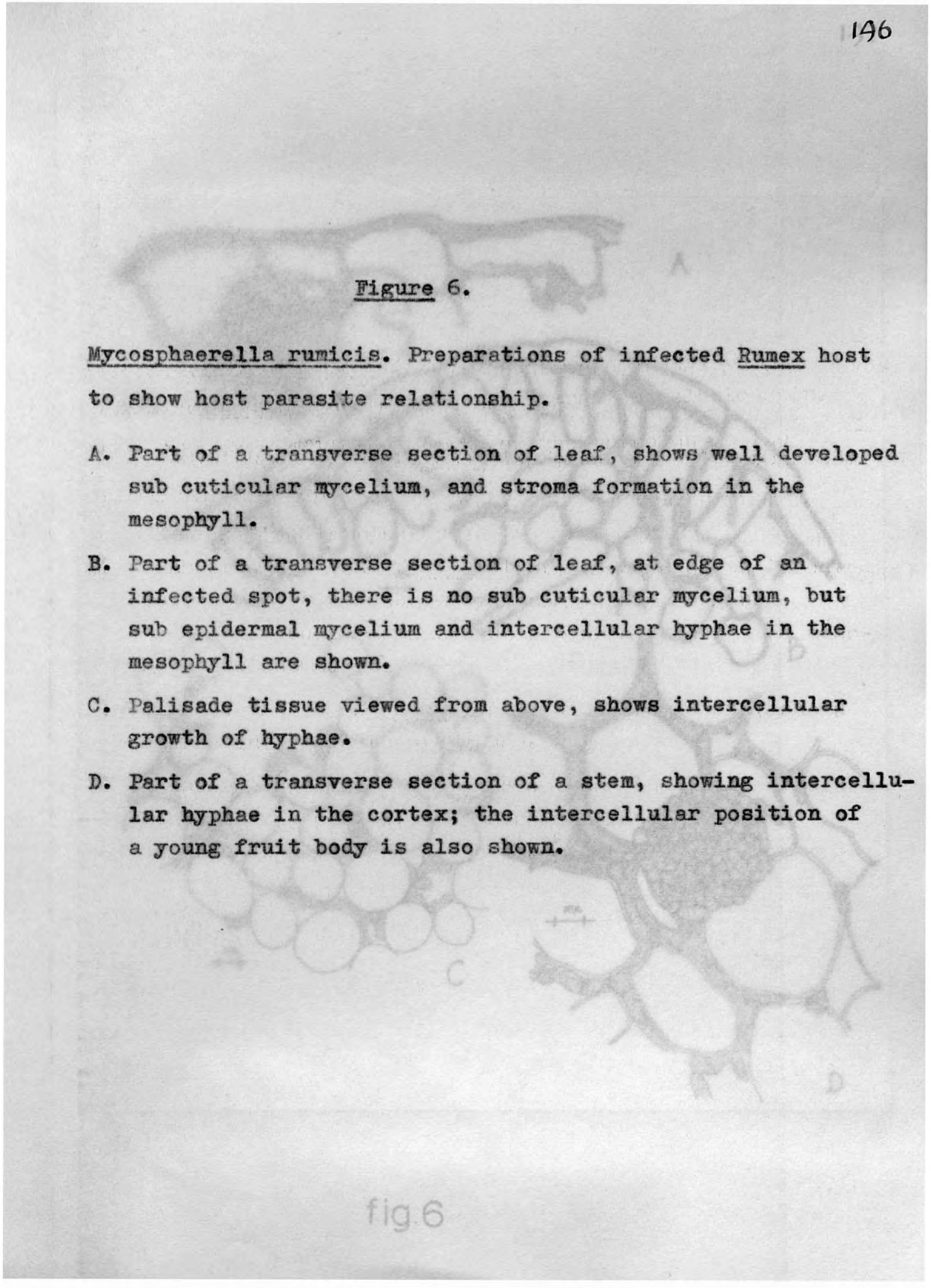
Mycosphaerella rumicis. Preparations of infected Rumex host to show host parasite relationship.

A. Part of a transverse section of leaf, shows well developed sub cuticular mycelium, and stroma formation in the mesophyll.

B. Part of a transverse section of leaf, at edge of an infected spot, there is no sub cuticular mycelium, but sub epidermal mycelium and intercellular hyphae in the mesophyll are shown.

C. Palisade tissue viewed from above, shows intercellular growth of hyphae.

D. Part of a transverse section of a stem, showing intercellular hyphae in the cortex; the intercellular position of a young fruit body is also shown.



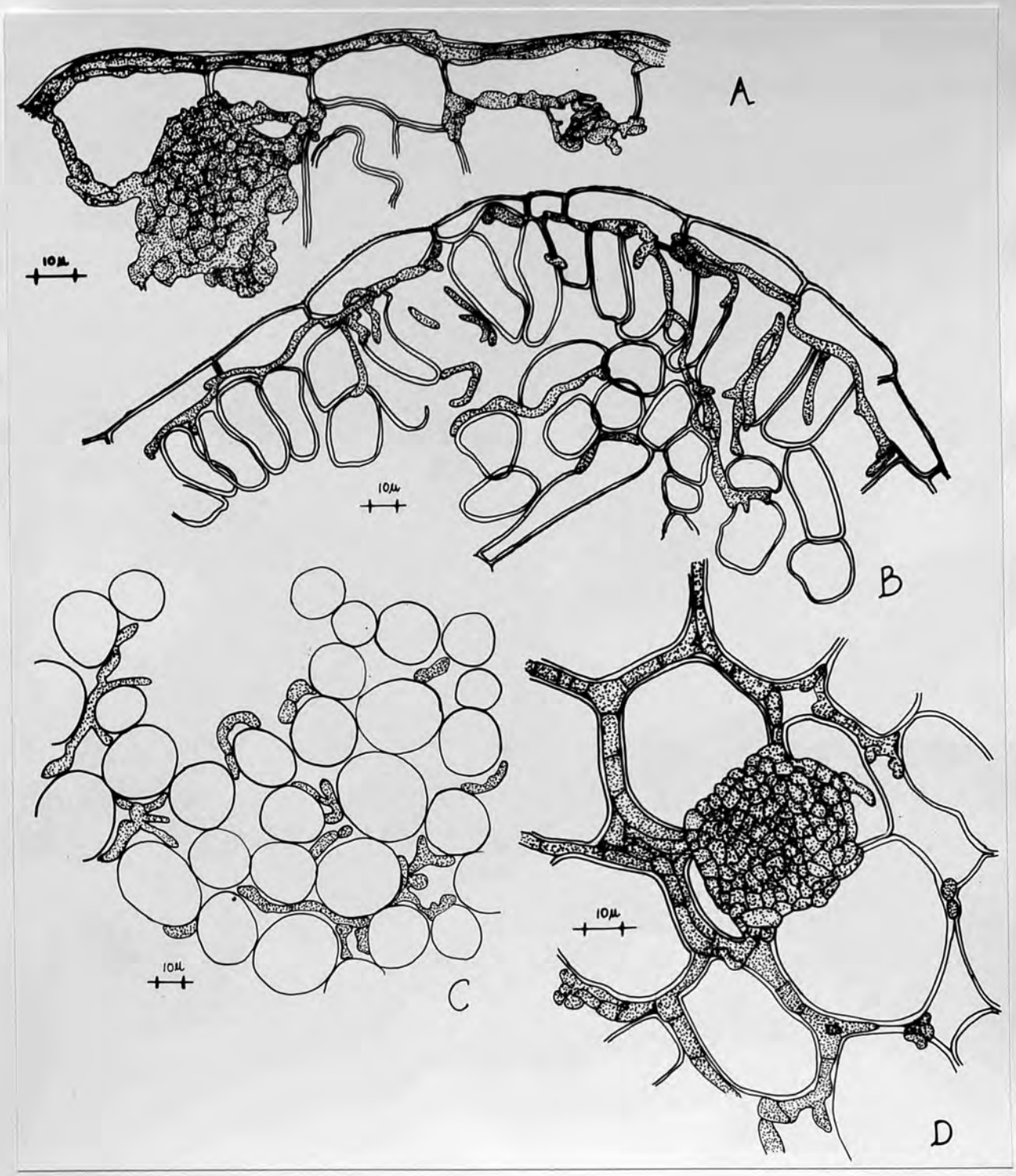



fig.6

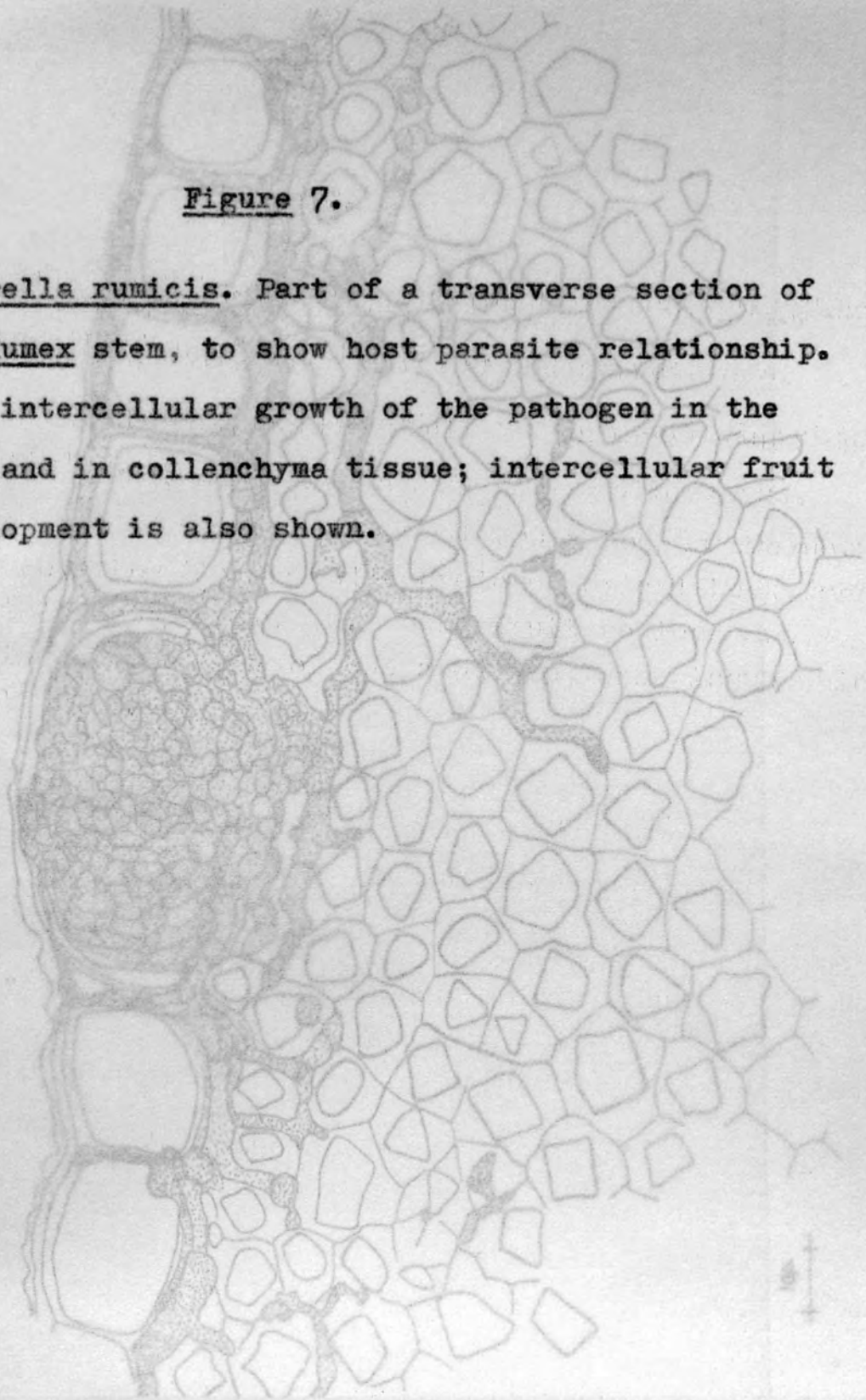


fig.7.

Figure 7.

Mycosphaerella rumicis. Part of a transverse section of infected Rumex stem, to show host parasite relationship. Shows the intercellular growth of the pathogen in the epidermis and in collenchyma tissue; intercellular fruit body development is also shown.

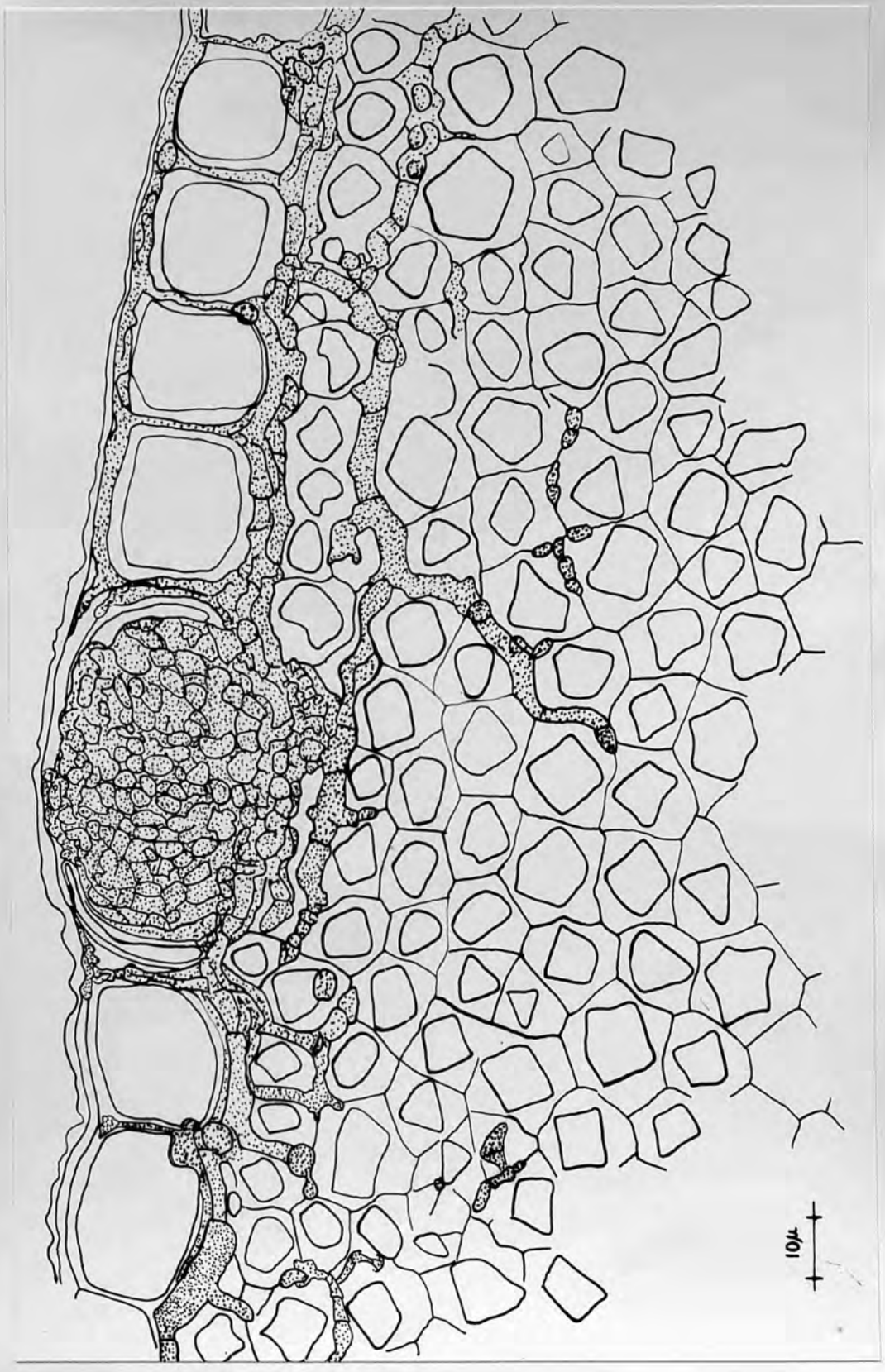



fig. 7

**Figure 8.**

*Mycosphaerella rumicis.* Two infection spots on *Rumex* leaves to show distribution of fruit bodies.

A. A red-bordered spot, centre fruit bodies are mature and have ostioles.

B. A younger spot than in A, central fruit bodies are not yet mature, they have a well developed apical beak (shaded), but ostiole not yet formed. Young fruit bodies are developing at the edge of the spot, in leaf tissue which is still green.

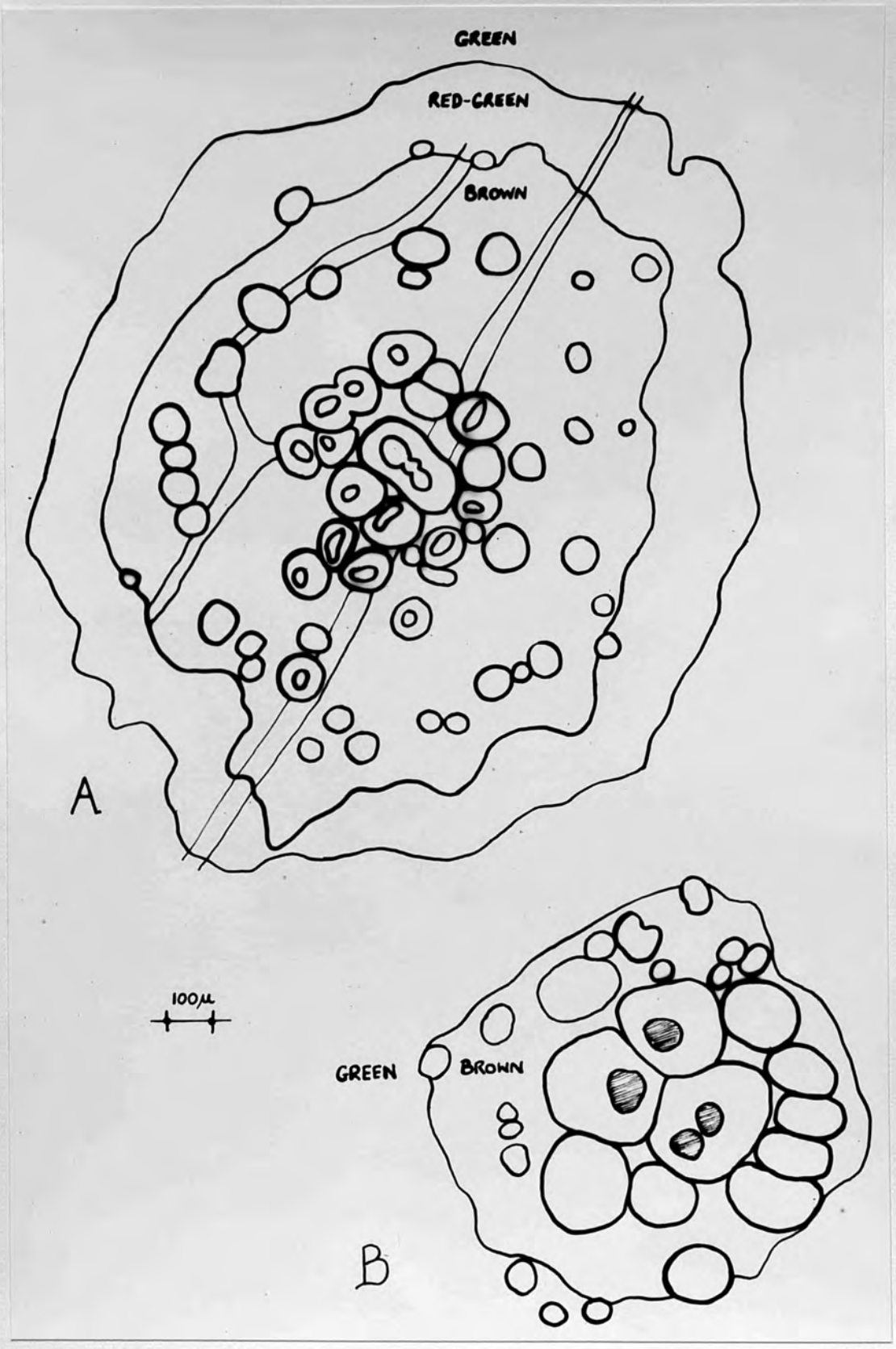
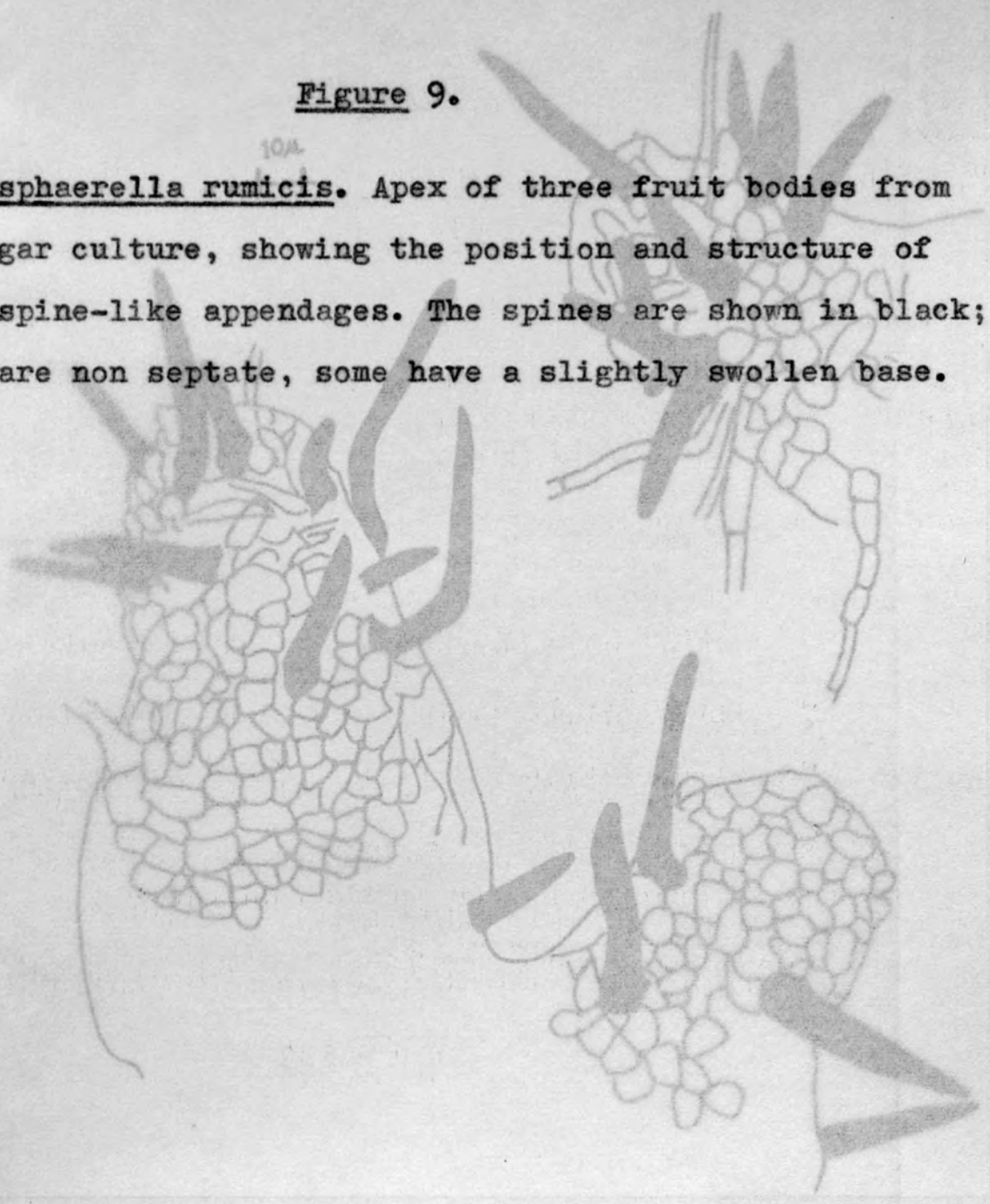



fig. 8

Figure 9.



