STUDIES ON THE GENUS *ENTORRHIZA* C. WEBER

(USTILAGINALES)

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by

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PREFACE

This study began when a number of collections of unknown but obviously related fungi in root-swellings of the Cyperaceae and Juncaceae were brought to me for identification. Attempts to identify the endophytes by reference to manuals on plant diseases were unsuccessful. Eventually a discussion of the group was found in Kelley's "Mycotrophy of Plants" (1950). This author stated that they were considered to be members of the genus Entorrhiza in the Tilletiaceae. In standard references on the Ustilaginales available in New Zealand (eg. Ainsworth & Sampson, 1950; Fischer & Holton, 1957) the genus was mentioned only briefly and according to Fischer (1951) almost all the relevant literature was in early German publications.

In 1969-1970 while on leave in the Mycology section of the Department of Special Botany at the Swiss Federal Institute of Technology, Zürich, I had the opportunity to study the literature on Entorrhiza and to examine early European herbarium collections. To a limited extent, I was also able to collect new material from type localities. Without this opportunity to assemble information on the genus, the work described in this thesis would have been difficult to carry out in New Zealand. Previous collections of Entorrhiza were almost entirely from Central Europe and Scandinavia; reports of Entorrhiza on the Cyperaceae and Juncaceae were rare for Britain and completely lacking for the North American continent and the Southern Hemisphere.

Initially the study was directed mainly towards identifying the New Zealand collections (Fineran, 1971). During this work it became clear that a detailed investigation of some aspects of this relatively little known genus would prove informative. This thesis presents the results from such an investigation.

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CHAPTER 1

GENERAL INTRODUCTION

The genus Entorrhiza was founded by Weber (1884) to describe fungi producing root swellings, or galls, on some members of the Cyperaceae and Juncaceae. These fungi had previously been assigned to the genus Schinzia Naegeli by Magnus (1878) who based his decision on an examination of the root swellings of Cyperus flavescens L. Magnus believed that the fungus present was similar to Schinzia cellulicola described by Naegeli (1842) in the roots of Iris, and named it Schinzia cypericola Magn. He also ascribed the fungus producing root swellings in Juncus bufonius L. to the same species.

Later Weber (1884) made a detailed investigation of the fungus in Juncus bufonius and concluded that it was a member of the Ustilaginales. As Schinzia had no known affinities with the Ustilaginales and included a heterogeneous assemblage of root-infecting organisms (among them Schinzia alni Woronin on Alnus glutinosa and Schinzia leguminosarum Frank on the Papilionaceae), Weber erected the genus Entorrhiza for the fungus on Juncus bufonius. Entorrhiza was characterised by having single, thick-walled, terminal spores which germinated by producing 1 - 3 germ tubes each bearing a single, falcate sporidium.

Weber's decision was criticised by Magnus (1888) and although de Toni in Saccardo's Sylloge Fungorum (1888) accepted Entorrhiza as the generic name for the group, Schinzia remained in the literature for some years (eg. Schellenberg, 1911; Bubák, 1916; Dietel, 1928).

Entorrhiza appears to infect only members of the glumiflorous monocotyledonous families Cyperaceae and Juncaceae. The genera Carex, Cyperus, Eleocharis, Scirpus, Juncus and possibly Eriophorum (Cameron, 1886) have been reported as hosts. Juncus species are the most commonly recorded host plants.

Seven species on the Cyperaceae and Juncaceae have been described or combined under Entorrhiza. These are:

E. aschersoniana (Magn.) Lagerh.
E. caricicola Ferd. and Winge
E. casparyana (Magn.) Lagerh.
E. cypericola (Magn.) de Toni
E. digitata Lagerh.
E. raunkiaeriana Ferd. and Winge
E. scirpicola (Correns) Sacc. and Syd.

Several species on the roots of other plants have been described or combined under Entorrhiza but most have not been generally accepted (see Ciferri, 1963; Thirumalachar & Whitehead, 1968). These are:

E. calospora Talbot on Limeum and Trianthema
E. cellulicola (Naeg.) de Toni on Iris
E. isoetes (Rostr.) Liro on Isoetes
E. solani Fautrey on Solanum
E. vaccinii Rostrup on Vaccinium

Very little material has been published on the biology of the genus. In this thesis one host-parasite combination has been studied in detail to provide information on the biology

of Entorrhiza; much of this information is required for an assessment of the taxonomic relationships of the genus and its species. The host-parasite combination chosen was originally identified as Entorrhiza digitata on Juncus articulatus (Fineran, 1971). However, as a result of the work carried out since then and reported in this thesis (Chapt. 7), it is clear that this species of Entorrhiza is synonymous with E. casparyana. This is therefore the name which has been used throughout the thesis when referring to biological studies of Entorrhiza on J. articulatus.

E. casparyana on J. articulatus was chosen as the material for study because it was available locally and also because it occurred in the Northern Hemisphere where it had been collected and examined by a number of early European mycologists. One of the advantages of studying such a host species was that the results obtained could be expected to have a wider application than if less cosmopolitan or endemic host species had been investigated.

The material of E. casparyana used for biological studies was collected mainly from the Groynes, at the source of the south branch of the Waimakariri River, near Christchurch.

In the thesis the structure of the galls induced by Entorrhiza is first considered (Chapt. 2). The biology of E. casparyana is then examined under three main headings:

a) The ultrastructure of the fungus in the host (Chapt.3).

b) Some general ecological and physiological aspects of infection (Chapt. 4).

c) Spore germination (Chapt. 5).

Much of the information obtained in these chapters provides a background for the taxonomic work which follows. This is divided into two sections. The first of these examines the use of the modern technique of scanning electron microscopy for elucidating taxonomic problems (Chapt. 6). The second is a taxonomic revision of the genus based on the knowledge obtained from the previous chapters and an examination of collections of Entorrhiza from New Zealand and from almost all herbaria in Europe known to have material (Chapt. 7).

CHAPTER 2

THE STRUCTURE OF GALLS INDUCED BY ENTORRHIZA

1. INTRODUCTION

The only detailed account of the structure of galls caused by Entorrhiza is that of Weber (1884) for E. aschersoniana in Juncus bufonius. Weber described the anatomy of the gall for the first time and his work still provides the most complete illustrations of the fungus in the host cells. Trail (1884), studying the same host-parasite combination, confirmed most of Weber's observations.

Magnus (1878, 1888, 1893) briefly described the structure of galls produced by E. cypericola on Cyperus flavescens and noted the distinctive corkscrewing of the intracellular hyphae. Observations by other workers since then eg. Correns (1897) on E. scirpicola in Scirpus pauciflorus and Schwartz (1910) on Entorrhiza in Juncus articulatus indicate that the galls are probably structurally similar for all species of Entorrhiza.

There are, however, some details of the development of the fungus that have not been confirmed. Brefeld (1912), in contrast to previous workers, noted for E. aschersoniana in Juncus bufonius that spores formed from intercalary swellings in septate mycelium. Ferdinandsen and Winge (1914) observed that the spores of Entorrhiza were terminal, which was in agreement with the observations of all previous workers except Brefeld, but believed that hyphal fusion probably occurred prior to spore formation.

These early observations were reviewed by Liro (1938). Since that time no further investigations appear to have been carried out.

The work in this chapter has been undertaken firstly to provide a complete description of infections caused by species of Entorrhiza and to elucidate the contradictory points mentioned above, and secondly to provide a better understanding of the host-parasite relationship at the cellular level.

2. MATERIALS AND METHODS

The following host-parasite combinations were studied:

a. Entorrhiza casparyana on Juncus articulatus L.

b. E. casparyana on Juncus gregiflorus L. Johnson

c. E. scirpicola on Scirpus basilaris (Hook. f.) C.B. Clarke

d. E. scirpicola on Scirpus cernuus Vahl.

Both hand-cut and microtome sections were examined. Galls from Juncus gregiflorus, Scirpus basilaris and Scirpus cernuus for microtomy were fixed in formalin-aceto-alcohol (FAA) or Crafs III (Johansen, 1940) and serial transverse and longitudinal sections were cut at 8 µm and 10 µm. The 10 µm sections were used routinely, however, as the 8 µm sections tended to wrinkle. Sections were stained in either haematoxylin and safranin (Johansen, 1940) or by the periodic acid - Schiff technique (Dring, 1955). Material stained by this latter method showed greater clarity and differentiation and

was used for most of the observations. FAA and Crafs III appeared to be equally suitable as fixatives. Material was mounted in DePeX.