Mycosphaerella rumicis. Apex of three fruit bodies from an agar culture, showing the position and structure of the spine-like appendages. The spines are shown in black; all are non septate, some have a slightly swollen base.

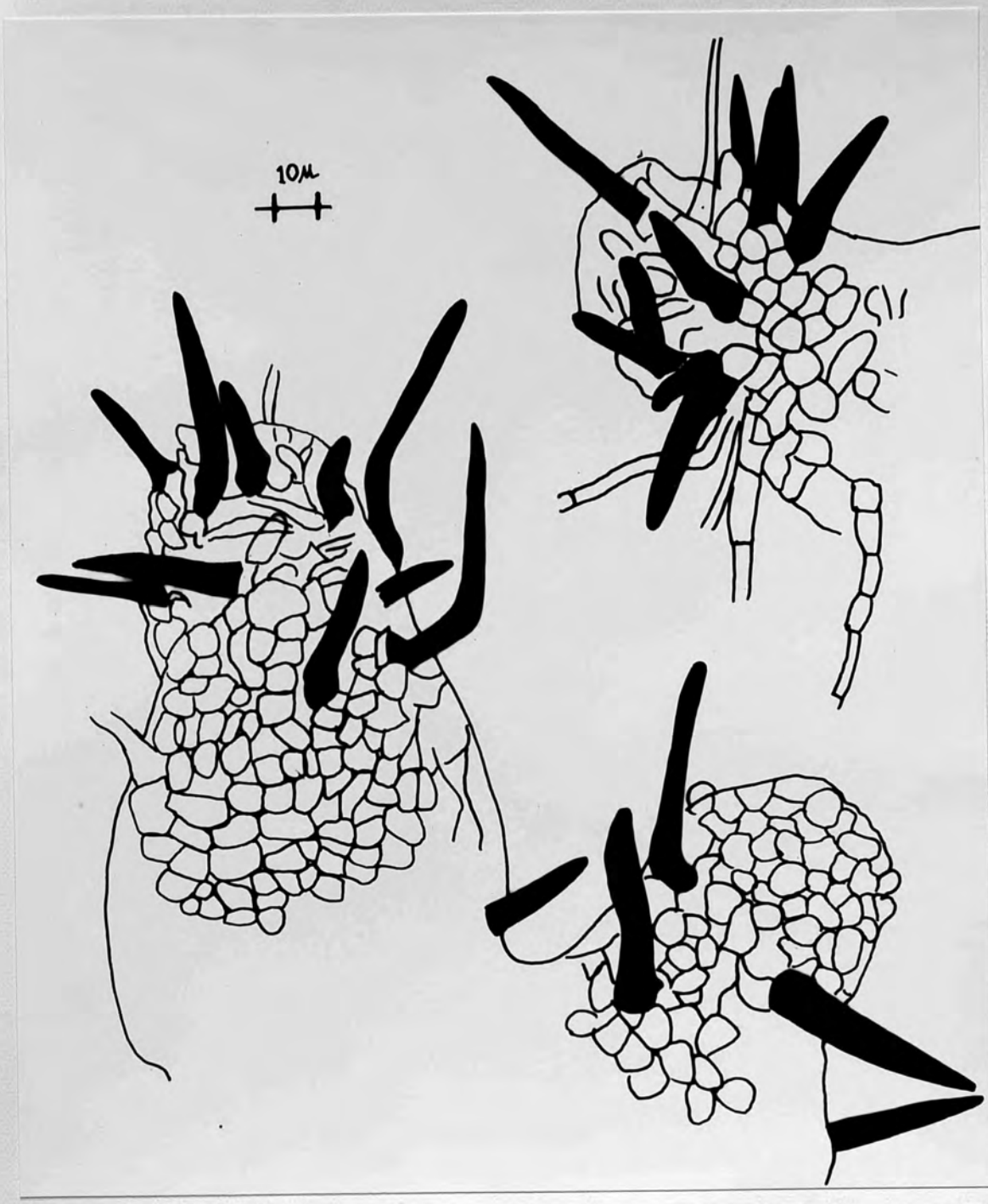
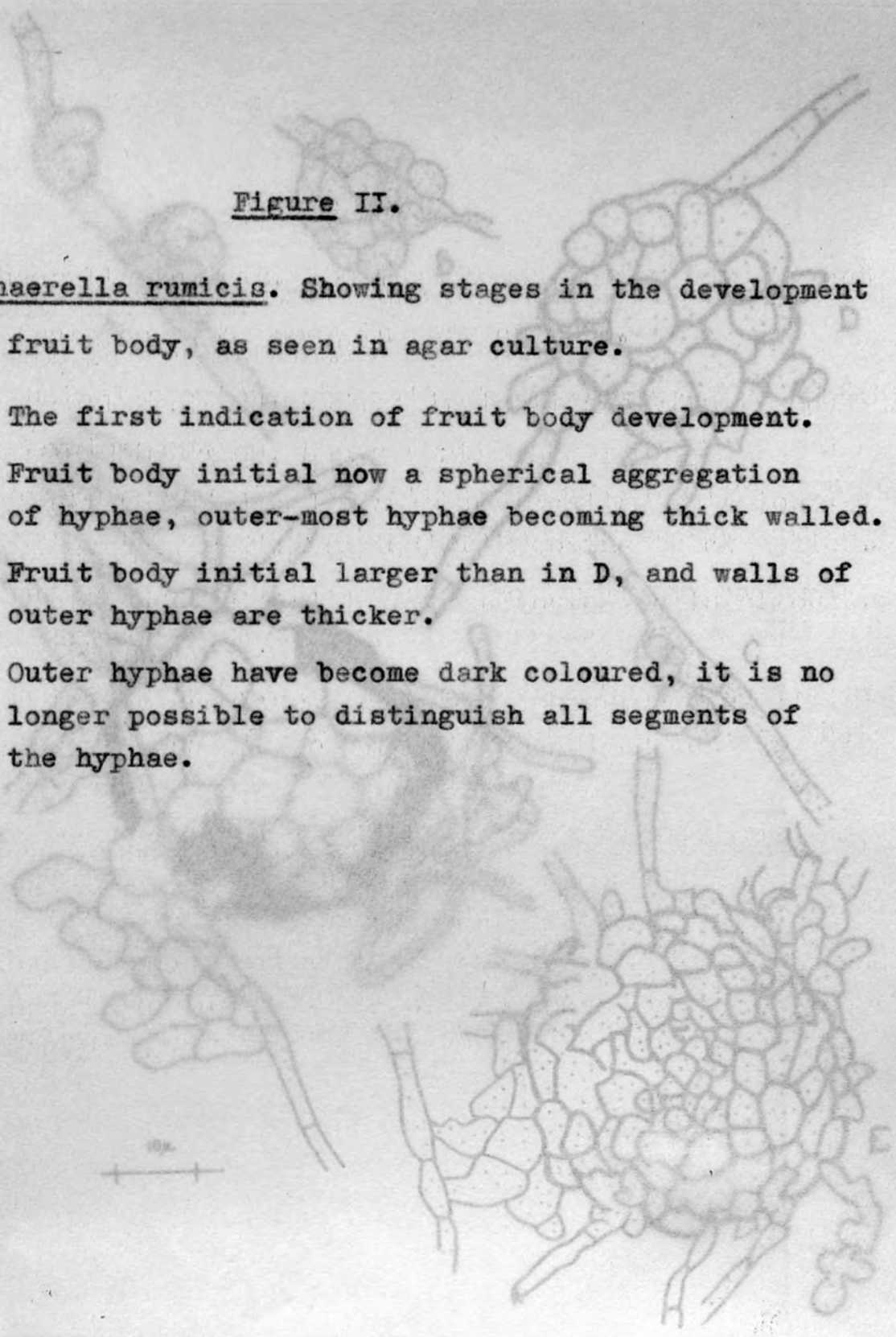



fig.9



## Figure II.

Mycosphaerella rumicis. Showing stages in the development of the fruit body, as seen in agar culture.

A,B,C. The first indication of fruit body development.

D. Fruit body initial now a spherical aggregation of hyphae, outer-most hyphae becoming thick walled.

E. Fruit body initial larger than in D, and walls of outer hyphae are thicker.

F. Outer hyphae have become dark coloured, it is no longer possible to distinguish all segments of the hyphae.

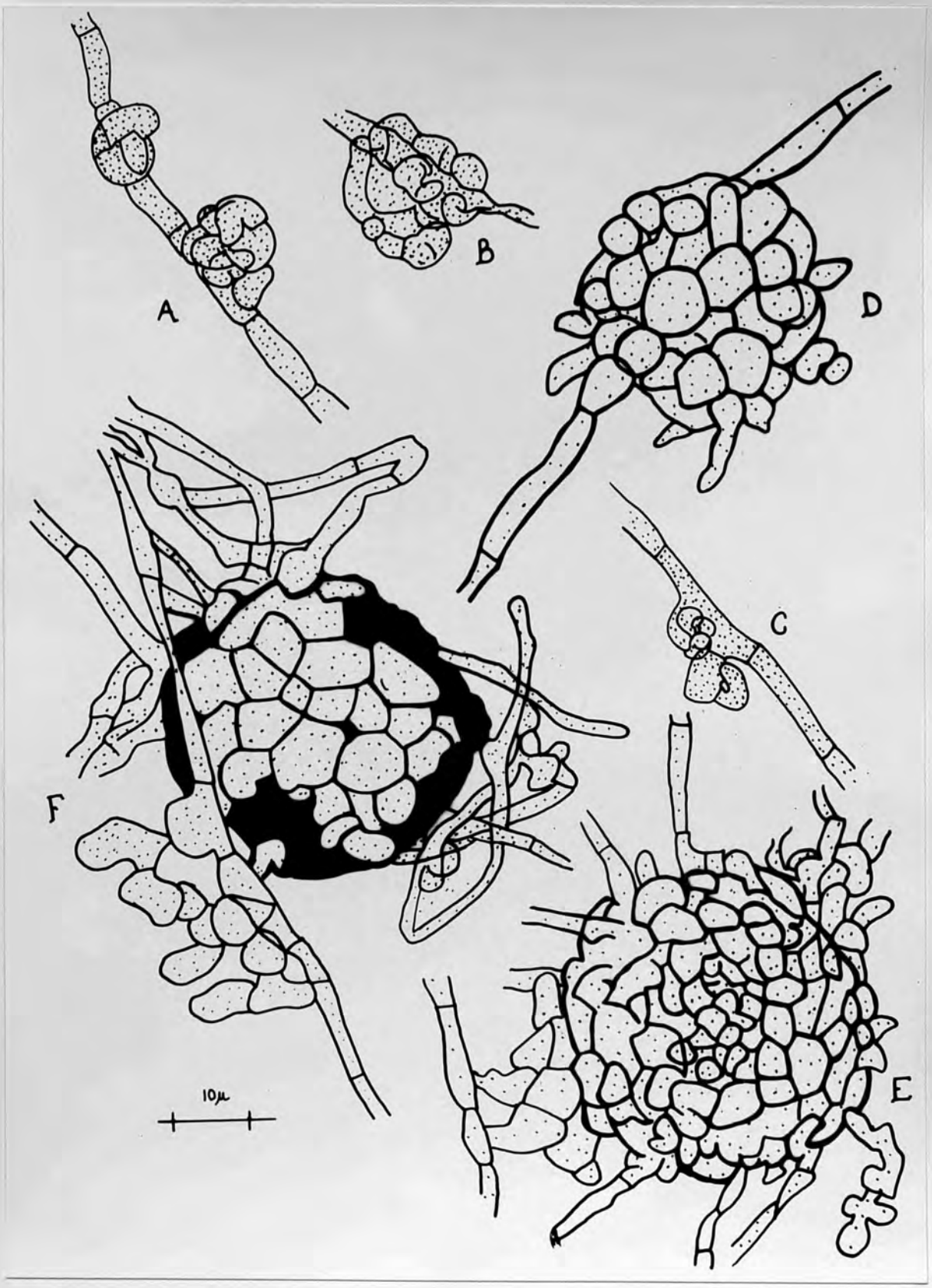



fig.11

Figure I2.

Mycosphaerella rumicis. Sections of young fruit bodies to show stages in development.

A. Longitudinal section, shows little internal differentiation.

B. Longitudinal section, shows vertically orientated pseudoparaphyses which are attached at both their apex and base; asci are not yet developed.

C. Longitudinal section, shows asci beginning to grow up among the pseudoparaphyses.

D. Longitudinal section, shows a slightly later stage than C; the pseudoparaphyses are prominent at the apex, their apical attachment is clearly shown.

E. Transverse section of fruit body which is at about the same stage of development as is the one shown in figure I3 A. Shows the arrangement of the asci and pseudoparaphyses. Few contents are left in the asci, and the inner layer of the wall of the ascus shows considerable swelling.

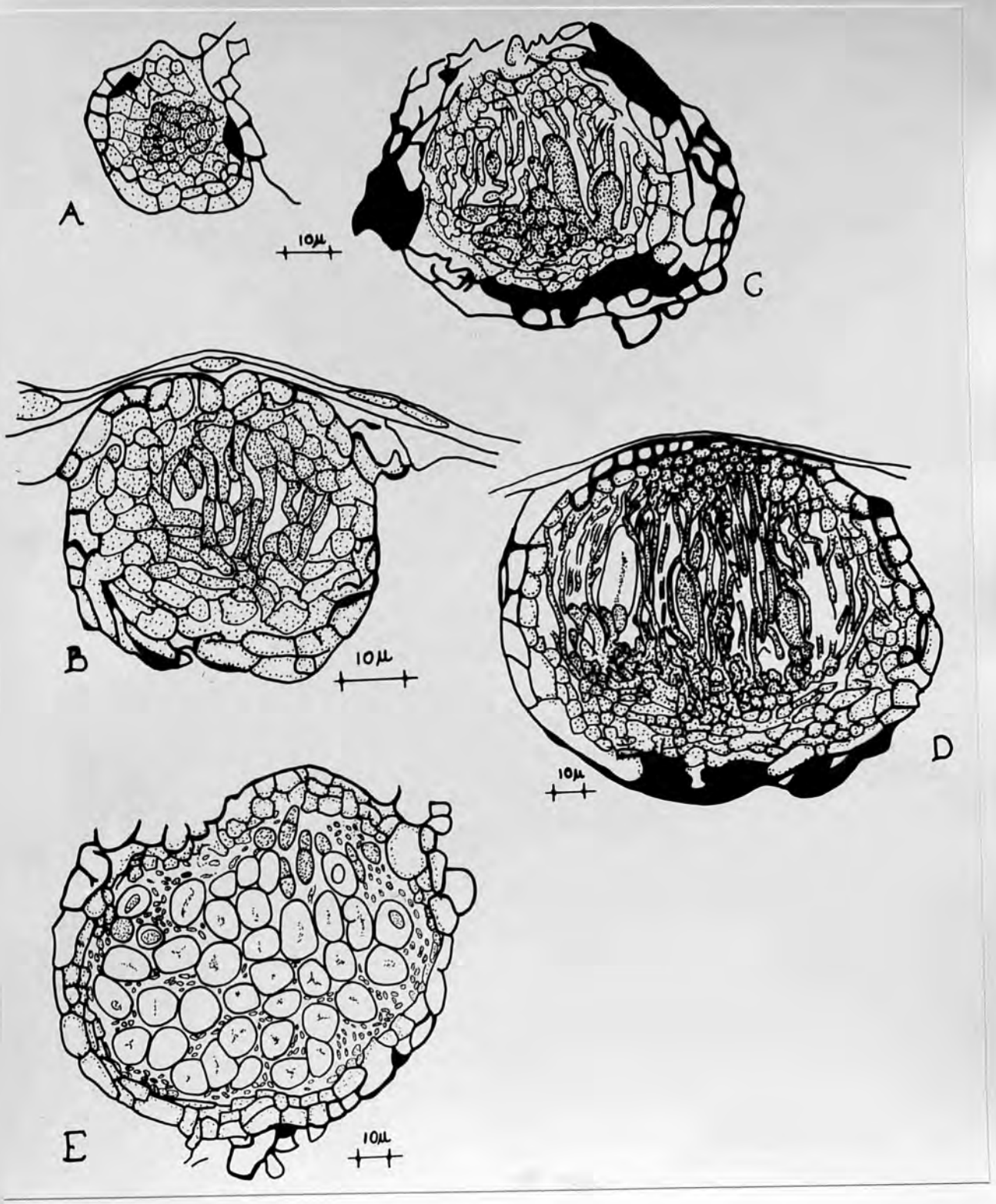



fig.12

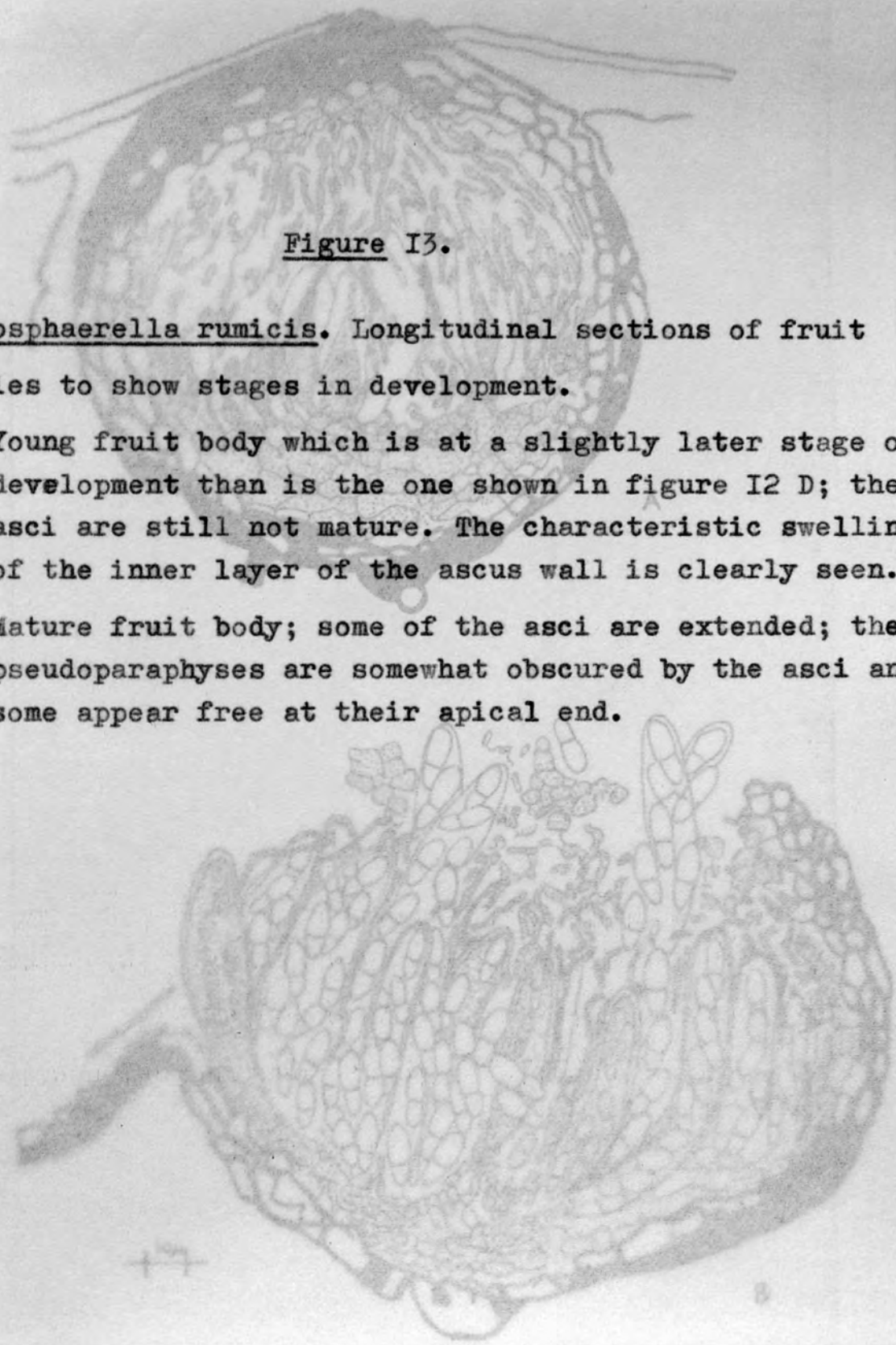


Figure I3.

Mycosphaerella rumicis. Longitudinal sections of fruit bodies to show stages in development.

A. Young fruit body which is at a slightly later stage of development than is the one shown in figure I2 D; the asci are still not mature. The characteristic swelling of the inner layer of the ascus wall is clearly seen.

B. Mature fruit body; some of the asci are extended; the pseudoparaphyses are somewhat obscured by the asci and some appear free at their apical end.

fig.13

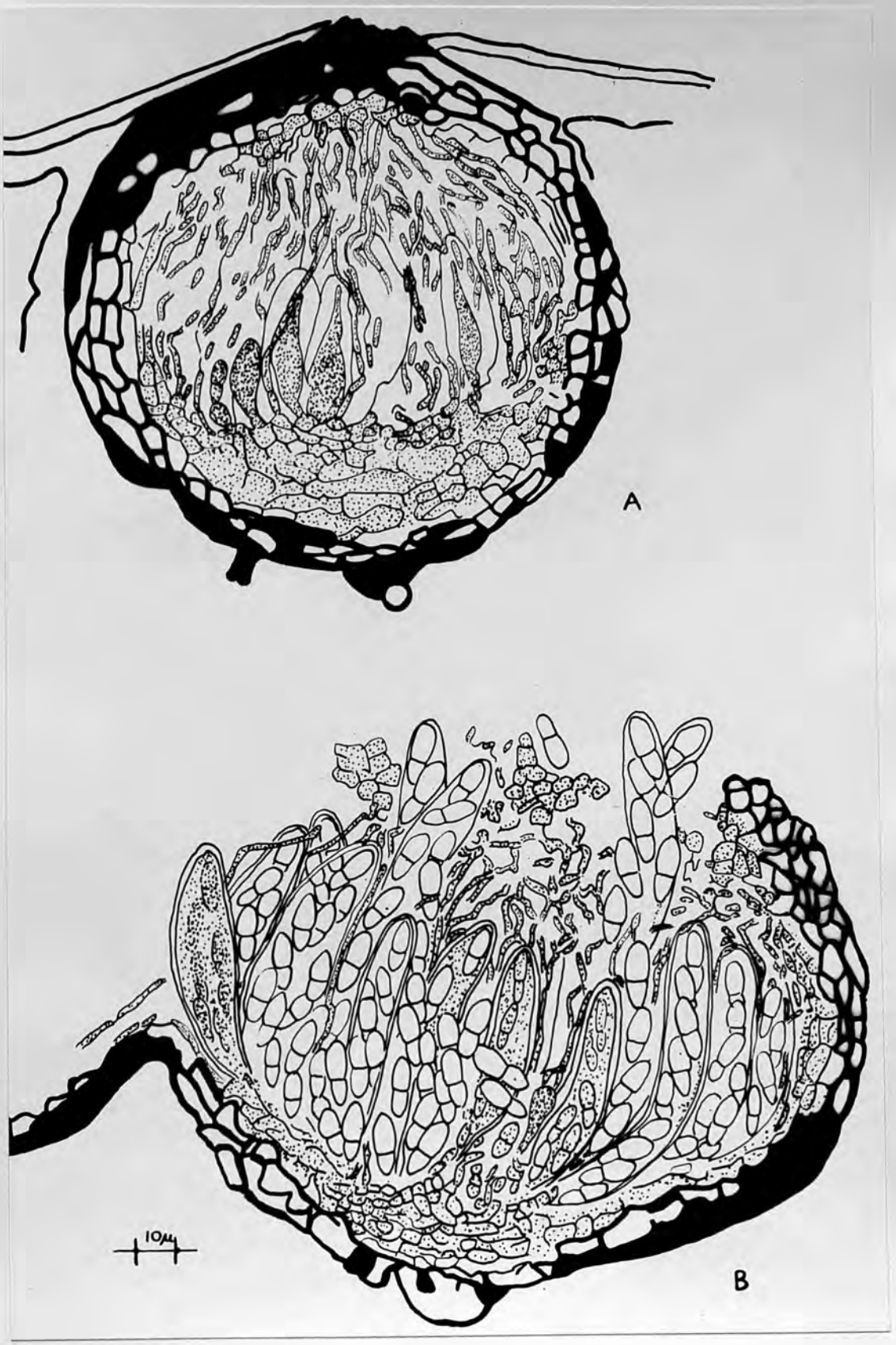



fig.13

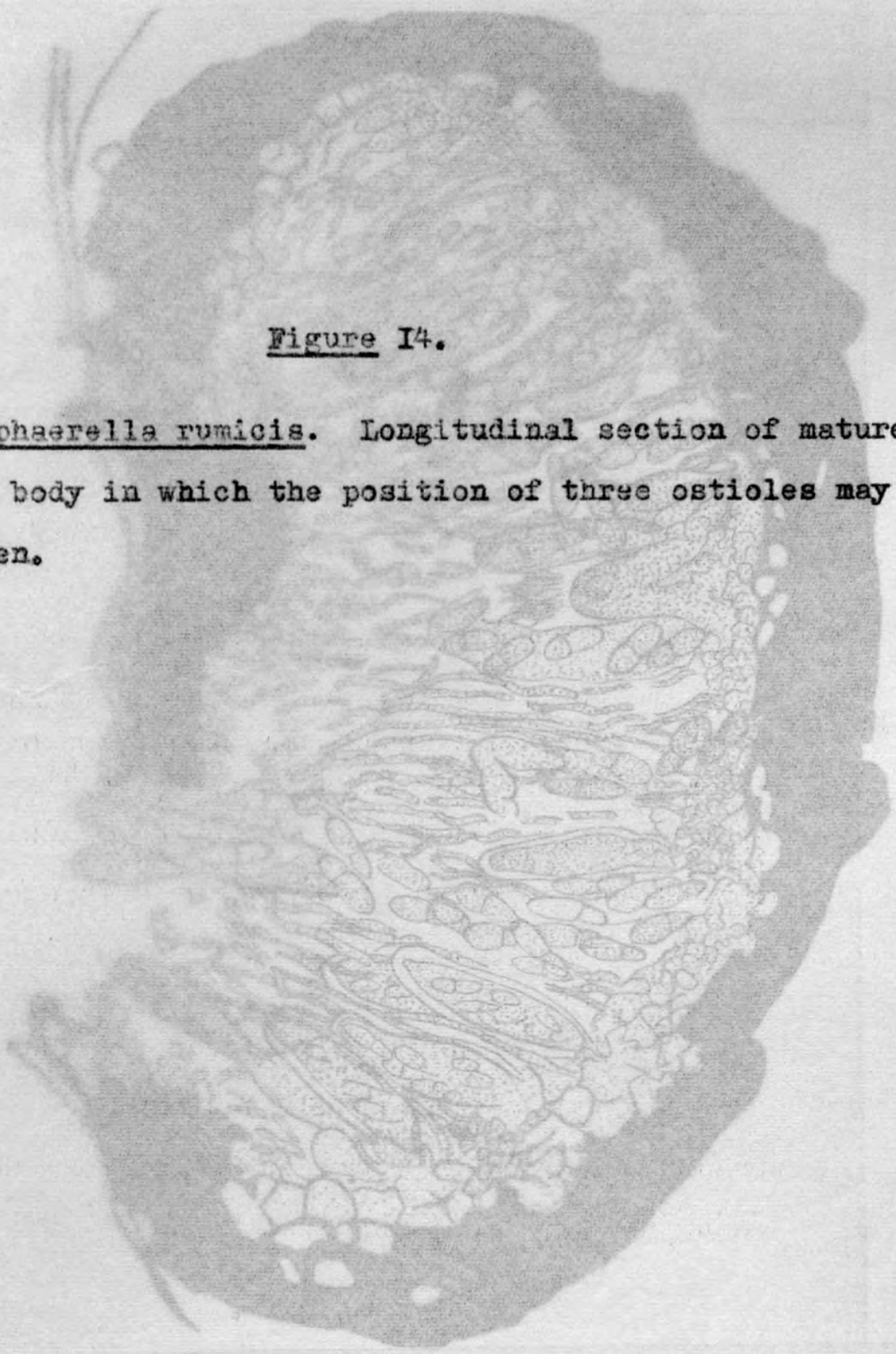


Figure I4.

Mycosphaerella rumicis. Longitudinal section of mature fruit body in which the position of three ostioles may be seen.

fig.14

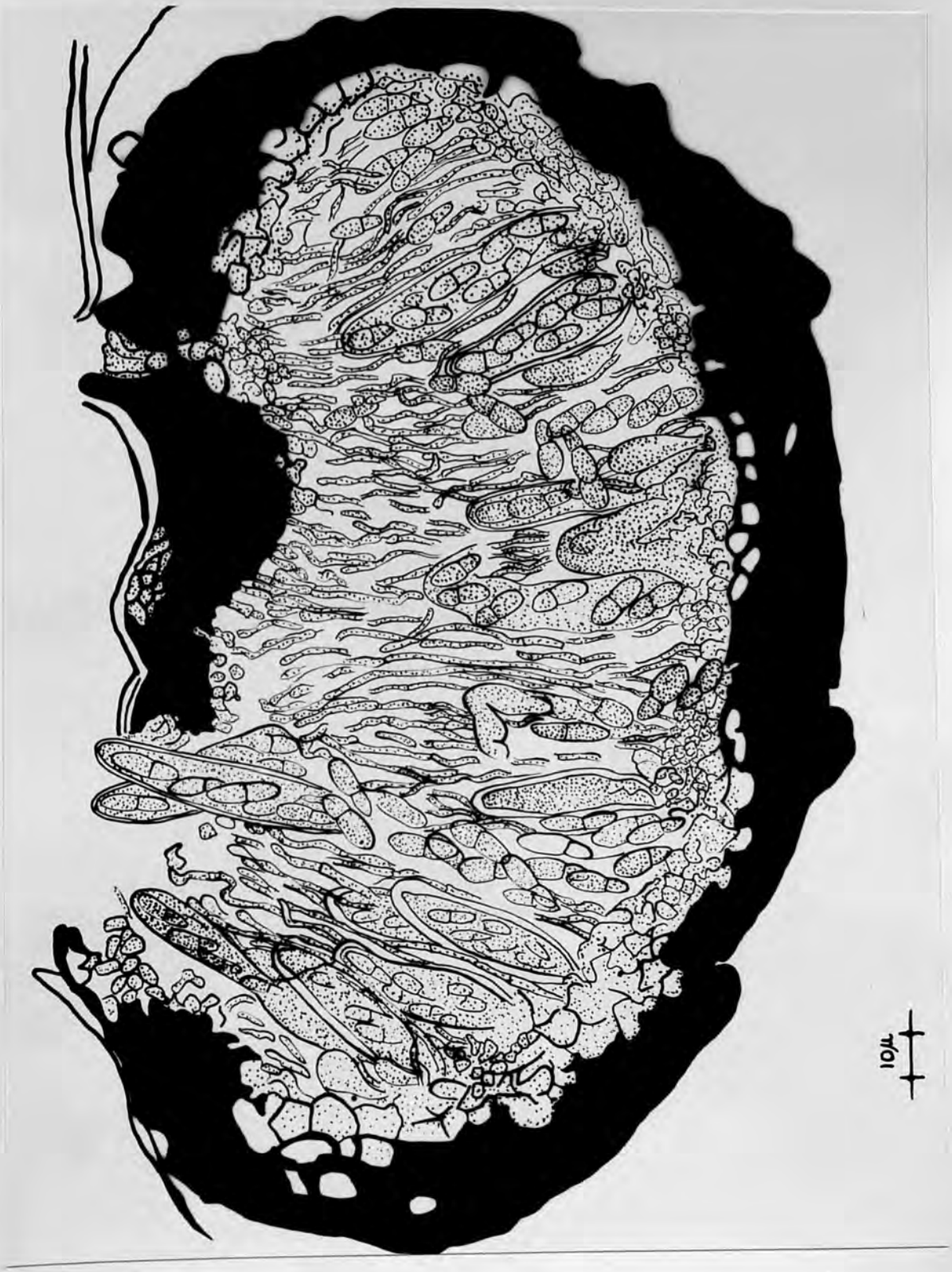



fig.14

Figure IO.

Mycosphaerella rumicis. Showing some characteristics of the vegetative hyphae in agar culture.

A. Hyphal anastomosis.

B. Characteristic branching of the vegetative mycelium.

C. Hyphae arising from a germinated ascospore.

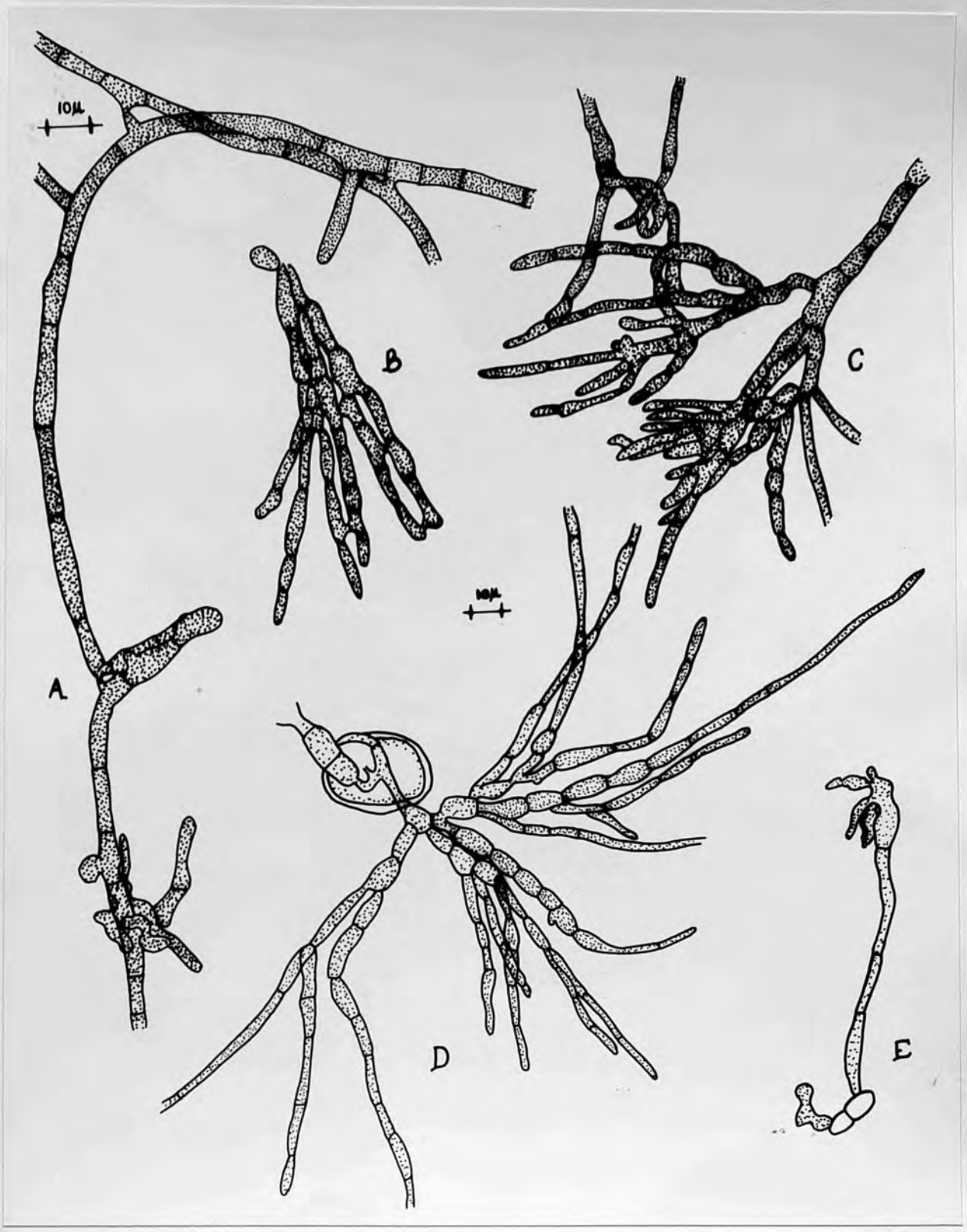



fig.10

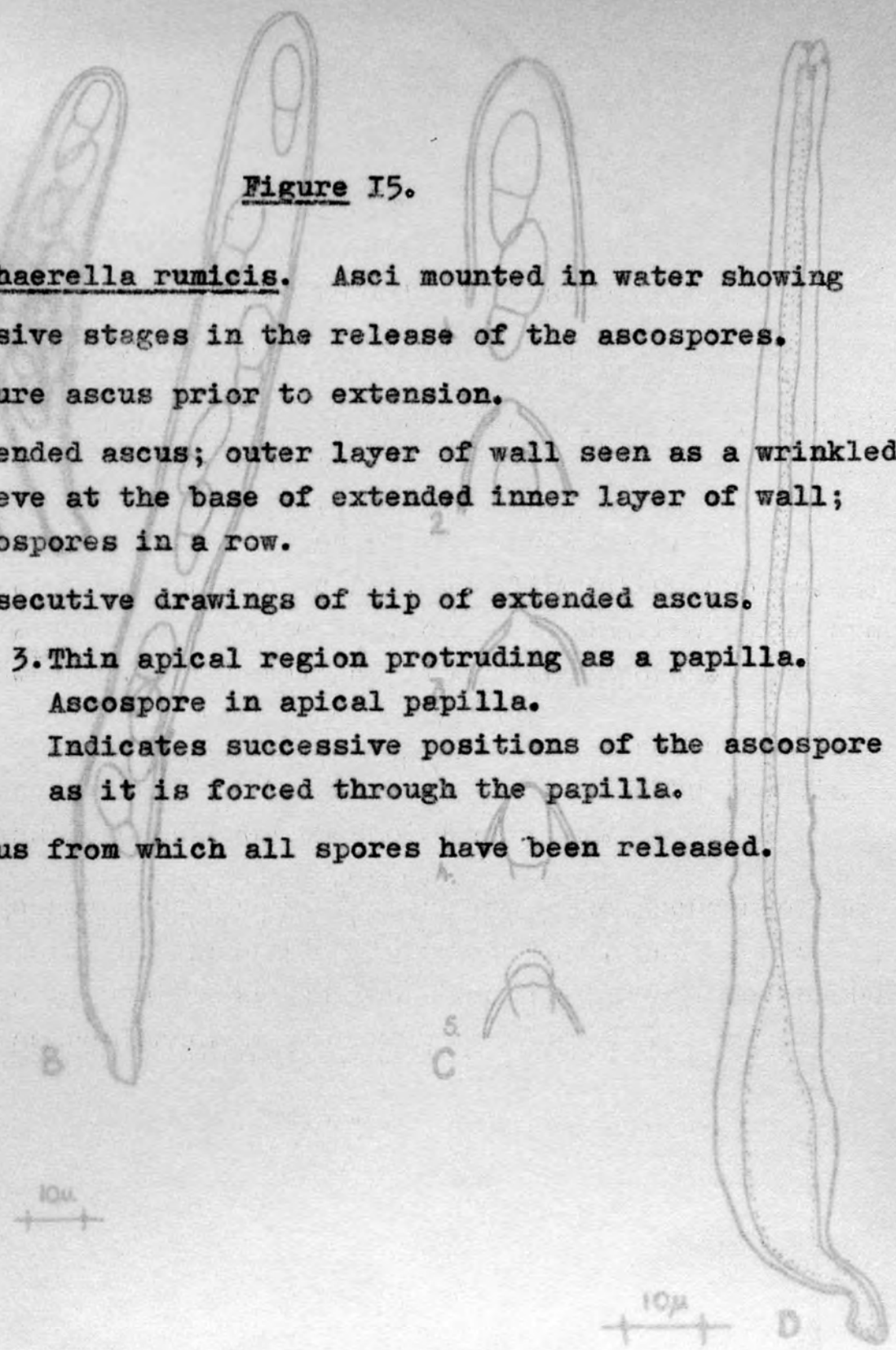



**Figure** I5.

**Mycosphaerella rumicis.** Asci mounted in water showing successive stages in the release of the ascospores.

A. Mature ascus prior to extension.

B. Extended ascus; outer layer of wall seen as a wrinkled sleeve at the base of extended inner layer of wall; ascospores in a row.