Hand-cut sections of galls from Juncus articulatus were examined using a variety of stains:

a. lactophenol cotton blue

b. 0.05% aqueous toluidine blue (O'Brien & McCully, 1969)

c. Melzer's reagent

d. Burke's iodine for starch

e. Sudan IV for total lipids : fats, oils and waxes (Jensen, 1962)

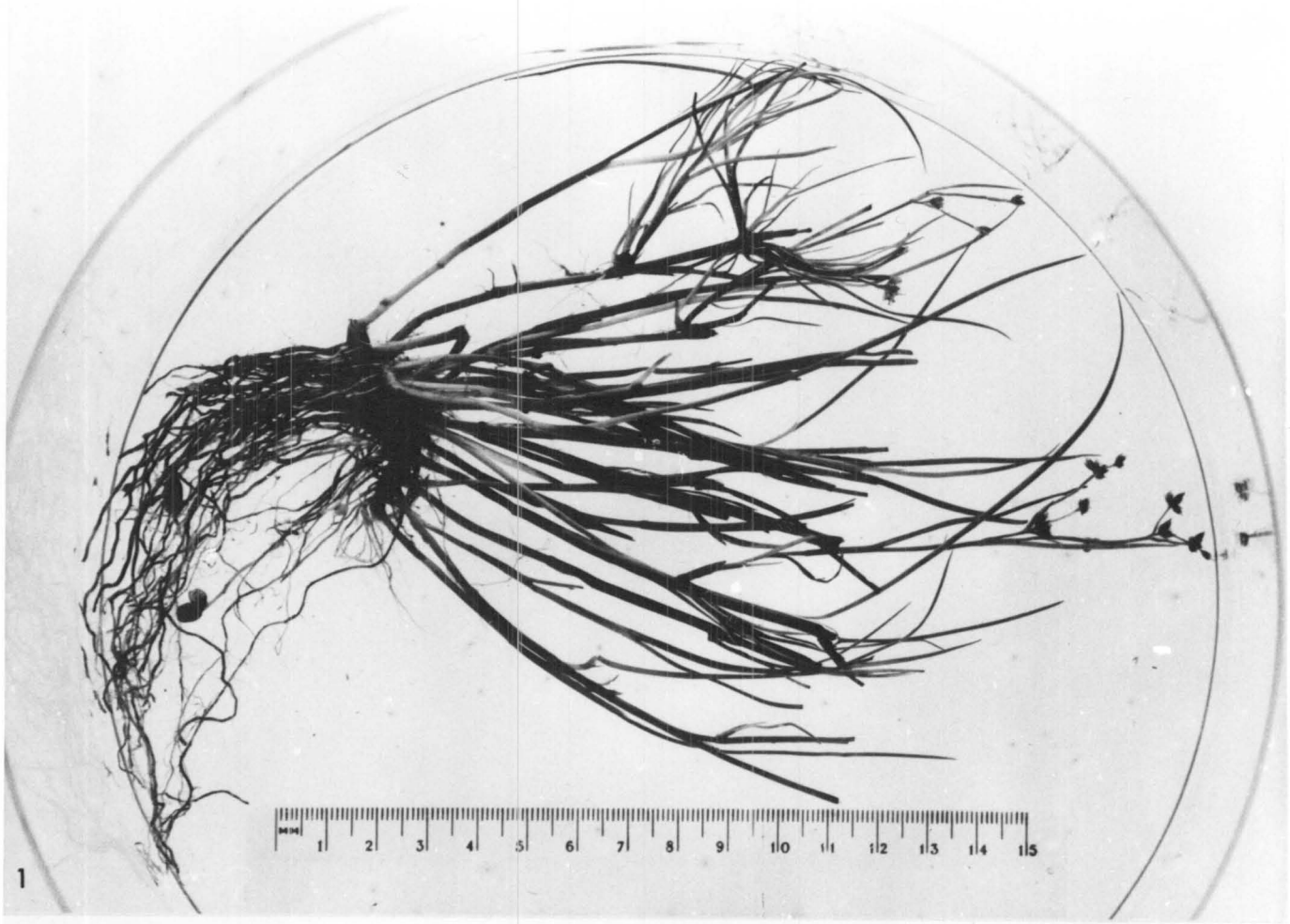
Several other stains were tested eg. 0.1% aqueous neutral red and picro-lactophenol (Dring, 1971) but they had no advantages over those listed above. Lactophenol cotton blue and toluidine blue were used for routine observations. Both stains had certain disadvantages : lactophenol cotton blue, although more commonly used as a fungal stain, was found to be too refractive for examining the fine hyphae of Entorrhiza; toluidine blue, on the other hand, gave greater definition but the staining time was critical and a period of 5 mins. was found to be more suitable than the shorter period recommended by O'Brien and McCully (1969).

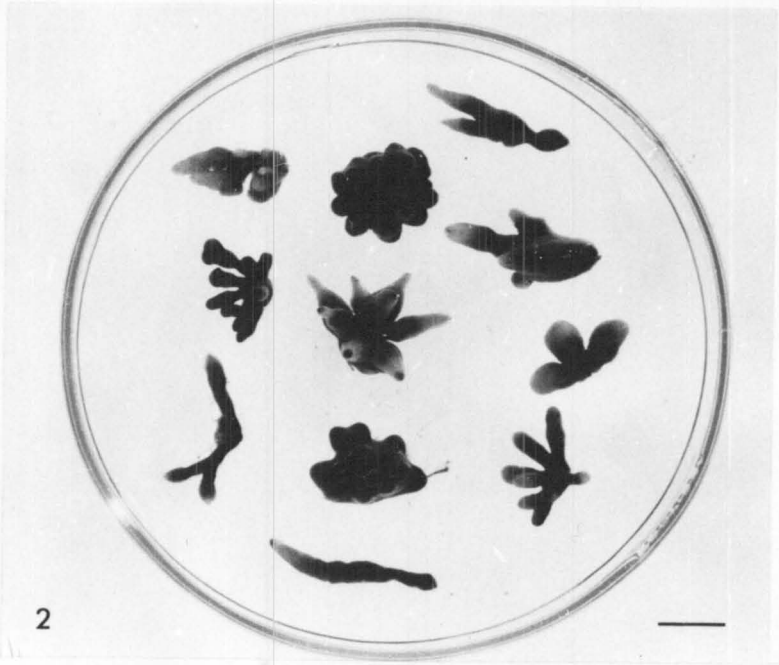
Most of the observations were carried out with a Leitz Orthoplan microscope; for all details a x10 ocular and x100 objective (oil immersion) were used. Photomicrographs were taken using an automatic camera attachment.

3. RESULTS

The galls of all host-parasite combinations studied have a basic similarity in structure. Most galls are modified root (Figs. 1-6) apices; however, occasionally, lateral galls are formed. Some galls which initially appear to be lateral and to completely surround a root are found on further investigation to be terminal in origin; in these cases, the gall had enveloped an adjacent root. Compound galls sometimes occur and are formed when a root tip is infected in several positions. In all the host species examined the uninfected root has an outer epidermal layer bearing root hairs, a 1-2 layered exodermis of thicker-walled cells, a 2-3 layered cortex and a well-defined endodermis surrounding the stele (Fig. 7). For more detailed information on the morphology of roots of the Cyperaceae and Juncaceae see Cutler (1969) and Metcalfe (1971).

The gall retains an active apical meristem (Fig. 8) and definite root cap but has no distinct epidermal layer and bears no root hairs (Figs. 8-10). Its swollen form is due mainly to hyperplasia and hypertrophy of the thin-walled, large, radially elongated cortical parenchyma cells. The outer 3-5 layers of tangentially orientated cells appear to correspond to the exodermis of the uninfected root and, together with the root cap tissues, they appear to be sloughed off readily, presumably due to the rapid growth of the gall. Unlike the cortical cells, the exodermal cells do not contain starch. They are tightly packed, with no intercellular spaces. Their staining reaction with toluidine blue indicates that the thickened walls of these exodermal cells are either lignified secondary walls or primary walls which have been impregnated with phenols.



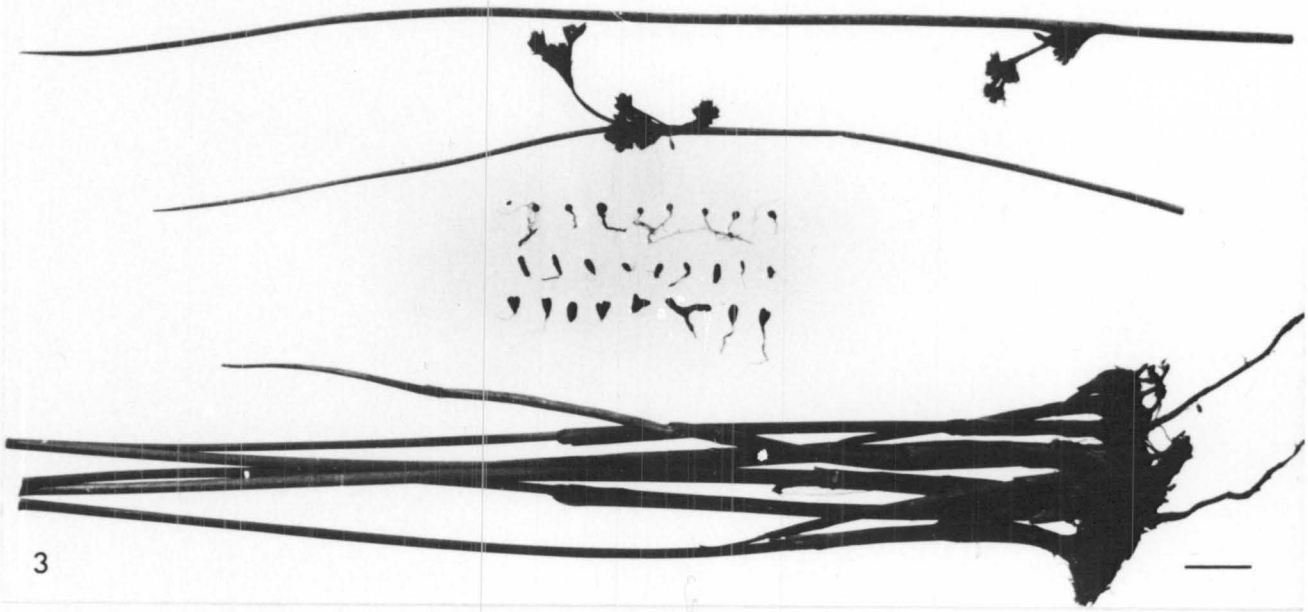


Scale for Figs. 2-6: line represents 1 cm.