C. Consecutive drawings of tip of extended ascus.

I - 3. Thin apical region protruding as a papilla.
4. Ascospore in apical papilla.
5. Indicates successive positions of the ascospore as it is forced through the papilla.

D. Ascus from which all spores have been released.

fig.15

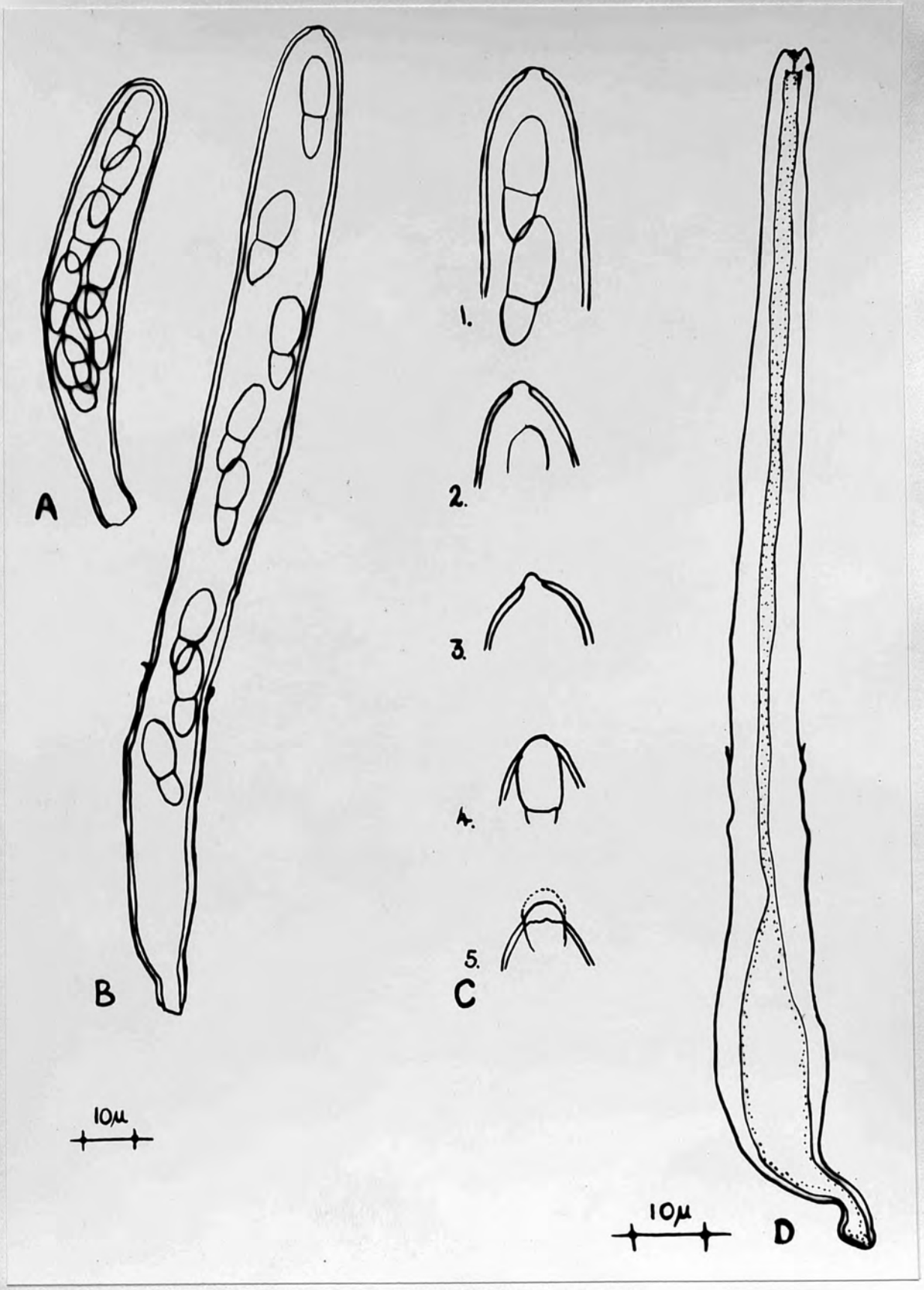



fig.15

Figure 16.

Mycosphaerella rumicis. Fruit bodies as seen in the ring moist chambers.

$^{A}_{I}$ - 2 Asci projecting from fruit bodies, in 2 the position of the ascospores is indicated.

$^{B}_{I}$ - 3 Consecutive drawings of one fruit body.
1. One ascus projecting, and liberating spores.
2. Two asci projecting, one of them liberating spores.
3. As 2, but both asci liberating spores.

$^{C}_{I}$ - 8 Consecutive drawings of an extended ascus showing the gradual collapse which occurred when the lid was removed from the moist chamber.

$^{D}_{I}$ - 5 Consecutive drawings of an extended ascus showing a similar gradual collapse as in C, but also a subsequent re-extension when the lid of the chamber was replaced.

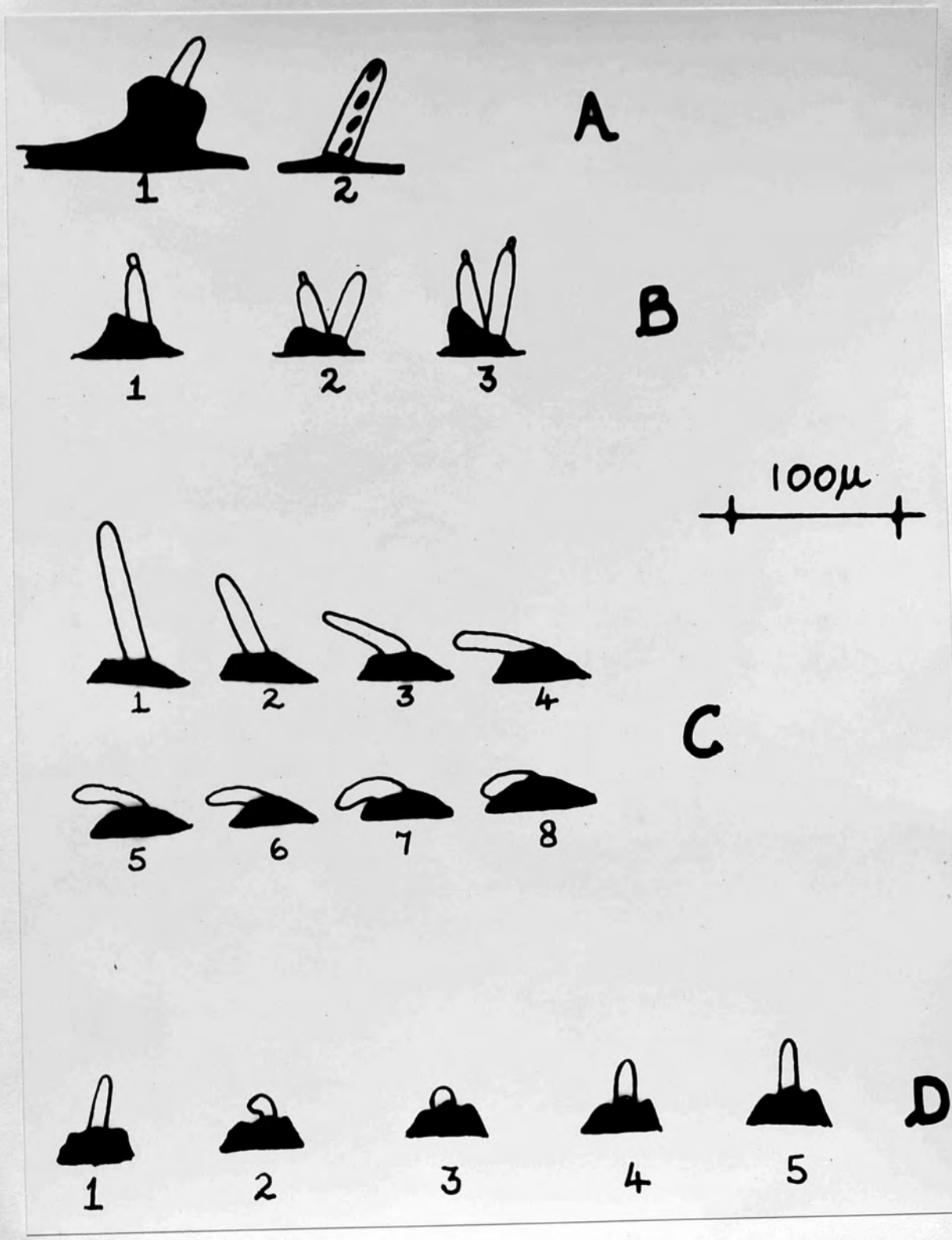



fig.16

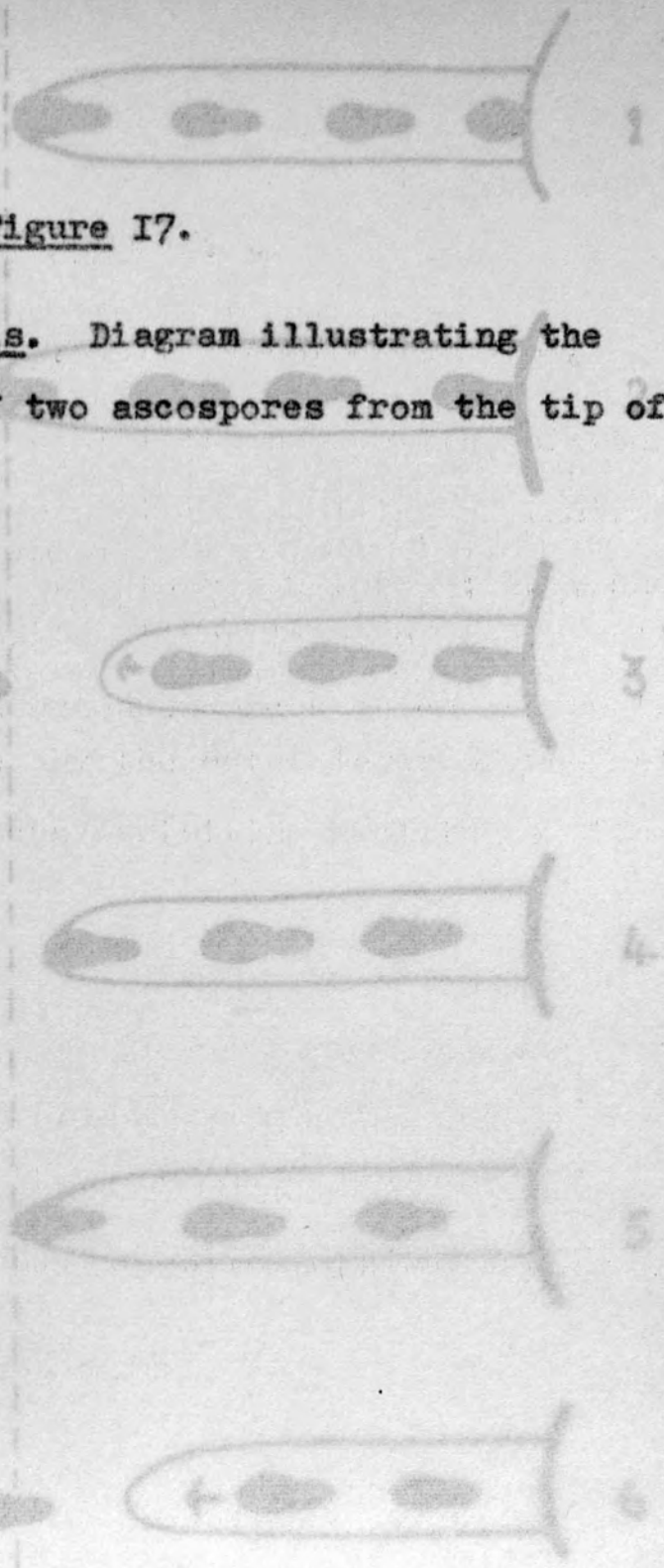



Figure I7.

Mycosphaerella rumicis. Diagram illustrating the successive release of two ascospores from the tip of an extended ascus.

fig.17

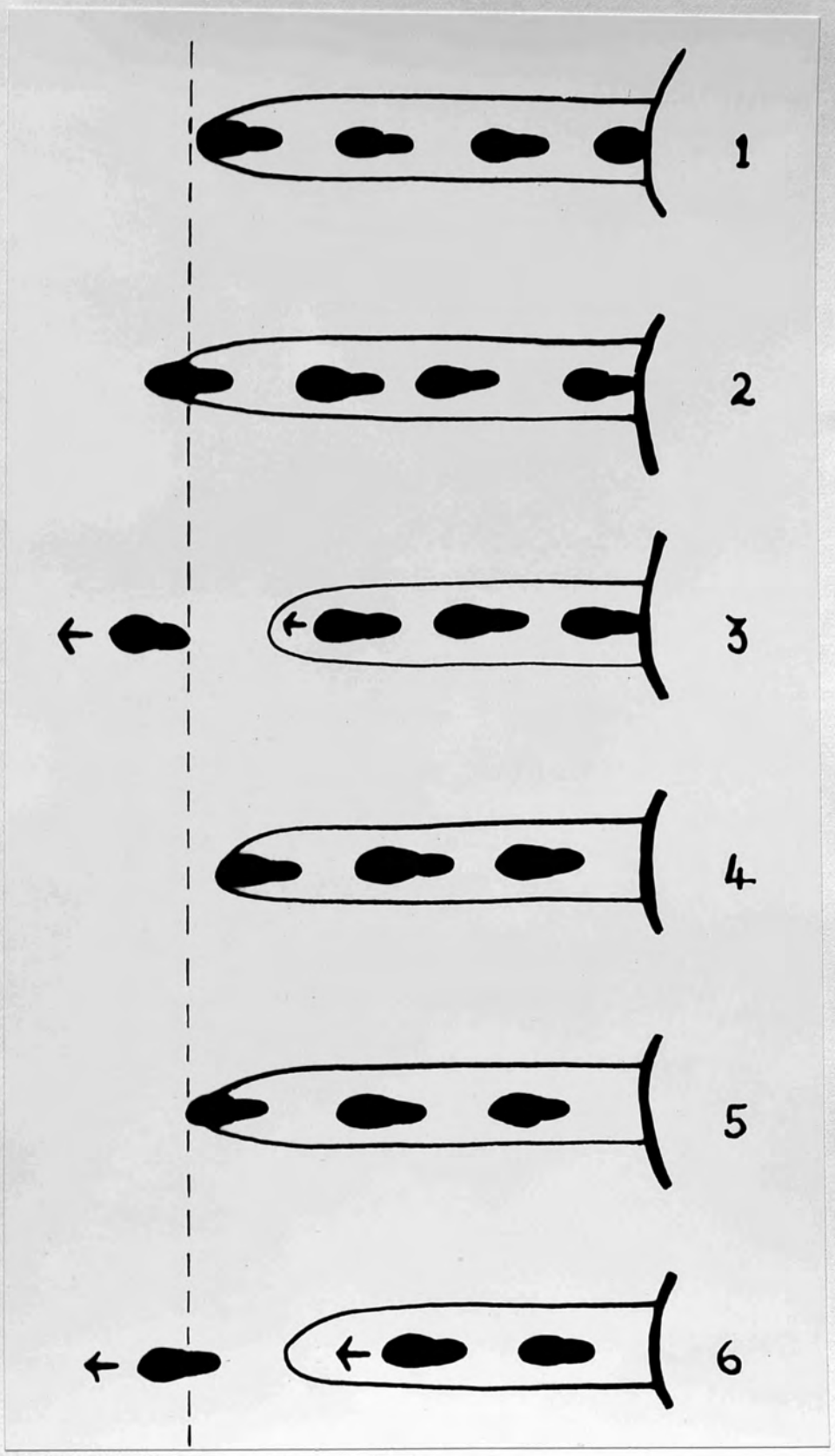



fig. 17

## Figure 18.

Mycosphaerella rumicis. Asci mounted in water.

A,B,C,F. Young asci exhibiting no swelling of their walls.

D,E. Young asci exhibiting swelling of their walls.

G,H,I. Young asci prematurely extended; H,I, show swelling of the inner layer of their wall.

J. Part of an extended ascus which has an 'apical cap' formed by the outer layer of the wall.

K. Damaged ascus exhibiting swelling of its wall.

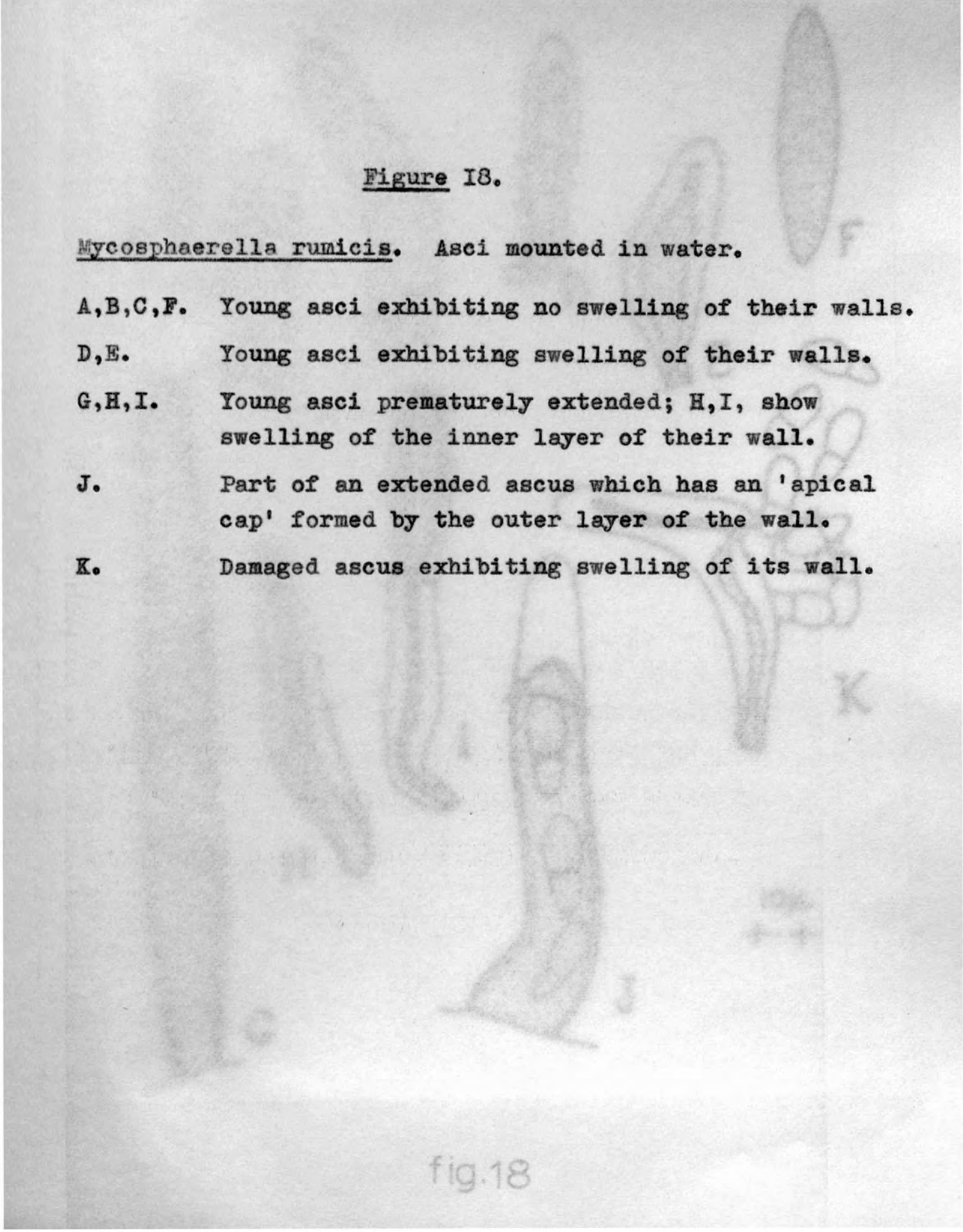



fig.18

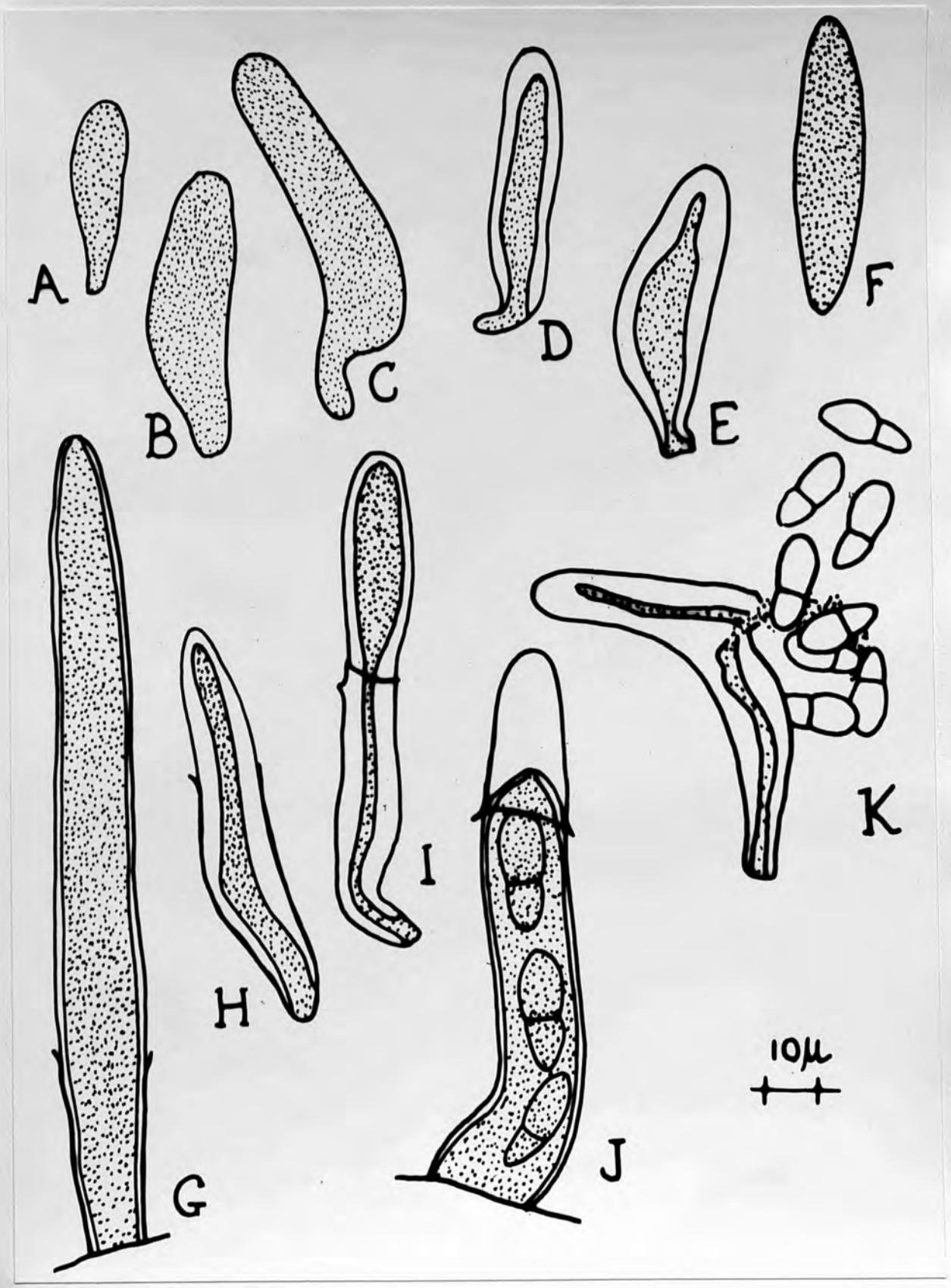



fig.18

Figure 19.

Pleospora herbarum. Asci mounted in water.

A. Mature ascus, spores shown only in outline.

B. Extended ascus, shows outer layer of wall as a wrinkled sleeve at the base of the extended inner layer. Septation of the ascospores shown.

C. Tip of extended ascus, showing the apical papilla.

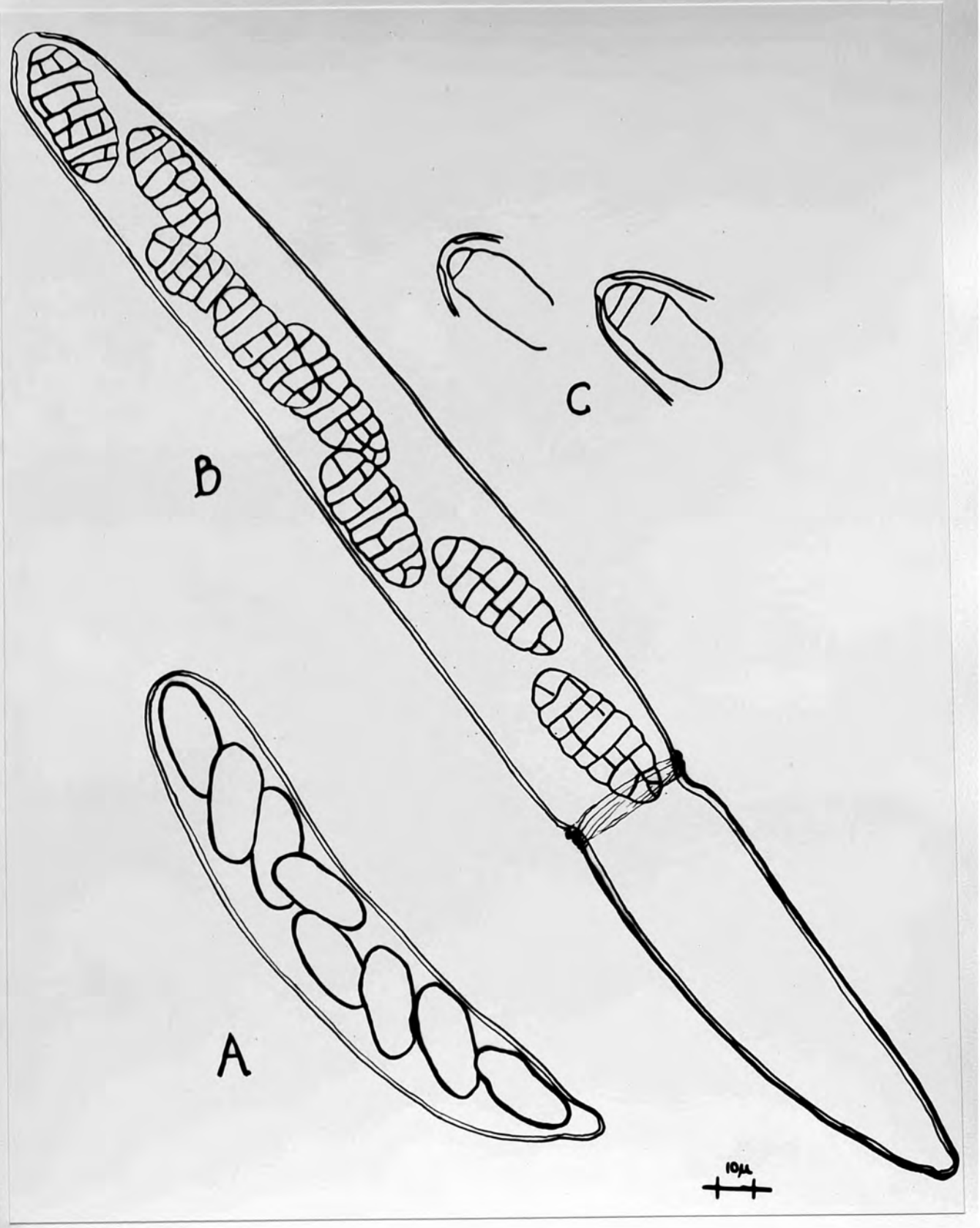



fig.19

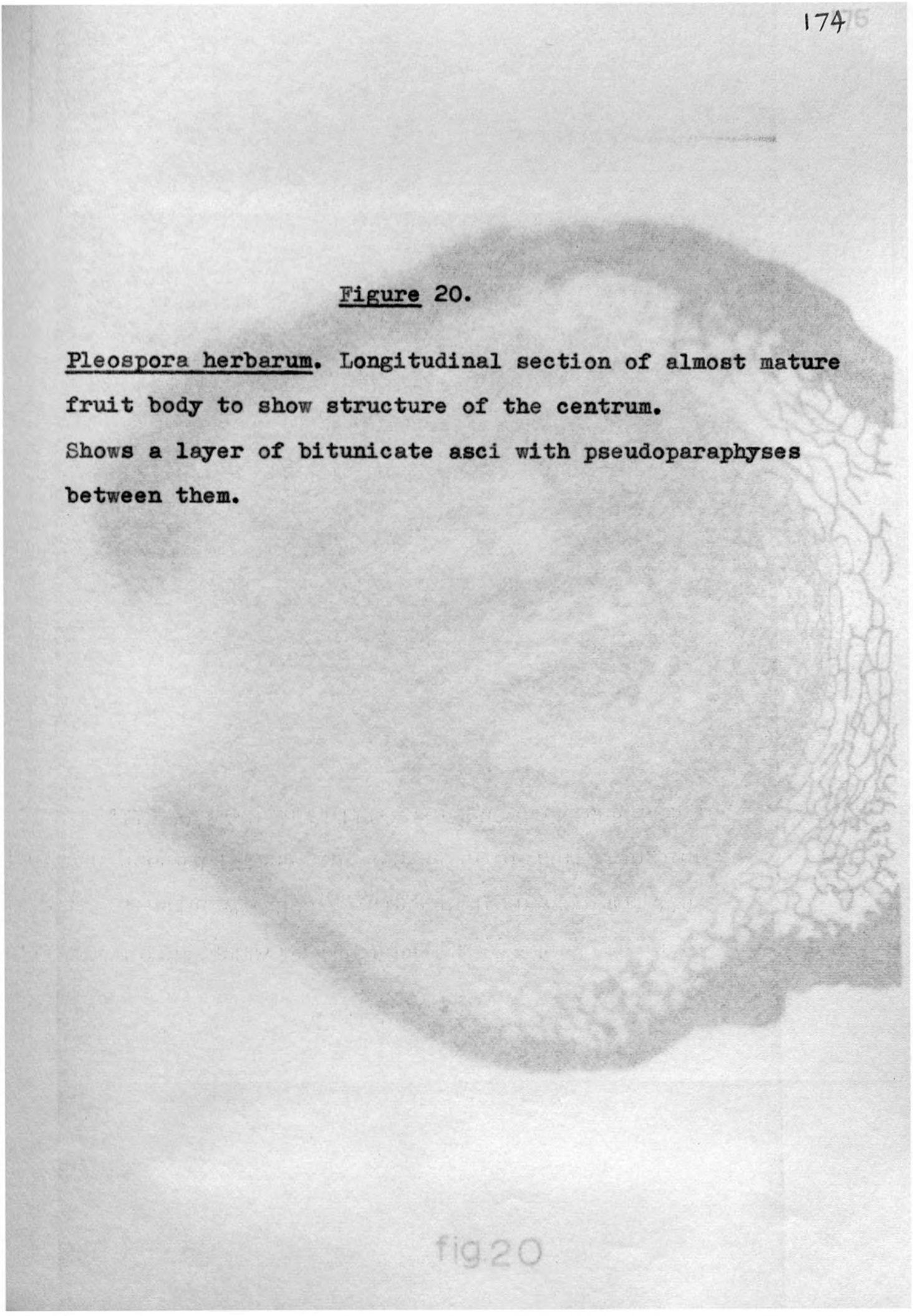



Figure 20.

Pleospora herbarum. Longitudinal section of almost mature fruit body to show structure of the centrum.
Shows a layer of bitunicate asci with pseudoparaphyses between them.

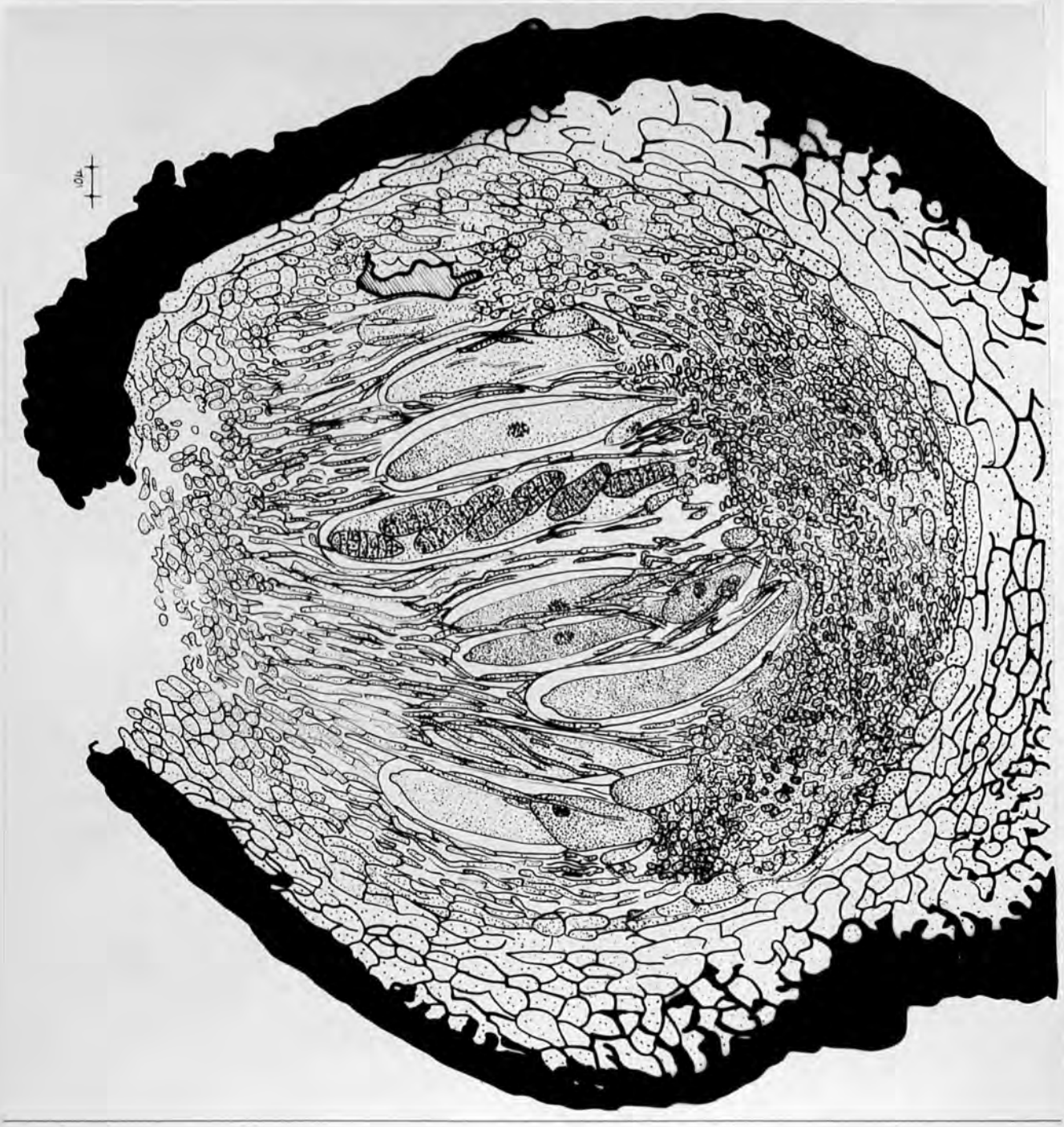


fig.20

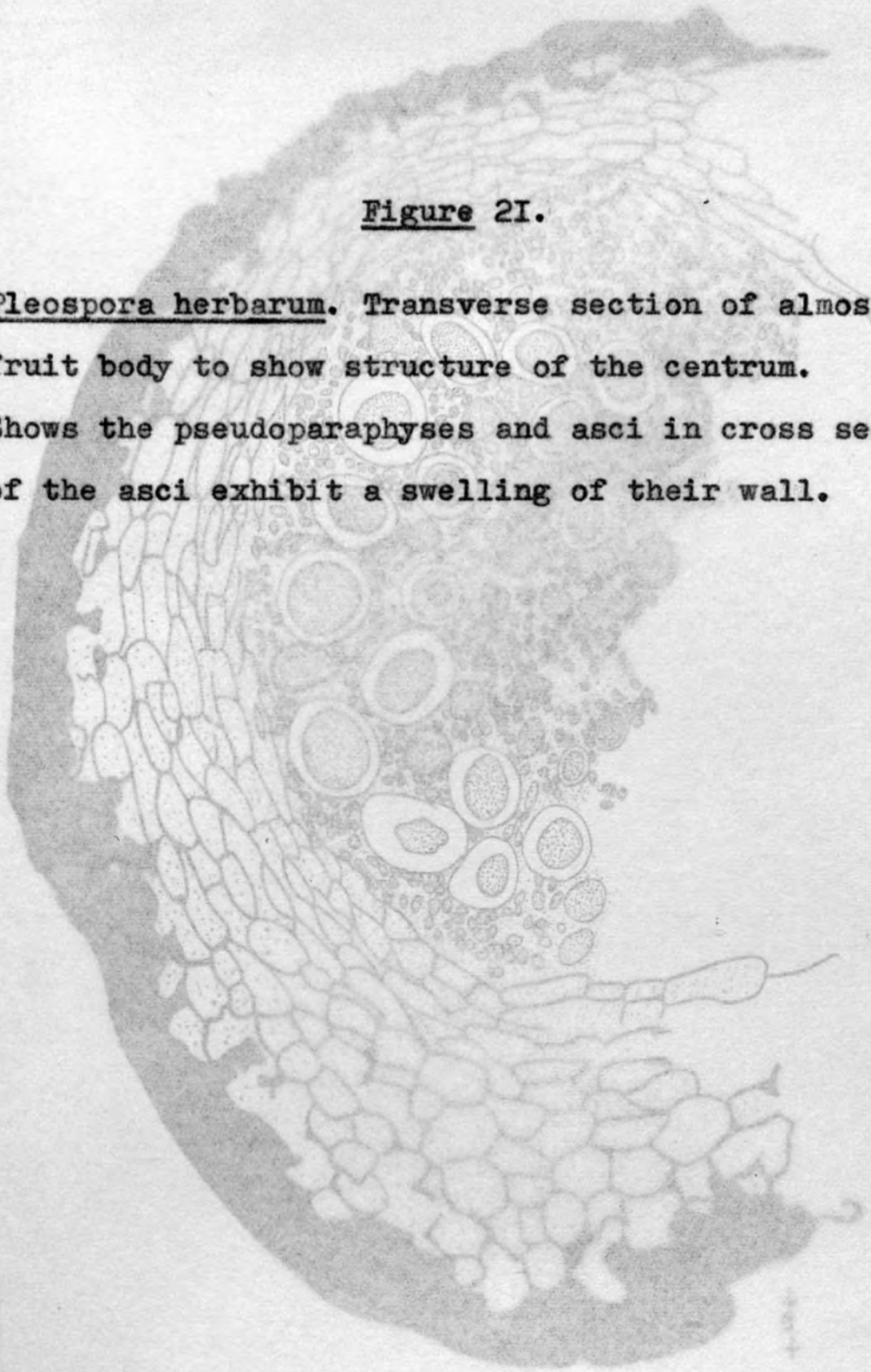


Figure 21.