Figs. 1 & 2 Juncus articulatus infected by Entorrhiza casparyana.

Fig. 1 Plant with attached galls.

Fig. 2 Large digitate galls from plants growing in infected soil for $8\frac{1}{2}$ months.



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Figs. 3 & 4 Juncus gregiflorus infected by Entorrhiza casparyana.

Fig. 3 Young galls beginning to branch; note size in comparison with host plant.

Fig. 4 Well-developed galls showing dichotomous branching (from Fineran, 1971).

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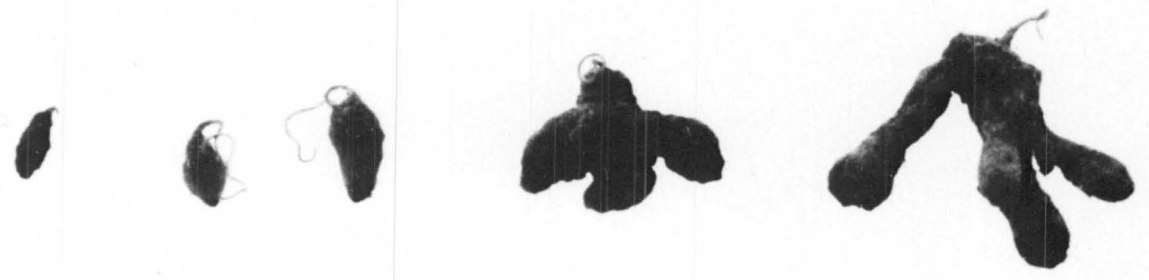

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Figs. 5 & 6 Scirpus spp. infected by Entorrhiza scirpicola (from Fineran, 1971).

Fig. 5 S. basilaris with unbranched galls.

Fig. 6 Branched galls of S. cernuus.

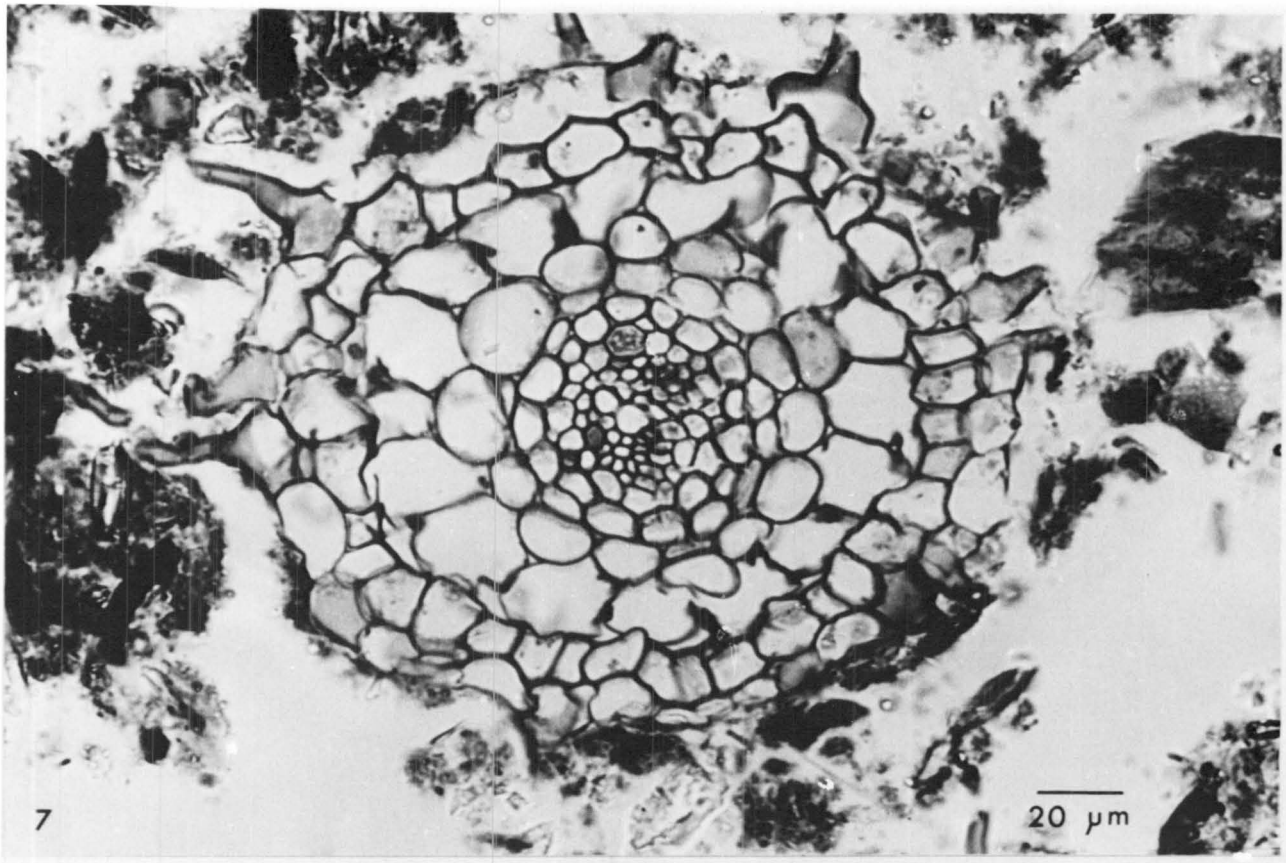


Fig. 7 T.S. of uninfected root of Juncus gregiflorus. Note epidermis, exodermis, cortex (2-3 layers), endodermis and stele.