Pleospora herbarum. Transverse section of almost mature fruit body to show structure of the centrum.
Shows the pseudoparaphyses and asci in cross section; many of the asci exhibit a swelling of their wall.

fig.21

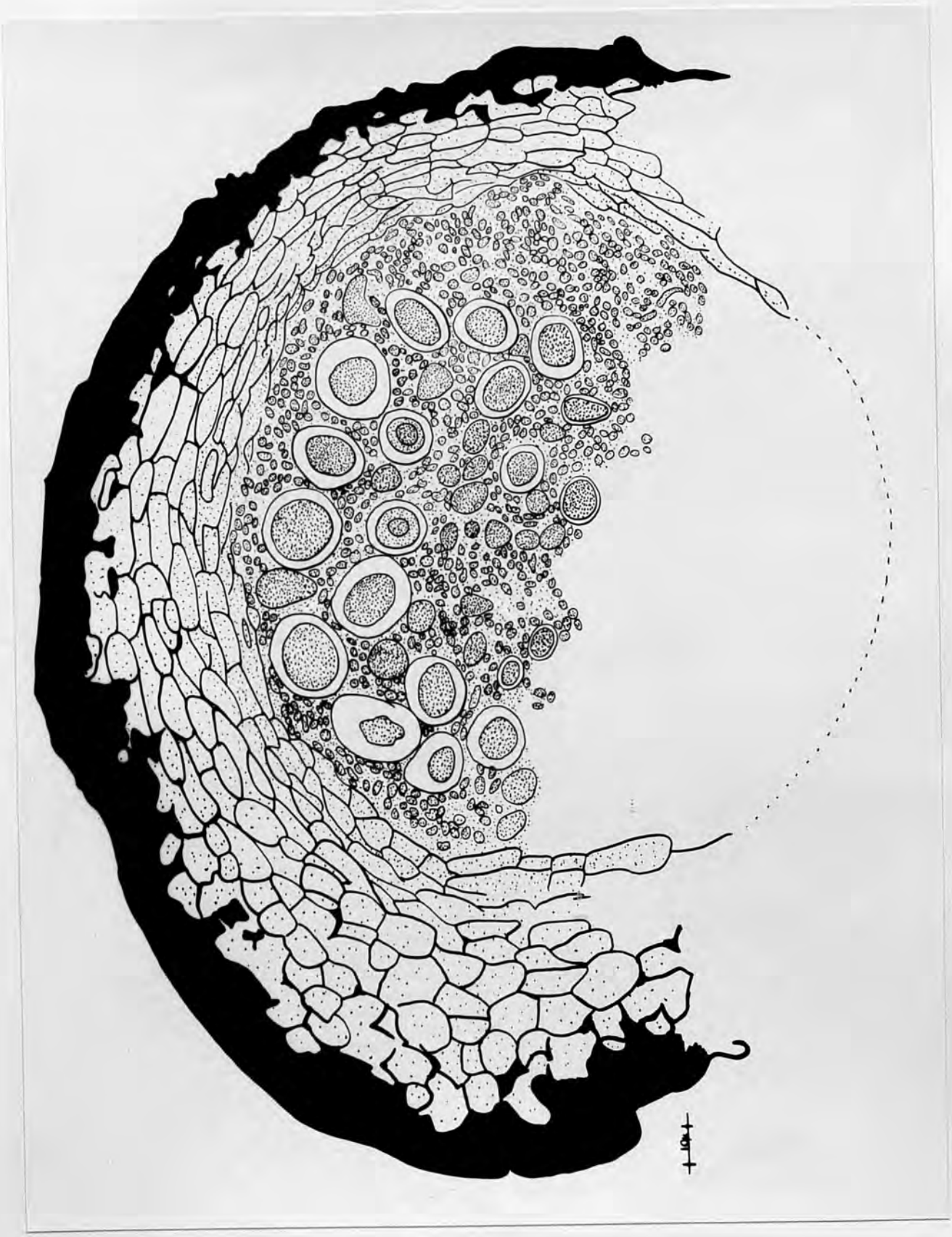


fig.21

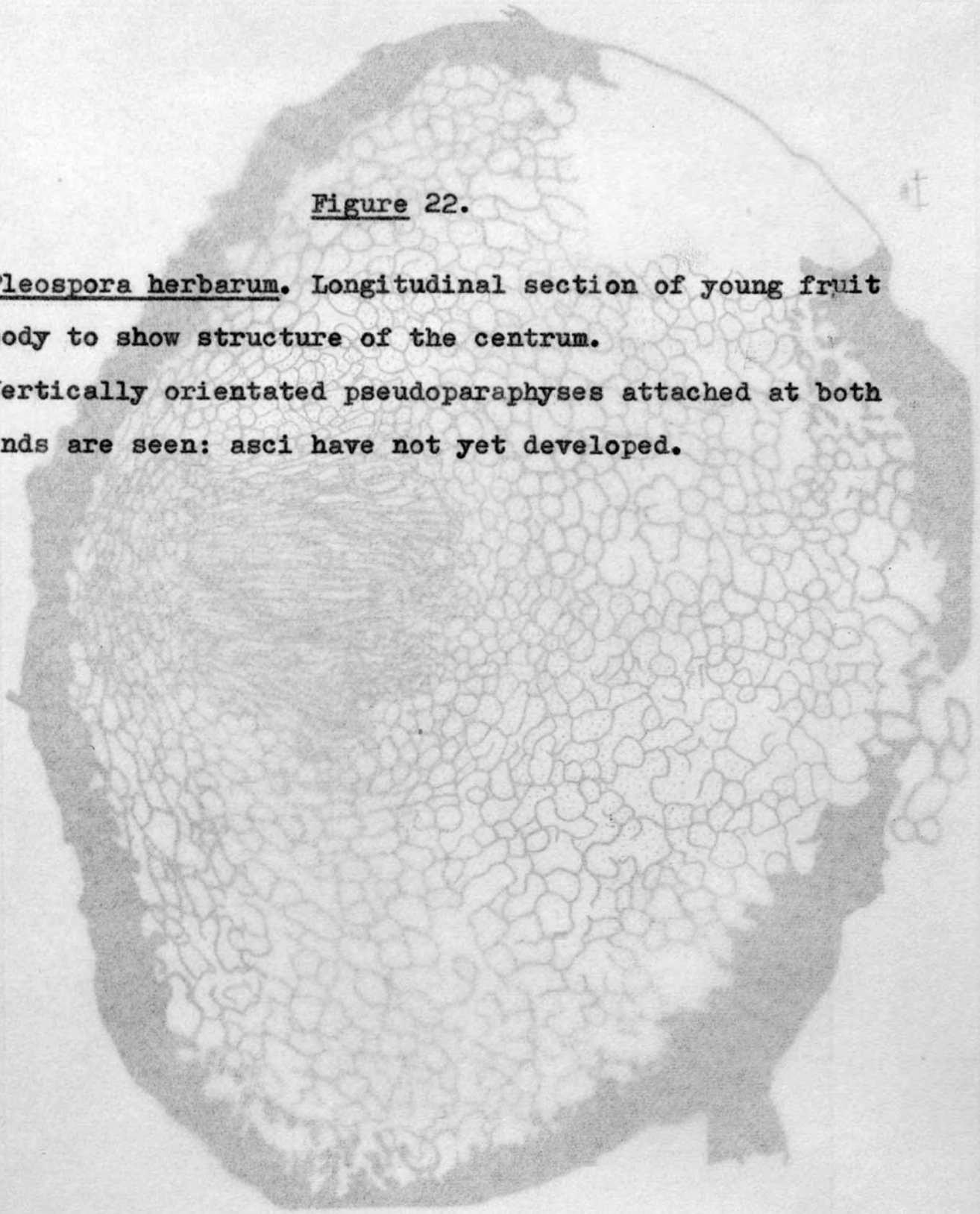


Figure 22.

Pleospora herbarum. Longitudinal section of young fruit body to show structure of the centrum.
Vertically orientated pseudoparaphyses attached at both ends are seen: asci have not yet developed.

fig.22

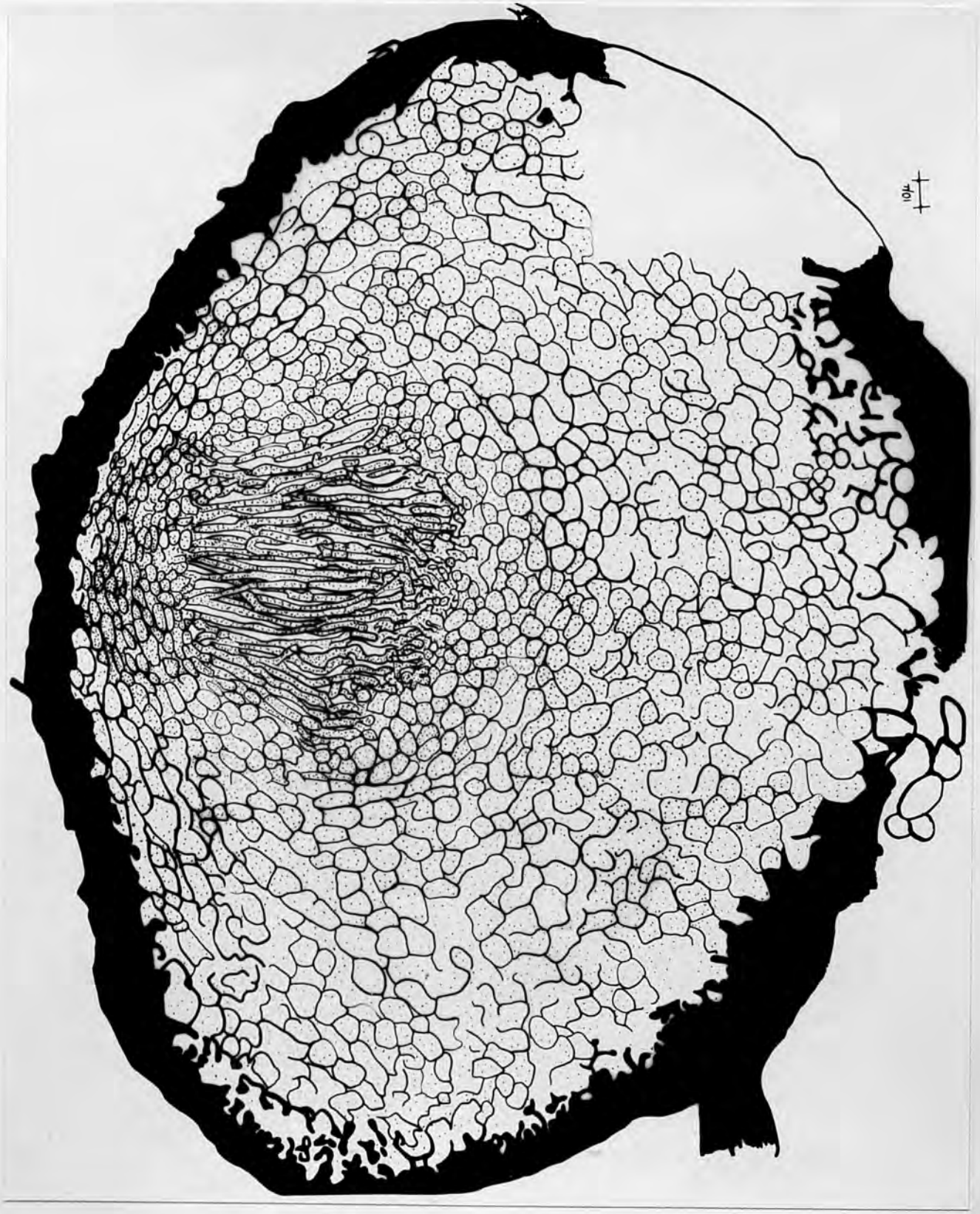


fig.22

Figure 23.

Pleospora herbarum. Longitudinal section of young fruit body to show the structure of the centrum. Edge of section only, is drawn; young asci and vertically orientated pseudoparaphyses are shown.

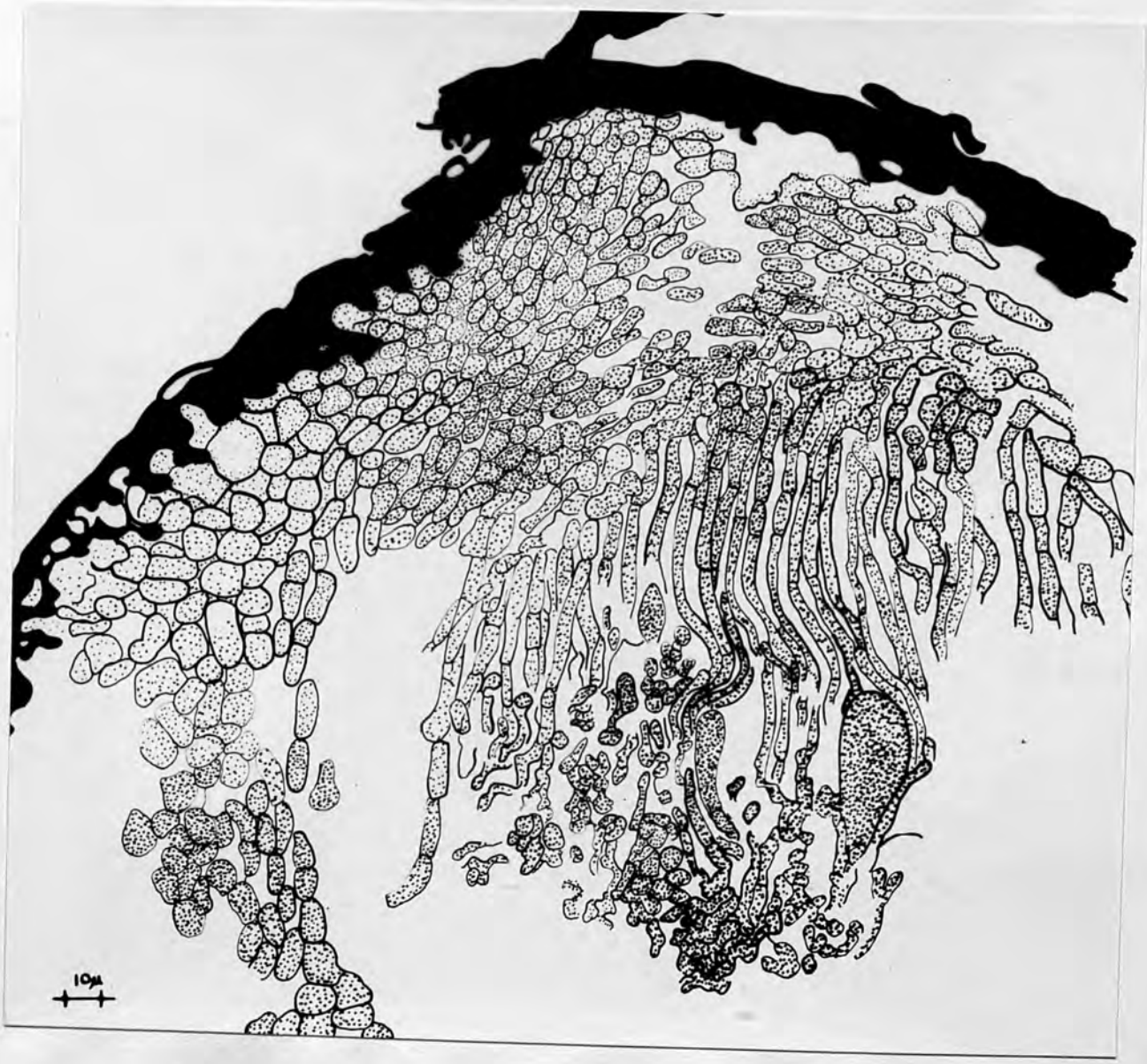



fig. 23

Figure 24.

Pleospora herbarum. Longitudinal section of young fruit body in which no asci are yet developed. The pseudoparaphyses have become folded in preparing the section, and some give the appearance of hyphae with free ends.
(The low power diagram is of the complete section, of which only part is drawn in detail)

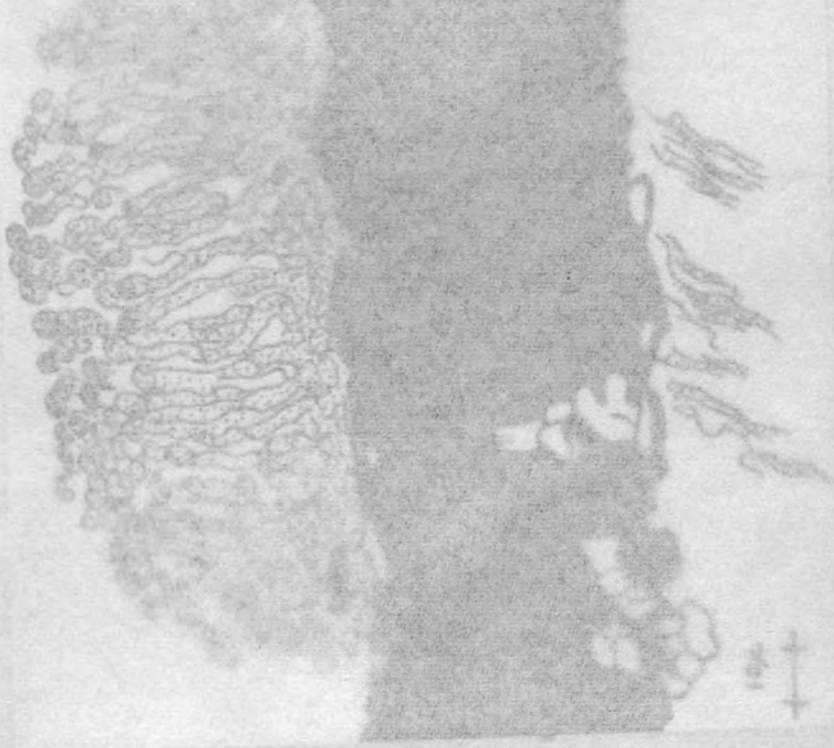


fig. 24

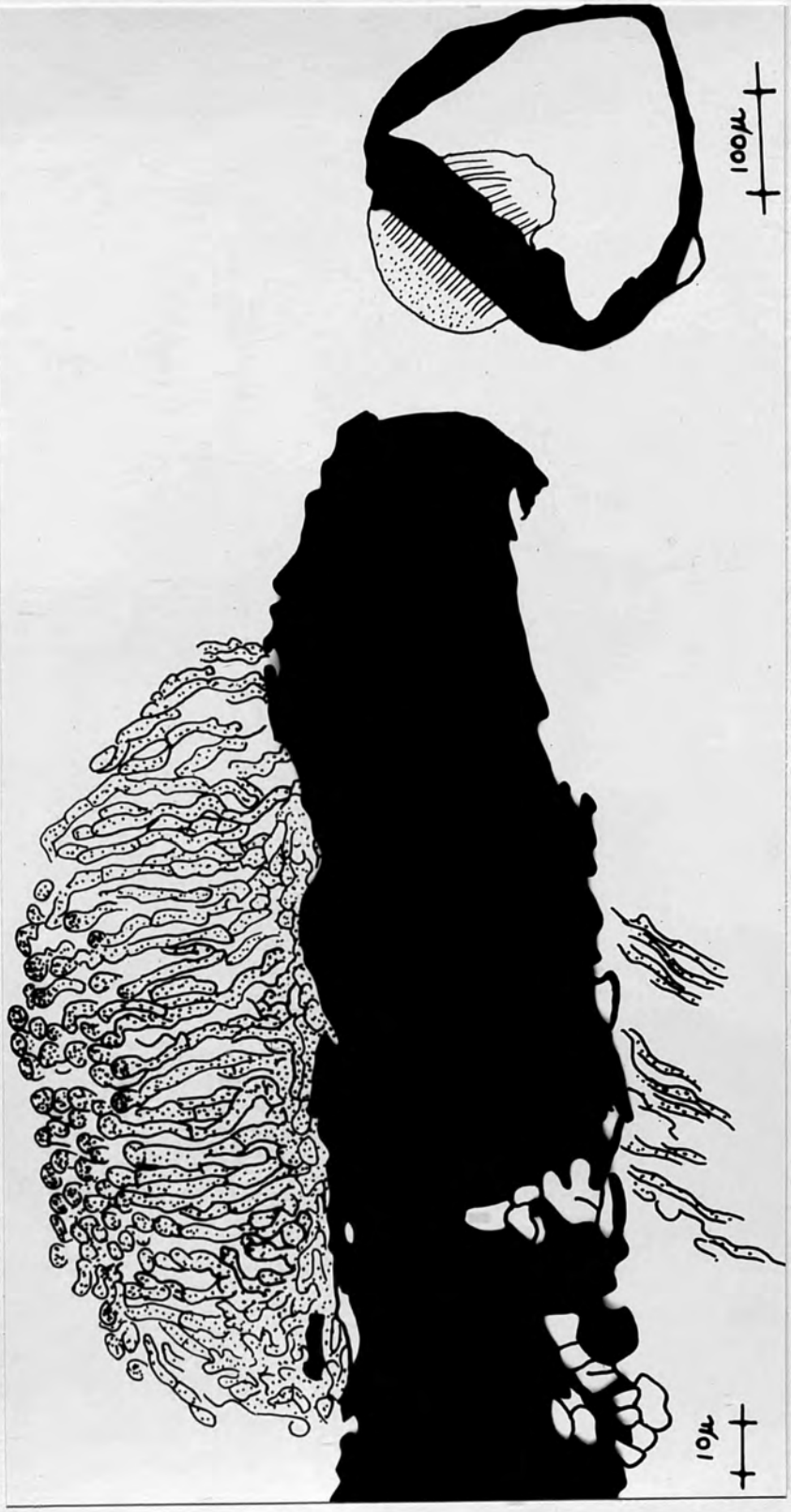



fig. 24

Figure 25.

Pleospora herbarum. Transverse section of young fruit body in which asci are not yet developed. Pseudoparaphyses are shown in cross section, but at the lower side of the section they have become displaced, and their cut length can be seen; they give the appearance of radial ingrowth.

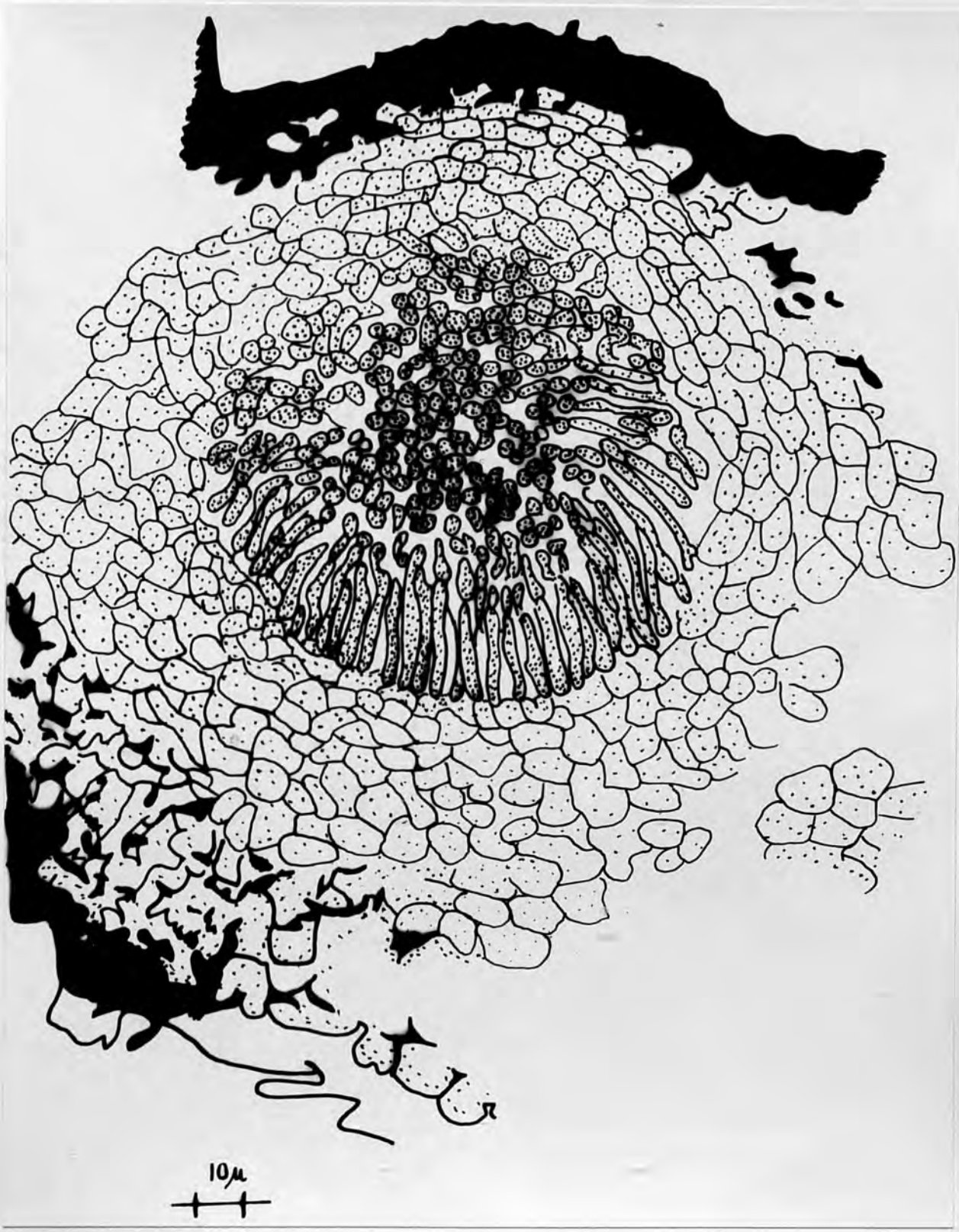



fig.25

Figure 26.