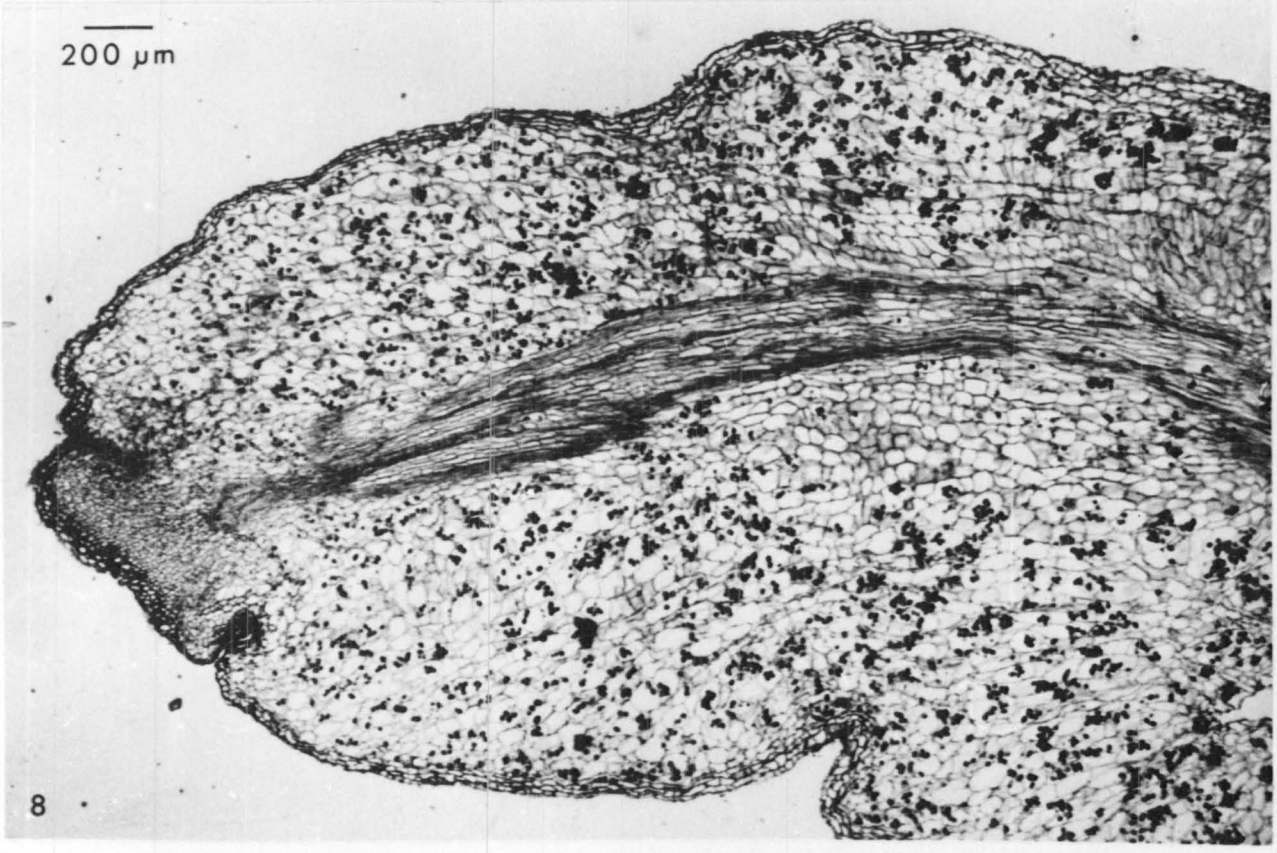
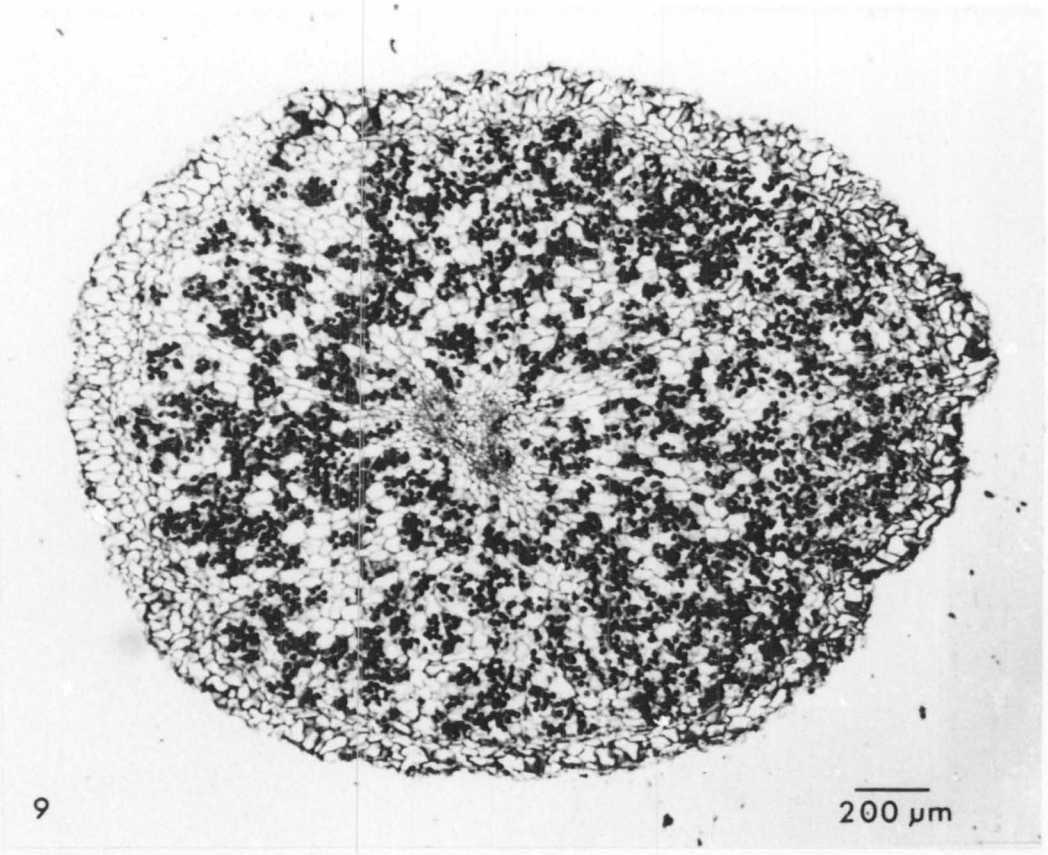
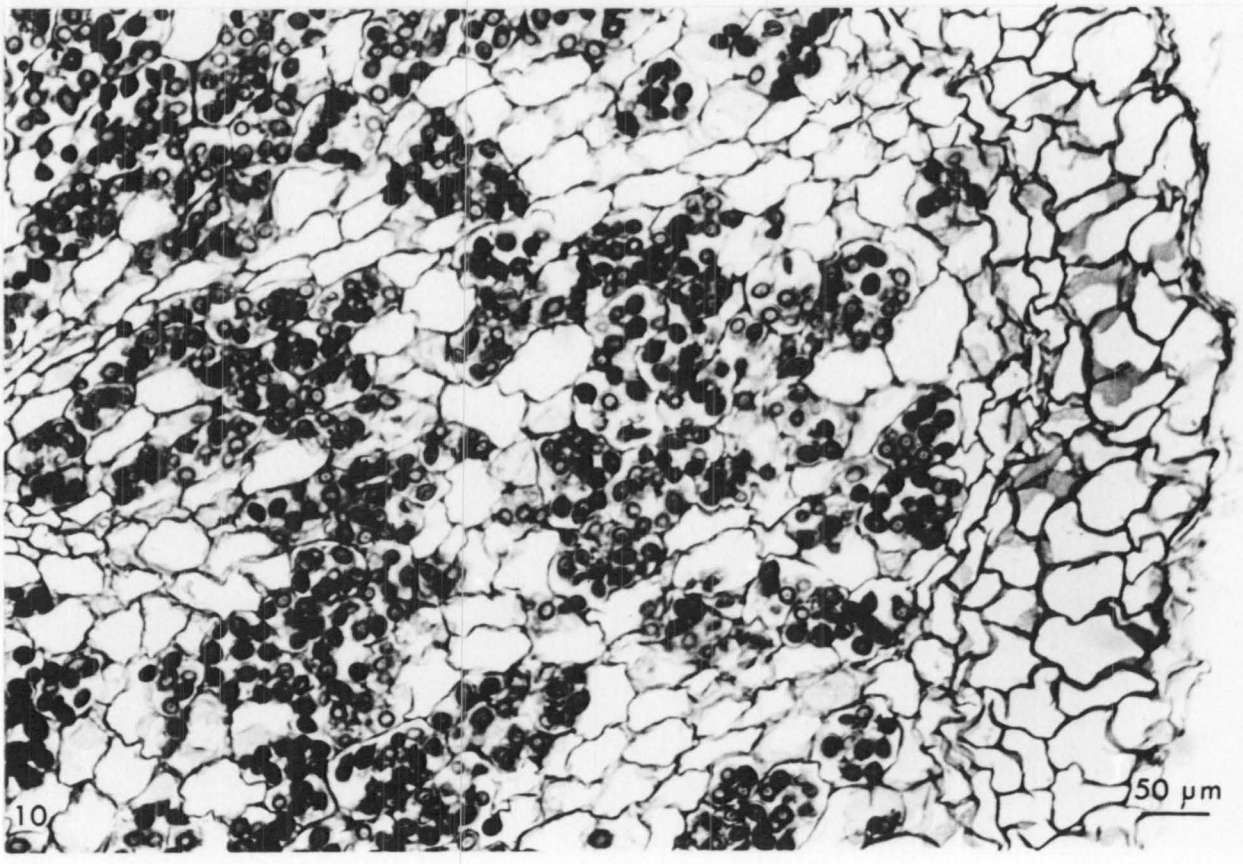


Fig. 8 L.S. gall of Scirpus basilaris infected by Entorrhiza scirpicola. Note active apical meristem, much enlarged infected cortex and uninfected exodermal layer and stele.



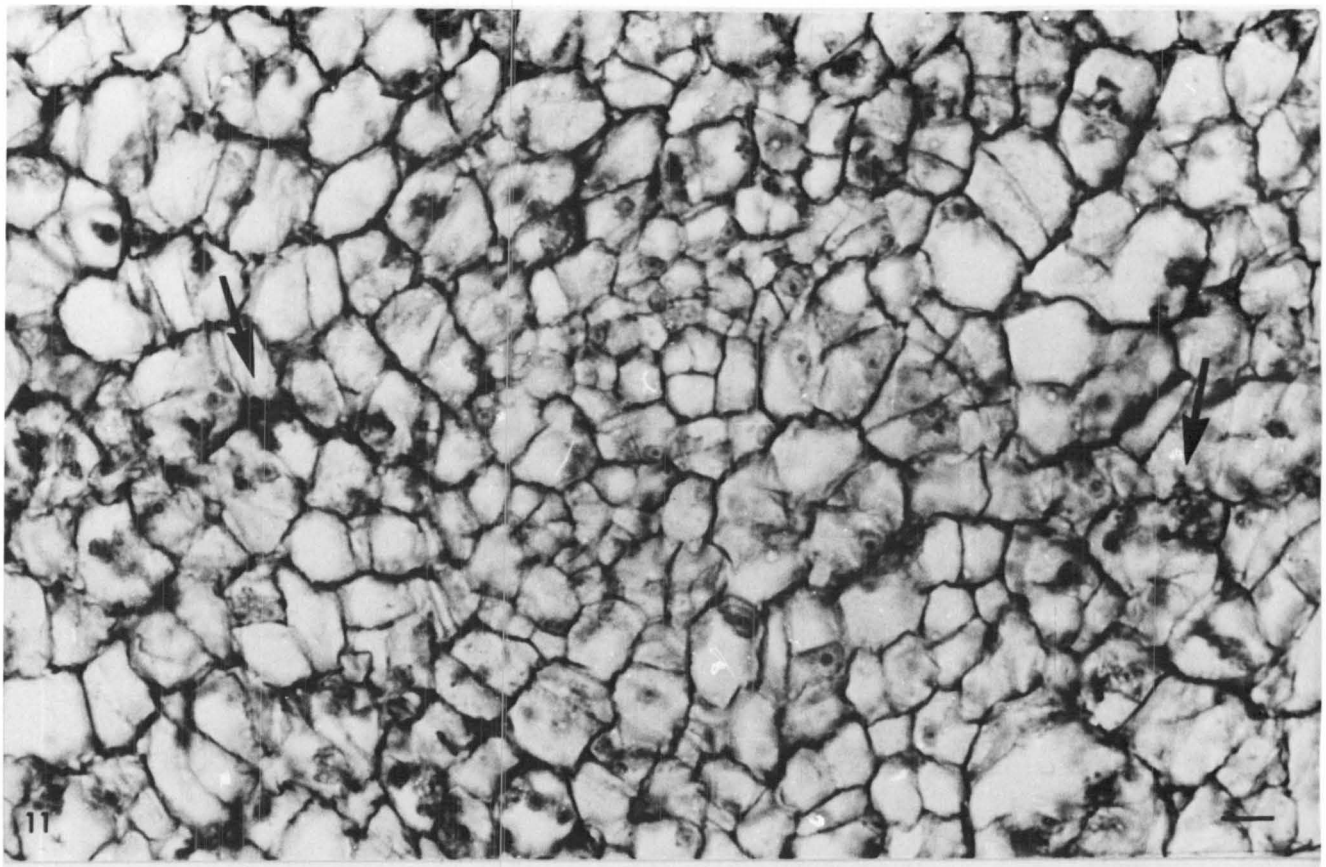


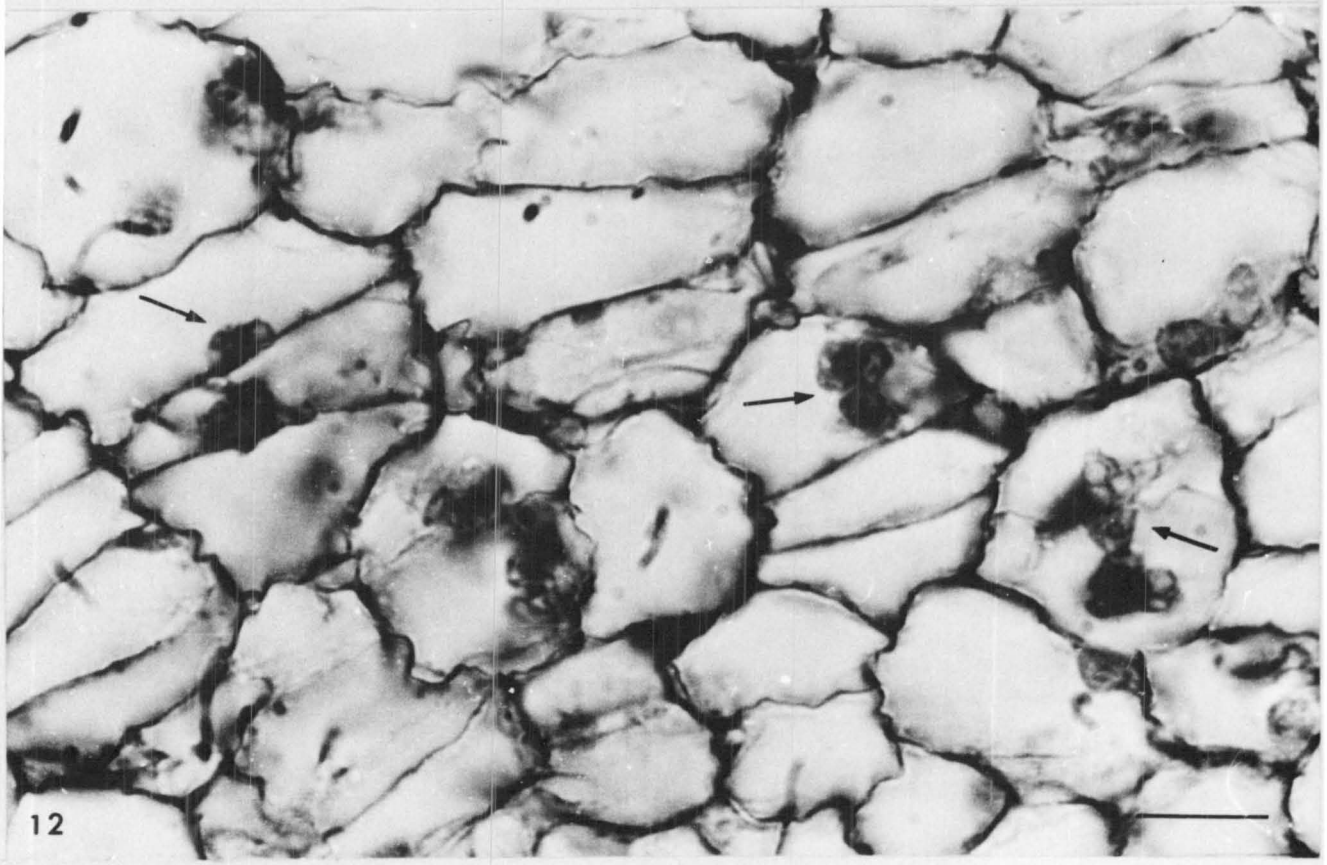
Figs. 9 & 10 T.S. gall of Scirpus basilaris infected by Entorrhiza scirpicola. Note much enlarged cortex, with most cells containing spores, and uninfected exodermal layer and stele.

The presence of the endophyte in some host species apparently stimulates branching of the root tip region as galls are often irregularly digitate as in Juncus articulatus (Figs. 1 & 2) or more regularly dichotomous as in Juncus gregiflorus (Figs. 3 & 4). Within the gall there are one to several vascular strands depending on whether the swelling is simple or branched. A conspicuous endodermis is usually present only towards the base of the gall.

The endophyte is present in the apex of the gall keeping pace with its development. In this region, intercellular branched hyphae, ramifying between the cells and penetrating intracellularly, appear to be the infective phase of the parasite (Figs. 11-14). The meristematic zone is not invaded but in adjacent areas where cells have undergone some differentiation intercellular hyphae are evident (Figs. 11,14). These hyphae are irregular, from 1.5 - 3.0 µm in diameter and septate. The endophyte is sometimes present in recently divided cells (Fig. 12).

The fungus is present mainly in the enlarged parenchyma cells of the cortex; however, not all cortical cells are infected (Figs. 8-10). The fungus does not normally occur in either the exodermal layer or in the stele (Fig. 10). Occasionally, however, xylem parenchyma cells near the base of a gall have been found to be infected. At first, abundant intracellular hyphae are present (Figs. 15 & 16) often in the distinctive regular coils first noted by Magnus (1878). These hyphae are usually between 1.0 µm and 1.5 µm in diameter and form coils about 3.0 - 3.5 µm across. They may, however, reach 2.5 µm in diameter. Septa, although difficult to detect, are present in these hyphae; they are most easily observed