Pleospora herbarum. Longitudinal section of young fruit body in which no asci are yet developed. The pseudoparaphyses at the top of the section give the appearance of radially ingrowing, or downwardly directed hyphae, with free ends: this may be attributed to the oblique nature of the section and the spherical shape of the centrum.

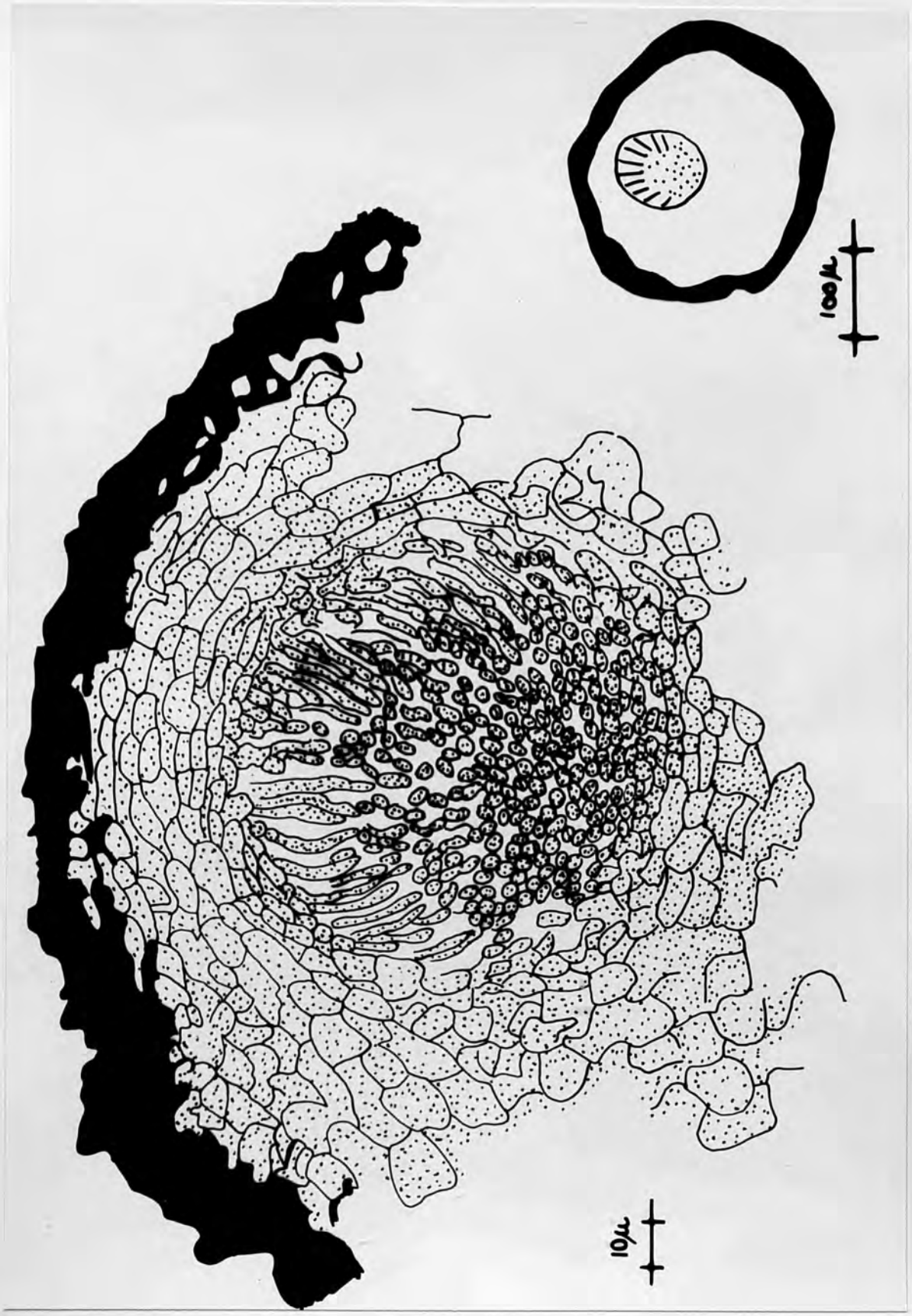



fig.26

Figure 27.

Ascospores mounted in water.

a. Pleospora herbarum

b. Venturia pirina

c. Venturia inaequalis

d. Mycosphaerella maculiformis

e. Mycosphaerella rumicis

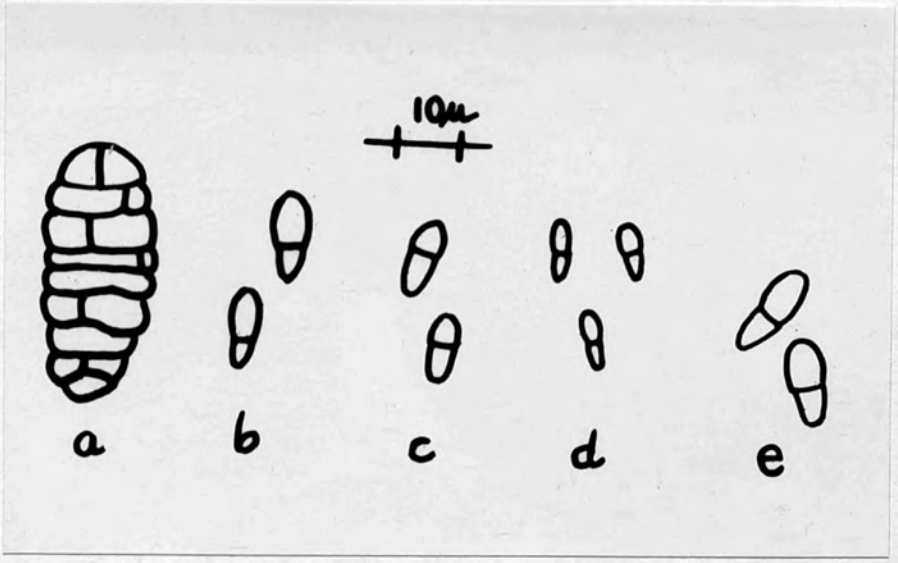
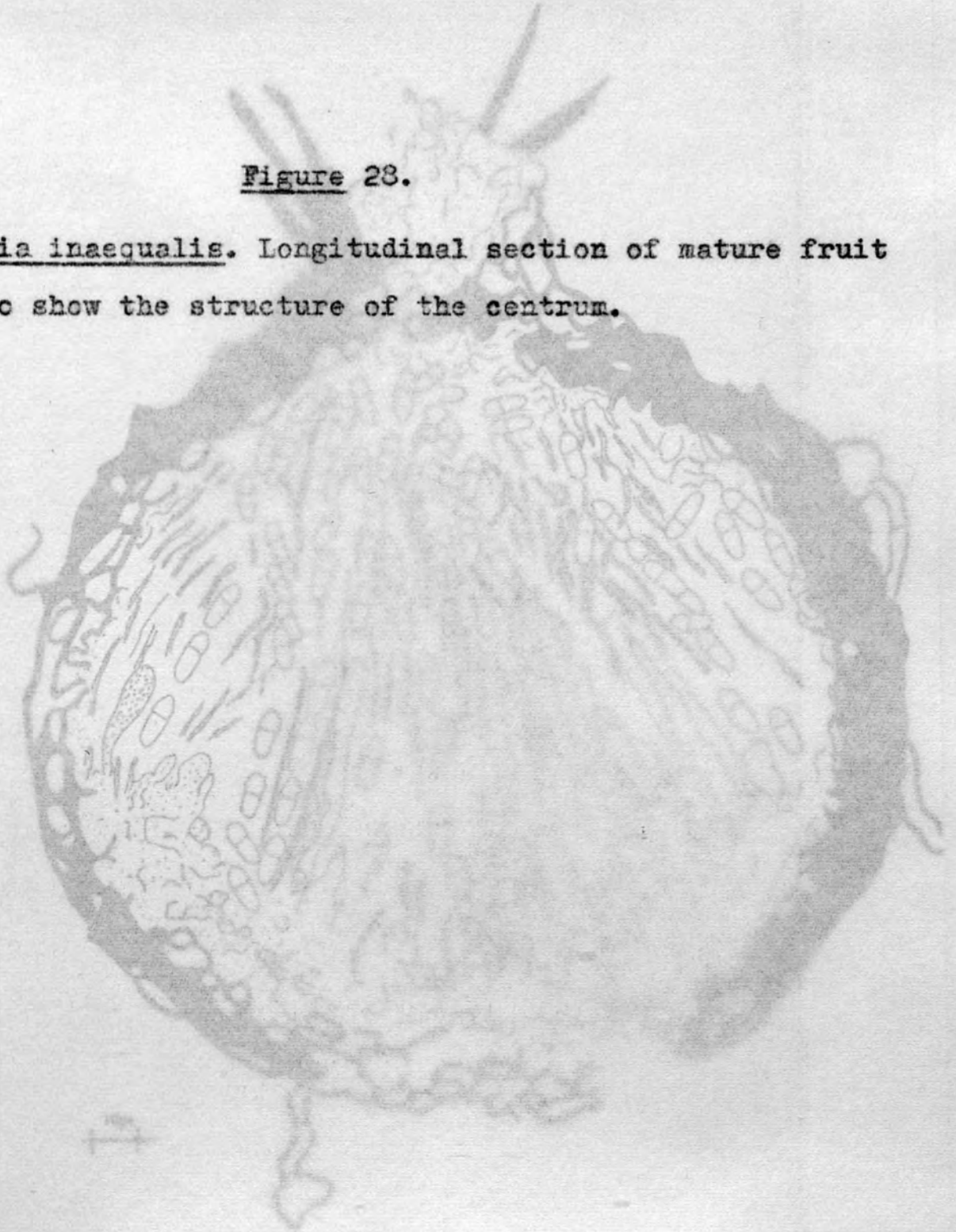



fig.27

Figure 28.

Venturia inaequalis. Longitudinal section of mature fruit body to show the structure of the centrum.



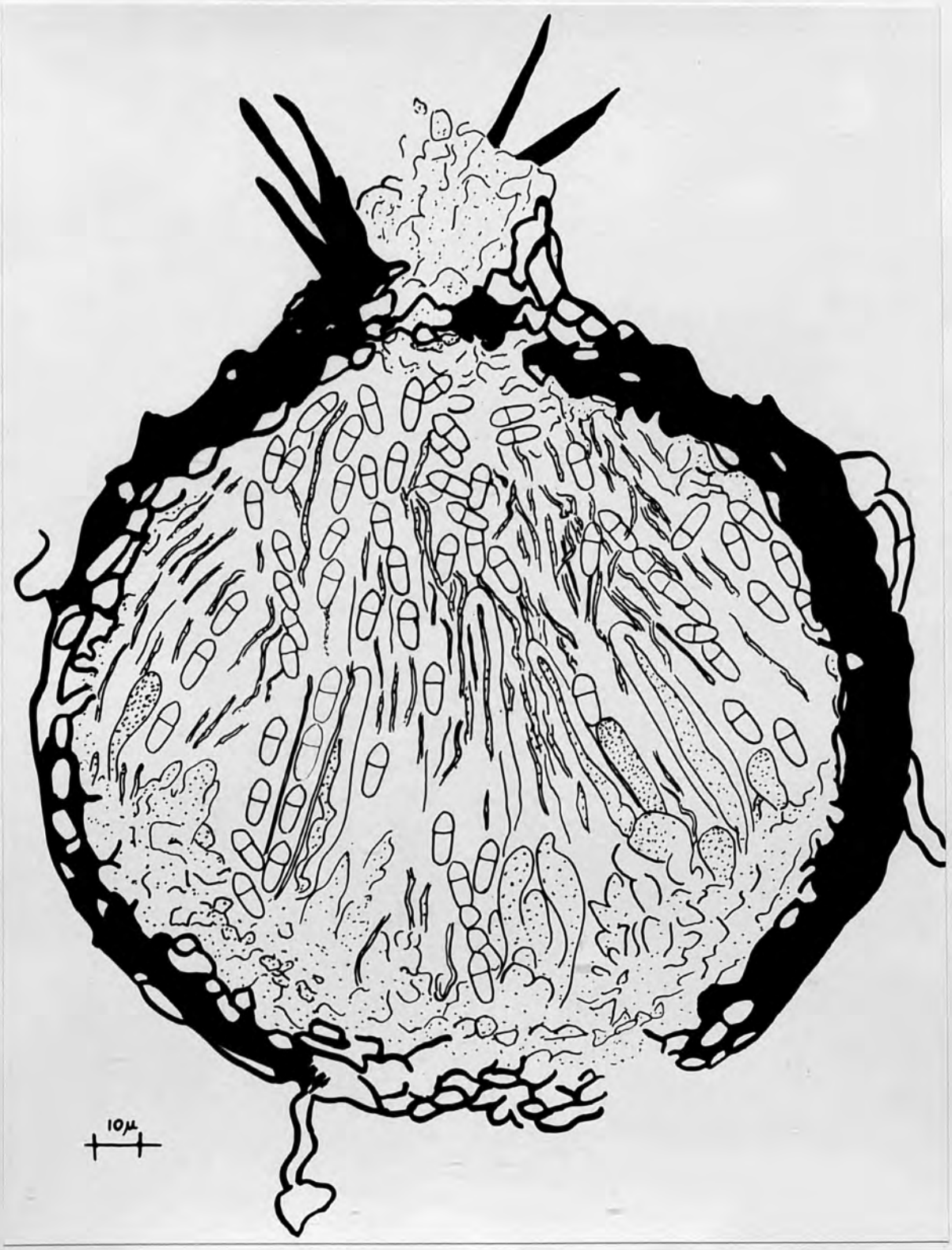



fig.28

Figure 29.

Venturia pirina. Longitudinal section of fruit body to show structure of the centrum. Some of the pseudoparaphyses appear free at their apex: the typical swelling of the wall of the bitunicate ascus is exhibited by some of the younger asci.


fig. 29

Figure 30.

Venturia inaequalis. Longitudinal section of young fruit body to show the structure of the centrum. The apical attachment of the pseudoparaphyses is clearly shown.