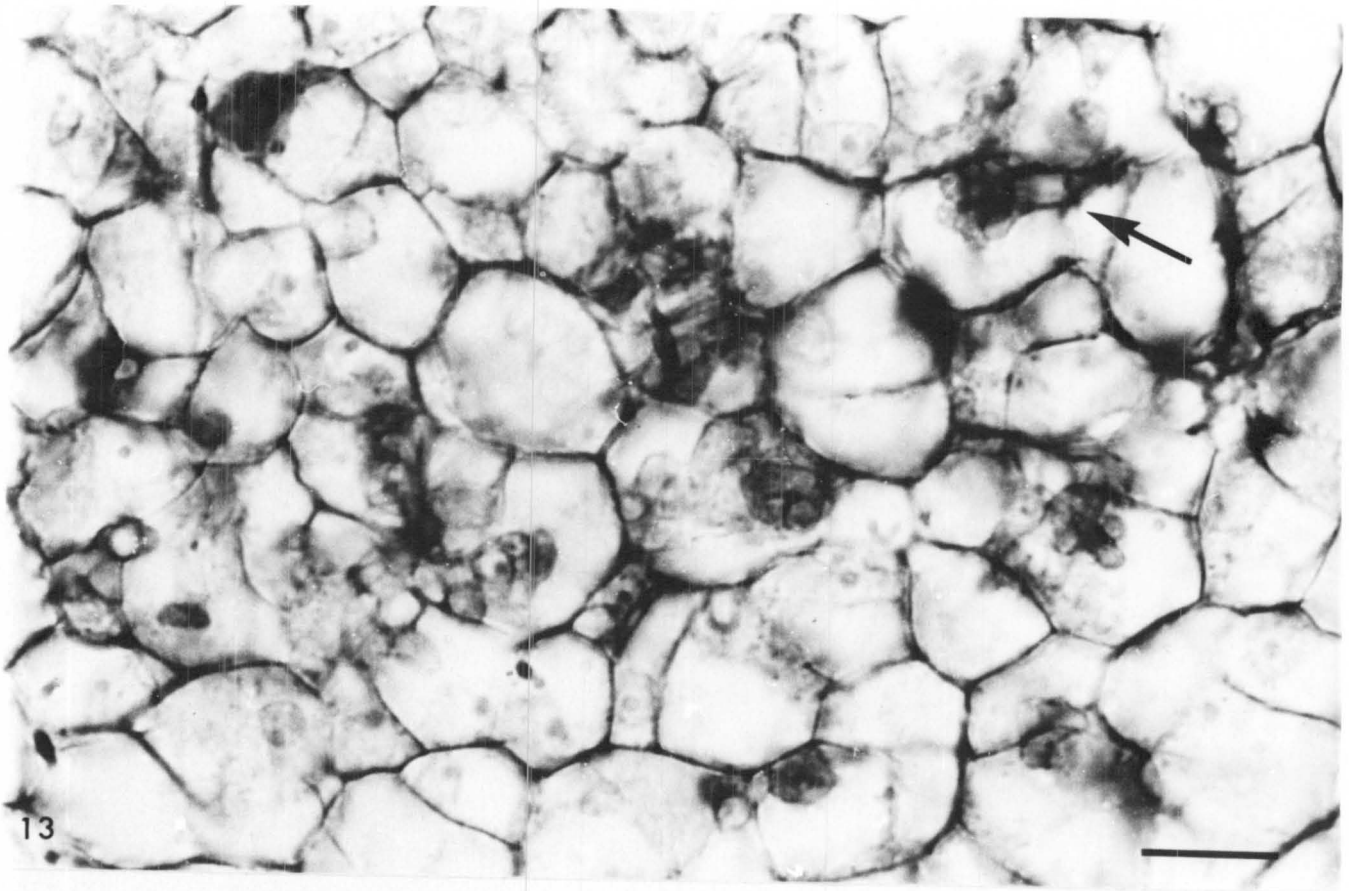


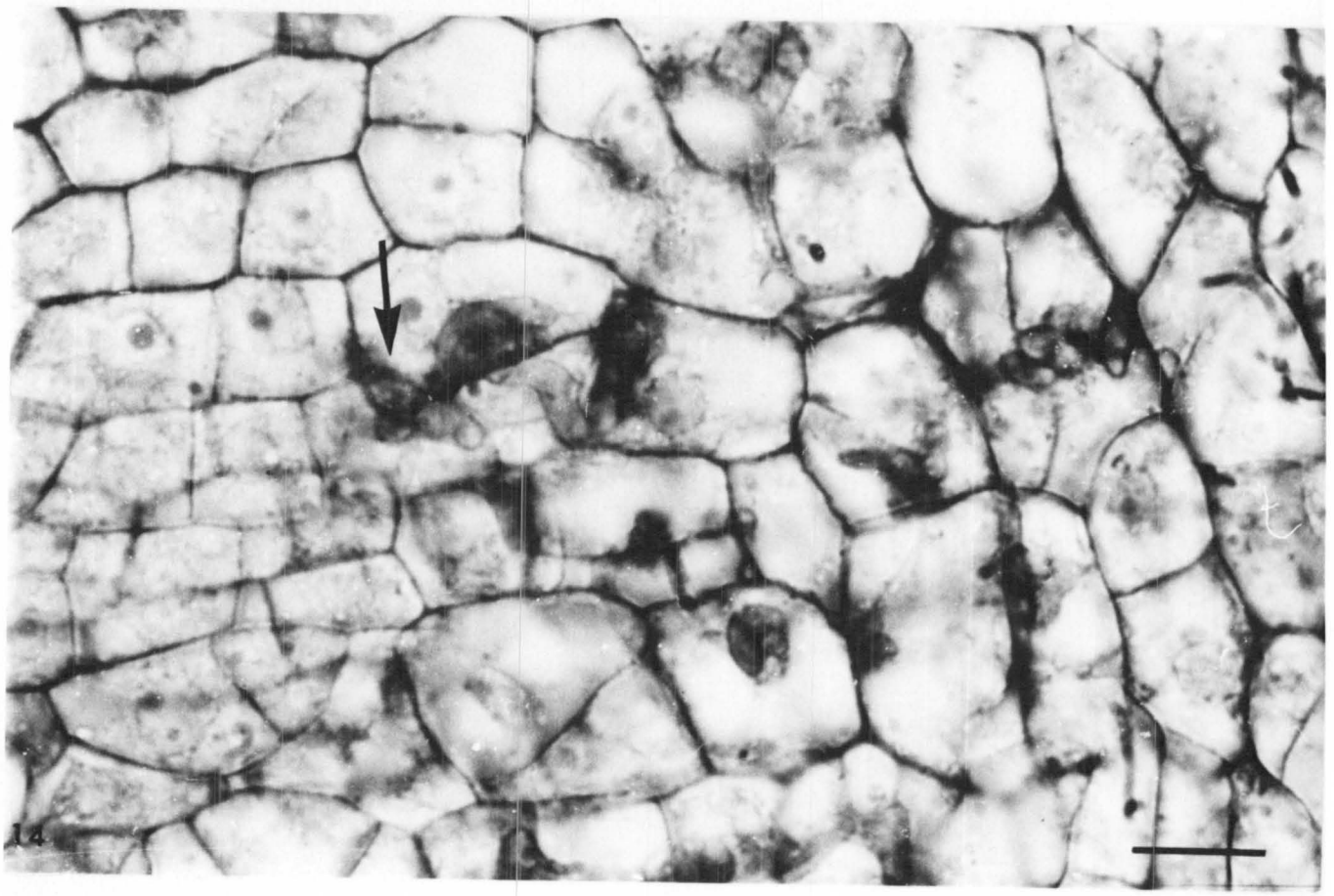
Scale for Figs. 11-18: line represents 10 μm.

Figs. 11 & 12 T.S. apex of gall of Scirpus basilaris infected by Entorrhiza scirpicola.

Fig. 11 Note hyphae (arrows) around periphery of meristematic region.

Fig. 12 Note hyphae (arrows) in recently divided cells.

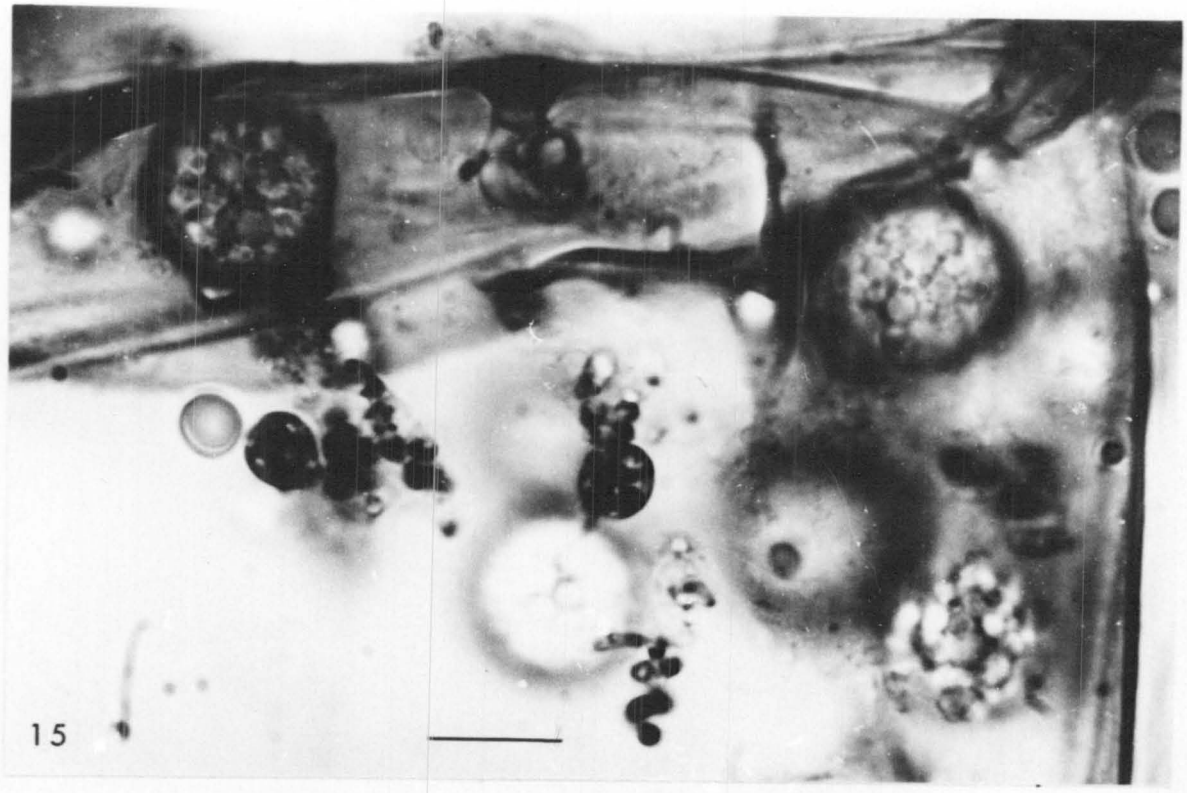


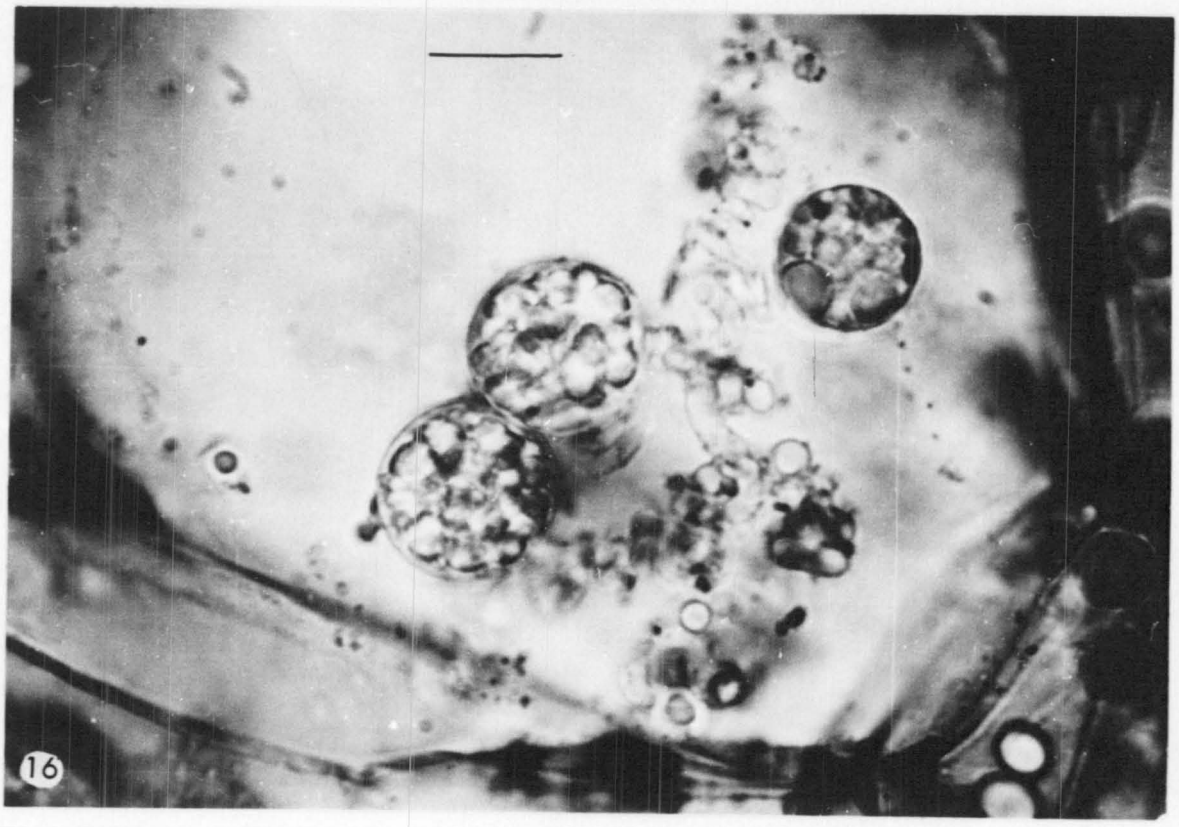


Figs. 13 & 14 Apex of gall of Juncus gregiflorus infected by Entorrhiza casparyana. Hyphae are probably mainly intercellular (arrows).

Fig. 13 T.S.

Fig. 14 L.S. Note hyphae behind meristematic region.





Figs. 15 & 16 Cells of Juncus articulatus infected by Entorrhiza casparyana. Note intracellular hyphae (often in coils) and developing spores.

with toluidine blue. The contents of the hyphae often stain poorly. Clamp connections have not been noted. There often appears to be an intimate association of hyphae with the host nucleus, a fact which was also recorded by Weber (1884).

With increasing distance from the apex, successive stages in spore development are found. The early stages are recognisable as small terminal swellings on the intracellular hyphae (Fig. 15). There is no evidence of hyphal fusion prior to spore formation. Moreover, spores have never been observed in an intercalary or intercellular position. Unlike many smut fungi, there is no gelatinous matrix surrounding spores and in the early stages the spore wall is merely an extension of the hyphal wall. With subsequent development the spore swells and the wall layers characteristic of the species concerned are produced. The number of spores per cell increases steadily towards the base of the gall so that cells in the lower portion of the gall are almost filled with teliospores (Fig. 17). Most of these spores are mature or almost mature but young developing spores are also present indicating the continuing activity of the intracellular hyphae, despite the fact that they are not nearly so conspicuous at this stage (Fig. 18).

The spores and young hyphae stain deeply with Sudan IV demonstrating their high lipid content. The iodine test for starch and schiffs reagent show that although starch is conspicuous near the apex of the gall and in uninfected areas throughout the cortex, it is much less conspicuous, in fact almost absent, in cells heavily infected with Entorrhiza. There was also no evidence of plastids in electron microscope preparations (see Chapt. 3).

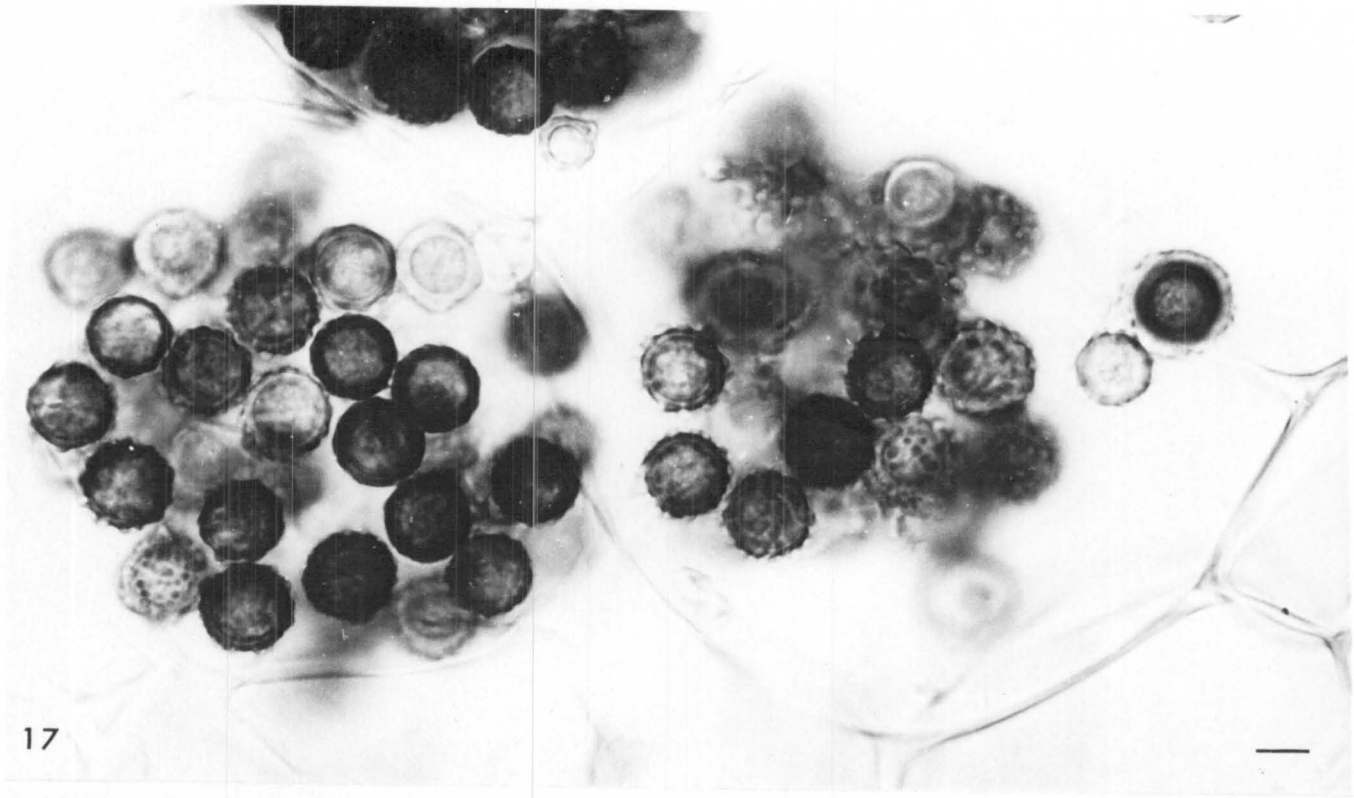


Fig. 17 Mature spores of Entorrhiza casparyana in cortical cells of Juncus articulatus.