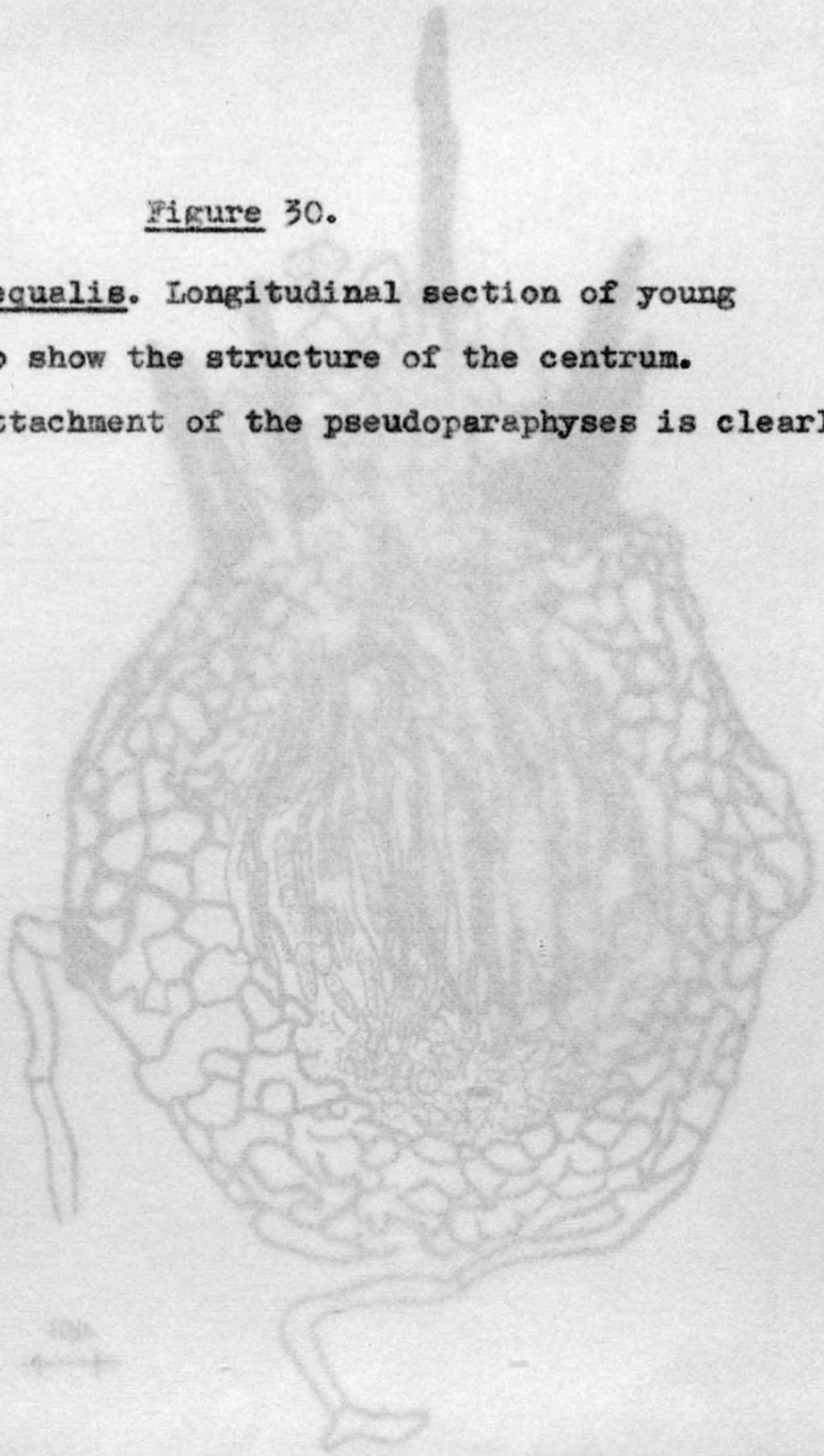


fig.30

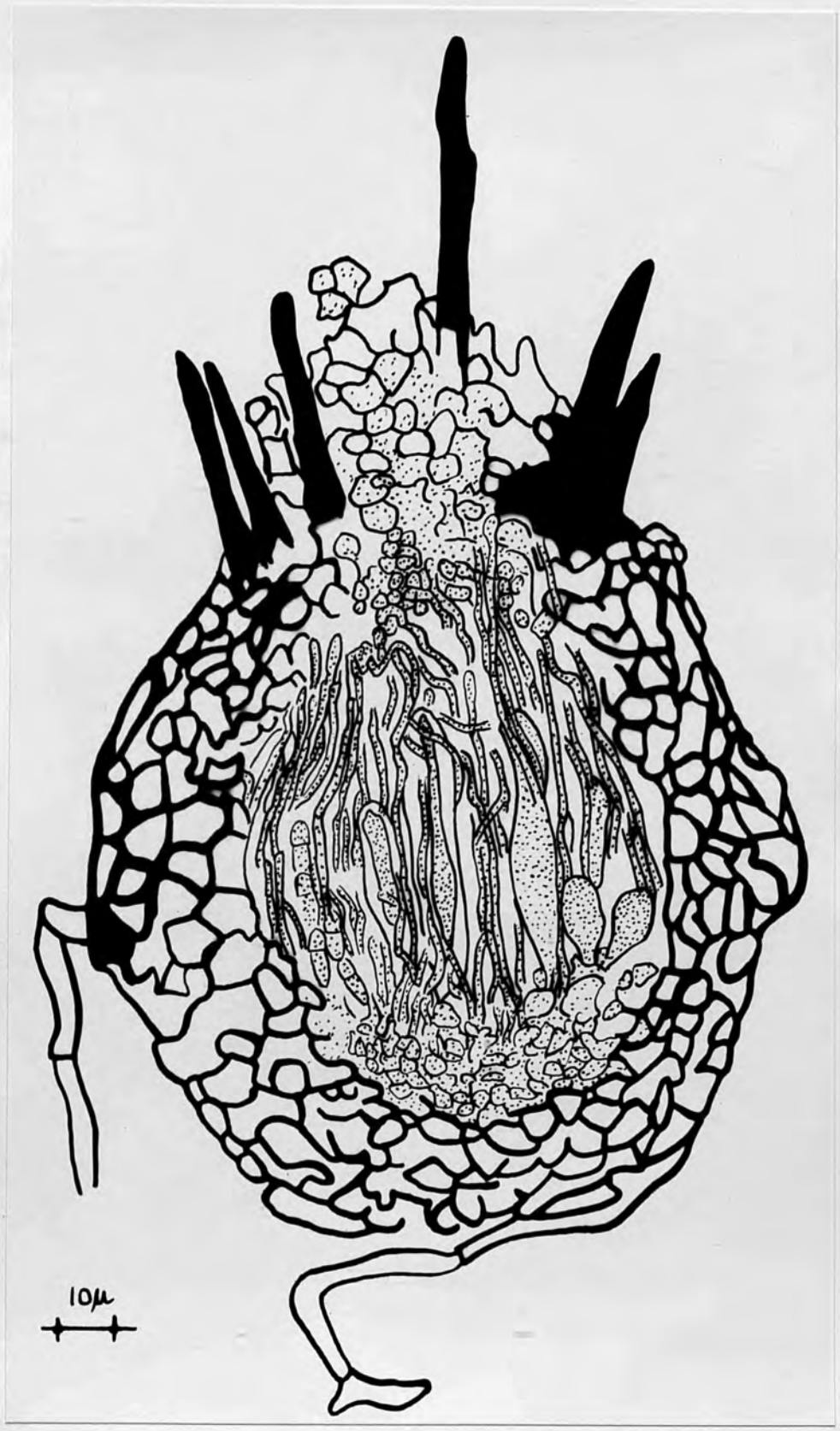



fig.30

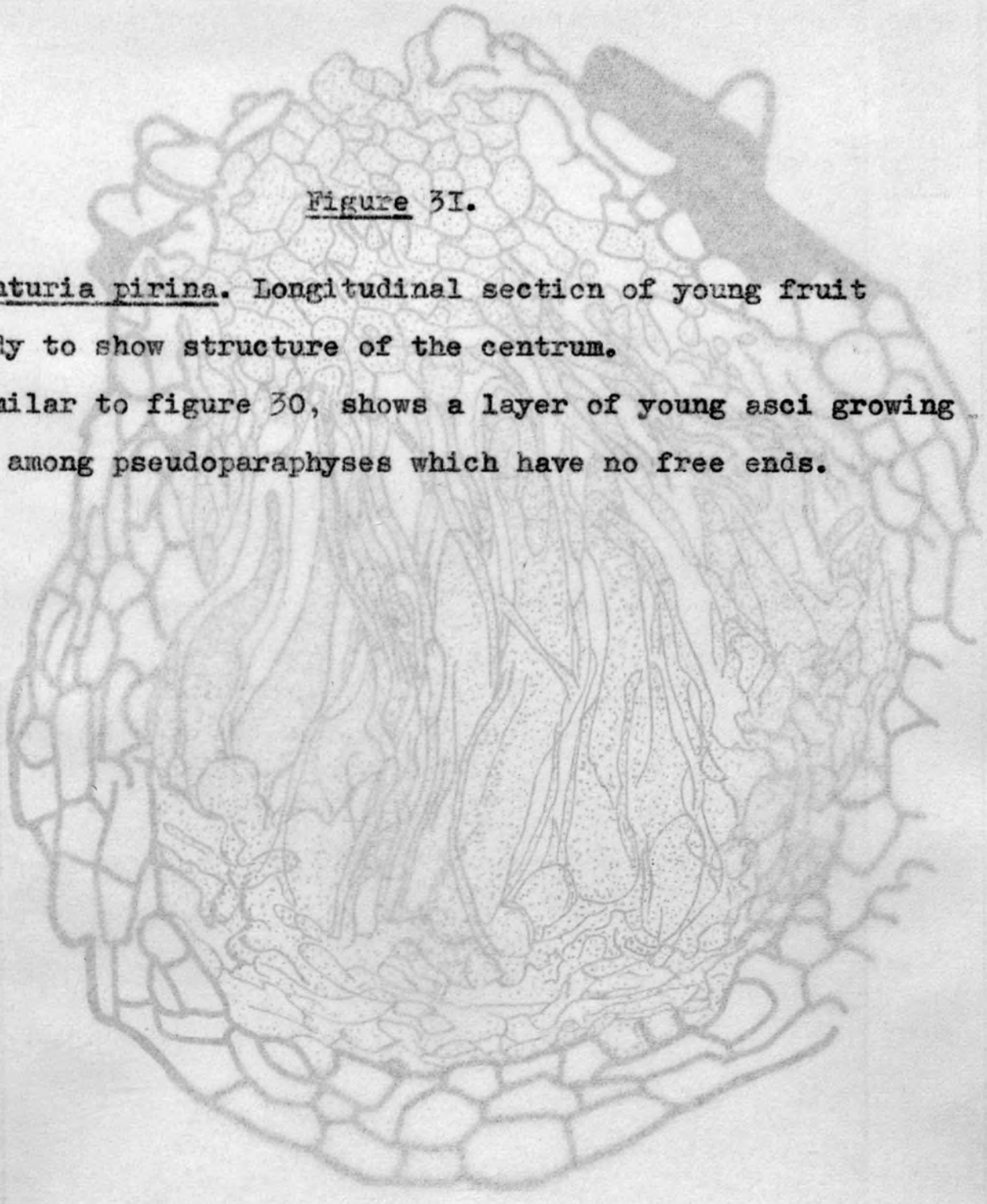


Figure 31.

Venturia pirina. Longitudinal section of young fruit body to show structure of the centrum.

Similar to figure 30, shows a layer of young asci growing up among pseudoparaphyses which have no free ends.

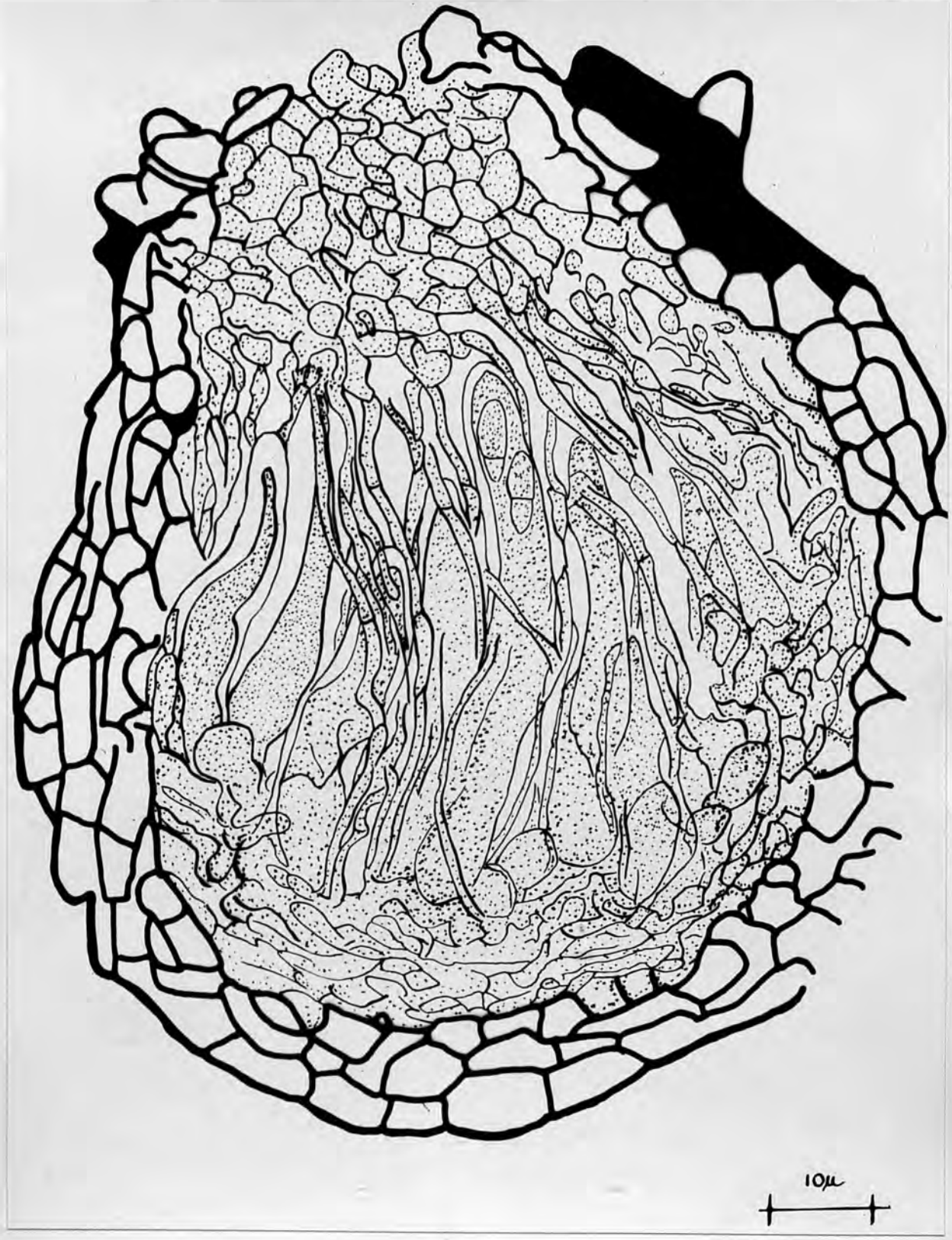



fig. 31

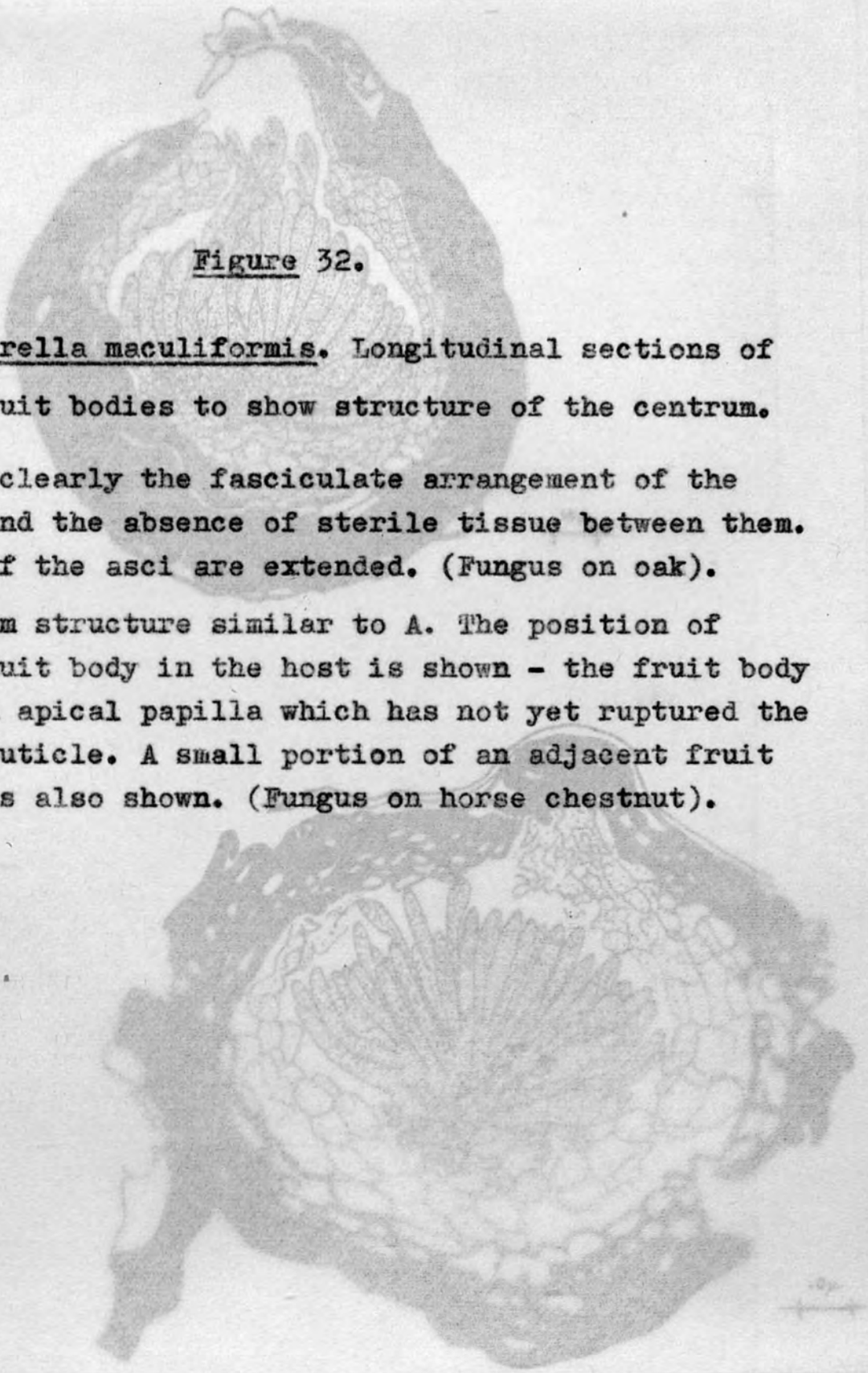


Figure 32.

Mycosphaerella maculiformis. Longitudinal sections of mature fruit bodies to show structure of the centrum.

A. Shows clearly the fasciculate arrangement of the asci and the absence of sterile tissue between them. Some of the asci are extended. (Fungus on oak).

B. Centrum structure similar to A. The position of the fruit body in the host is shown - the fruit body has an apical papilla which has not yet ruptured the host cuticle. A small portion of an adjacent fruit body is also shown. (Fungus on horse chestnut).

fig.32


fig.32

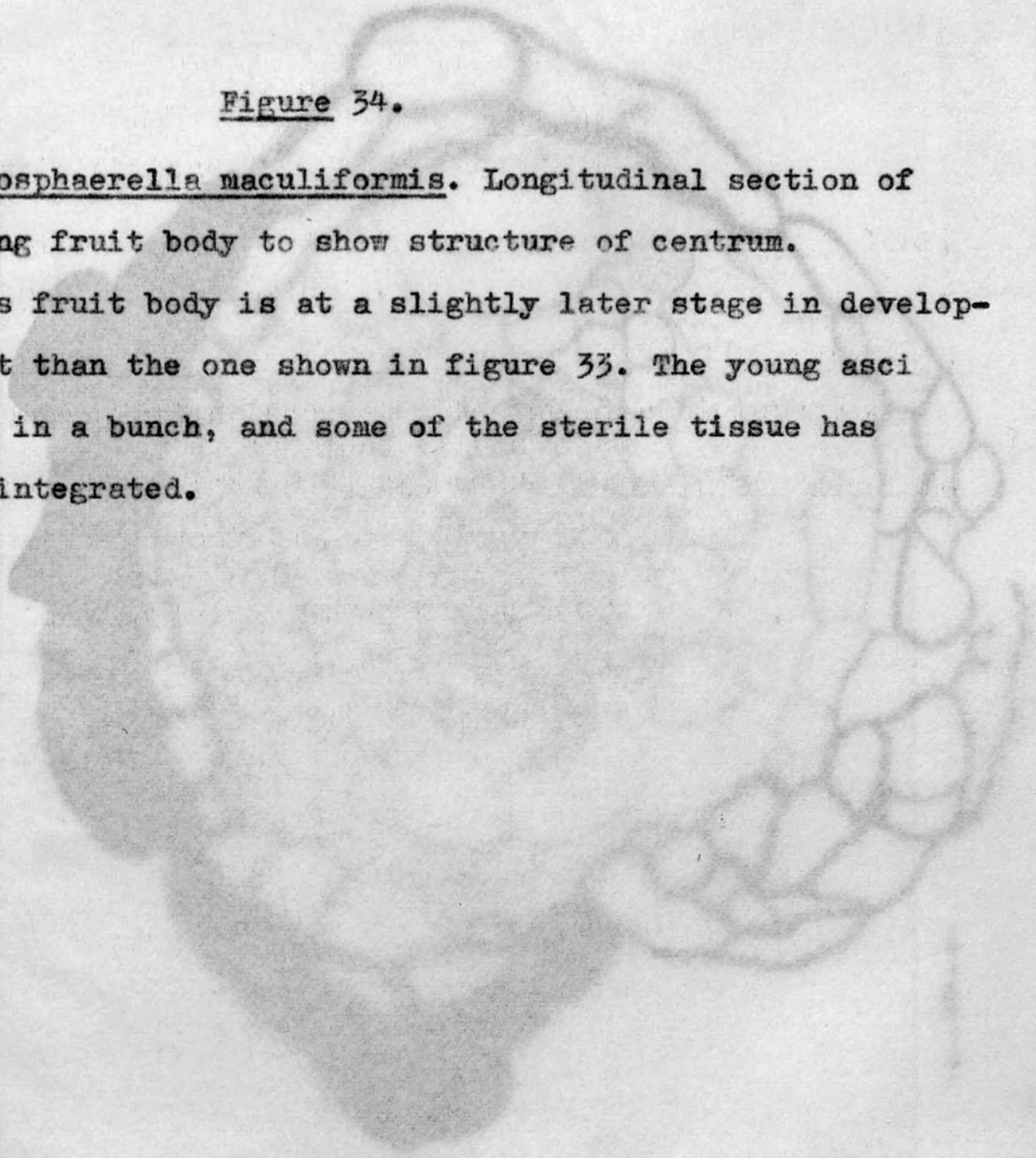
Figure 33.

Mycosphaerella maculiformis. Longitudinal section of young fruit body to show structure of the centrum. Thin walled pseudoparenchymatous tissue present, and asci just beginning to develop (deeply stained in the centre).


fig. 33

Figure 34.

Mycosphaerella maculiformis. Longitudinal section of young fruit body to show structure of centrum.
This fruit body is at a slightly later stage in development than the one shown in figure 33. The young asci are in a bunch, and some of the sterile tissue has disintegrated.



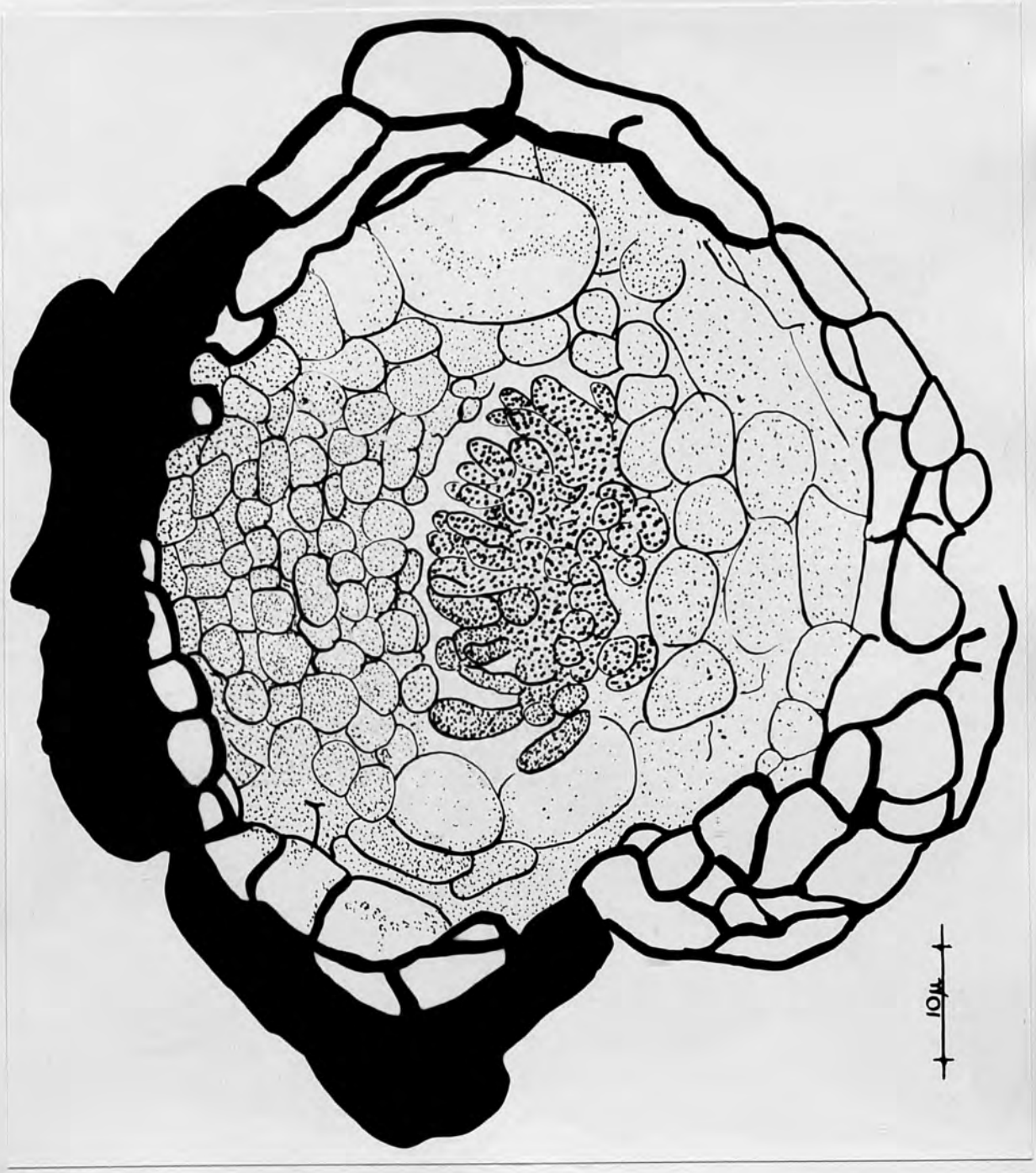



fig.34