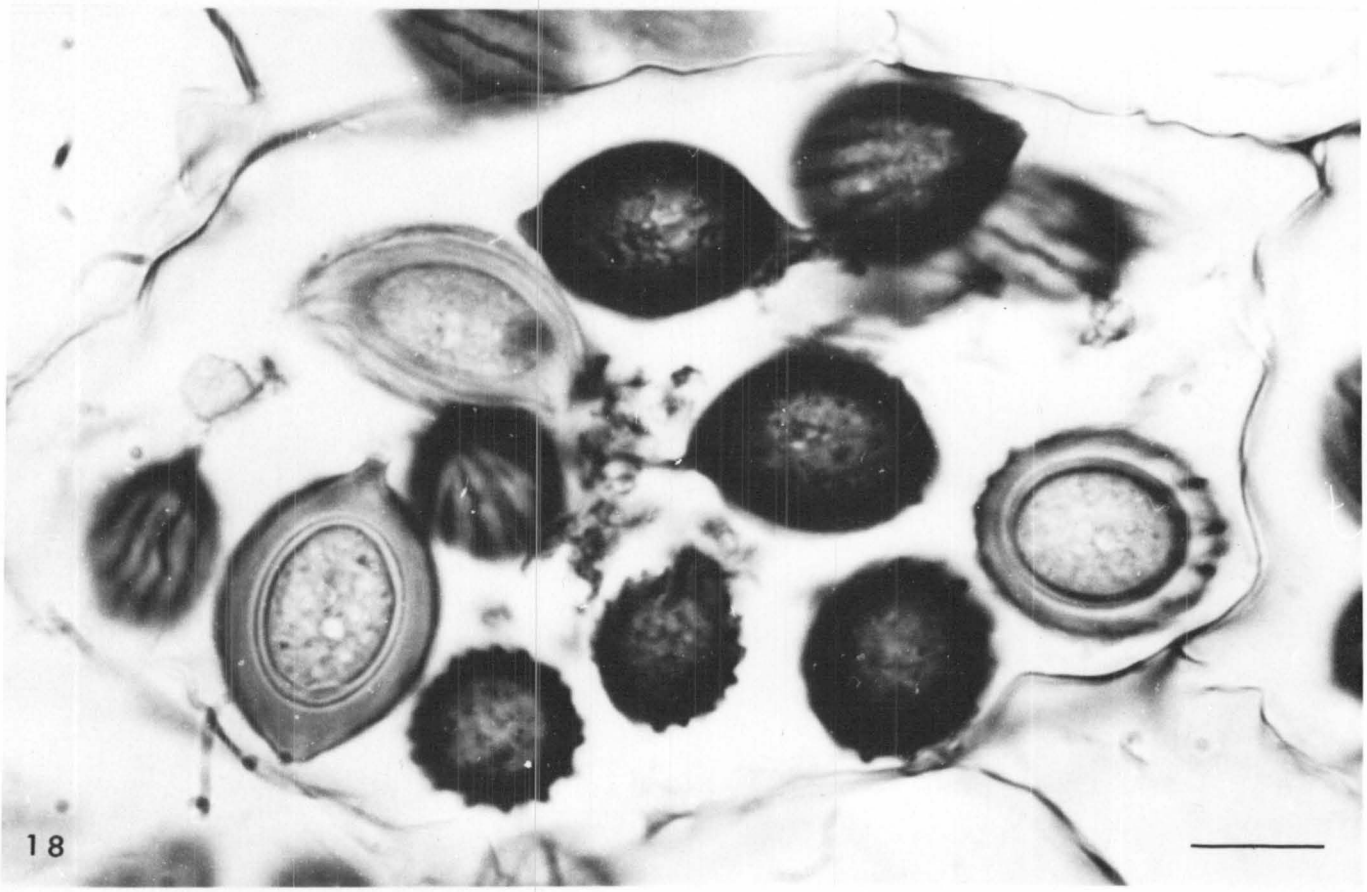


Fig. 18 Scirpus basilaris infected by Entorrhiza scirpicola: spores at varying stages of development, intracellular hyphae and host nucleus.

Sometimes hyphal coils and spores are enveloped by a layer of sheathing material (see also Weber, 1884). These sheaths are most apparent when material is stained in either Melzer's reagent or toluidine blue. The sheaths often surround hyphae either at their point of entry into a cell or subtending a spore. However, not all such hyphae are ensheathed. The sheath may be quite large eg. an ensheathed hyphal coil may have a diameter of 11.5 µm, the actual diameter of the hyphal coil being ca. 3.0 µm. The sheathing material appears to be of host wall origin (see also Chapt. 3) but the conditions controlling its formation are not known.

Entorrhiza is confined to the galls. It is absent from the subtending root and observations on the rest of the root system indicate that the fungus is absent from all parts of the root mass showing a normal morphology.

4. DISCUSSION

All observations indicate that Entorrhiza infections have a basic similarity, morphologically and cytologically. Only the roots are infected and infection is localised usually in the apex of the root but occasionally laterally. The only comparable root - infecting smut fungus is Urocystis coralloides Rostrup (syn. U. brassicae (Rostrup) Mundkür) which produces galls on the roots of Brassica species. However, these infections differ in detail from those of Entorrhiza. The mycelium is strictly intercellular in the cortex and there appears to be more disruption of the host tissue. According to Mundkür (1938)

"the host parenchyma looked as though it had been

> torn asunder by the invading hyphae, and large intercellular cavities full of mycelial filaments were evident here and there in the cortex".

Spore-balls formed in these intercellular spaces. In older infections the stelar parenchyma became infected as in some Entorrhiza galls but the stele always retained its individuality. The galls formed by U. coralloides are considerably larger than those caused by Entorrhiza. They may measure up to $2\frac{1}{2}$-4 cm. in diameter on mustard, Brassica campestris L. (Mitra, 1928) and on other cruciferous plants eg. Turritis glabra L. (Rostrup, 1881 cited by Mitra, 1928).

A few other smut fungi invade subterranean parts of plants, eg. Melanotaenium species on the upper portions of roots or on underground stems, and Thecaphora solani in potato tubers (Barrus, 1944). In addition, some fungi normally invading the aerial parts of plants will occasionally develop in underground organs eg. Ustilago striiformis and Urocystis agropyri on grass rhizomes (Duran, 1968).

According to Braun's (1959) classification, the root swellings induced by species of Entorrhiza are a type of gall and this terminology has been used in this thesis. The swellings are localised overgrowths caused by the presence of the fungus and are self-limiting as distinct from the tumorous growths apparent, for example,in the crown gall disease. They are structurally quite highly organised and morphologically, but not physiologically, probably most closely resemble the nitrogen - fixing root nodules of leguminous and non-leguminous plants. Both hyperplastic and hypertrophic activity is involved. The induced branching of the root apex in some infections is not unlike the effect produced in Pinus or Nothofagus roots by ecto-mycorrhizal fungi. Dichotomous

branching in some species of Pinus can be induced in sterile culture by IAA (indole acetic acid); as various mycorrhizal fungi have been shown to produce IAA the altered morphology is attributed to the fungus in the symbiotic association (Slankis, 1971). Growth promoting substances are also implicated in the rapid cell division and enlargement of legume nodules (Dixon, 1969). In Entorrhiza infections, an hormonal growth-promoting substance that is acting directly on the host cells or a regulatory enzyme that may be controlling or interfering with the host cell processes is also probably involved. Ustilago maydis, the most closely studied gall forming smut fungus, has been shown to produce the growth promoting hormone IAA in culture (Moulton, 1942; Wolf, 1952). Moreover, infected maize plants, particularly the galls, contain more IAA than do normal uninfected plants. Other smut fungi produce morphogenetic effects of a different kind but these are not relevant to the present discussion. The subject of morphogenetic effects on growth as a result of parasitic invasion has been reviewed by Braun (1959).

Within the gall Entorrhiza produces its sorus of spores. Because infection is localised and spores are formed throughout the cortical tissue of the gall, and because each gall is probably normally the result of a single invasion, a gall essentially represents a sorus of the fungus. Only a small region at the apex of the gall remains entirely mycelial. These sori differ from those of most smut fungi, other than Melanotaenium and Entyloma, in that the spores are embedded in the host tissue at maturity. The spores are passively released when the cortex breaks down and the sheath of the sorus ruptures. This soral sheath corresponds to the exodermal layer of the gall and is thus of host, rather than

fungal, origin. The vascular system remains largely intact during spore release.

Detailed observations of the development of Entorrhiza within the sorus agree with those already recorded for three species viz. E. aschersoniana (Weber, 1884; Trail, 1884), E. cypericola (Magnus, 1878, 1888, 1893) and E. scirpicola (Correns, 1897). Although hyphal fusion prior to spore formation has been suggested for Entorrhiza by Ferdinandsen and Winge (1914) there is no indication of fusion in any of the species studied during the present investigation. Furthermore, Brefeld's (1912) observations of intercalary and intercellular spores could not be substantiated.

In view of the lack of knowledge of the parasitic mycelium of the smut fungi (Fischer & Holton, 1957), it is informative to compare Entorrhiza infections with those caused by other smut fungi.

Entorrhiza resembles many other smut fungi in its penetration of meristematic tissue; such tissues present less resistance to infection than older parts of the plant. However, in contrast to the majority of smut fungi, which infect meristematic areas of aerial parts of the plant eg. cotyledons, nodes, ovaries etc., Entorrhiza penetrates cells close to the root apex. The fungus moves forward with the development of the gall and, in cells which are no longer dividing, spores are formed and mature. It is not known whether the hyphae penetrate meristematic cells but they have been seen in adjacent cells which have recently divided. This indicates that the fungus does not seriously interfere with mitotic division. This was also noted by Lutman (1910) for Ustilago hordei infections of oats. Entorrhiza does, however, appear to have

some effect on the meristematic activity of the root and this is reflected in the types of branching that may occur in the galls of different species; it is also possible that the rate of growth of the root is altered.

In Entorrhiza infections the hyphae are mainly intracellular except in the meristematic region. Fischer and Holton (1957) state that the majority of smut fungi have intercellular hyphae and that the penetration of cells is usually by haustoria and this is re-iterated by Ainsworth (1971). This may, however, be too much of a generalisation; Batts (1955) cites several authors who have reported smut fungi with intracellular hyphae. In addition, Fullerton (1970) has studied several species of the genera Sorosporium, Sphacelotheca and Ustilago which are systemic inflorescence fungi and which have intracellular hyphae developed to varying degrees.

Yet, although other smut fungi possess intracellular hyphae, only Entorrhiza appears to produce its spores intracellularly. The spores are formed terminally as is characteristic of the Tilletiaceae (Fischer & Holton 1957).

Clamp connections have not been detected in Entorrhiza hyphae. However, this may be because of difficulties of resolution with the light microscope. They are known to occur in some smut fungi eg. Ustilago striiformis (Osner, 1916 cited by Blizzard, 1926) but have not been detected in others eg. Urocystis cepulae (Blizzard, 1926). They are of common occurrence in the smut fungi according to Savile (1955).

The sheathing of intracellular hyphae of Entorrhiza is a feature which is common to many smut fungi; in some smut fungi the sheath is stratified. Rice (1927) and Kolk (1930) cite many of the early references. Woolman (1930) studied

Tilletia caries and noted sheaths 8-12 µm in diameter around invading hyphae of only 1 µm diameter. Batts (1955) describes sheaths around penetrating and intracellular hyphae in Ustilago nuda and discusses the origin and function of the sheath. The sheathing material is generally believed to be a host response to infection and to originate from host cell wall material. Guttenberg (1905) (cited by Kolk, 1930) believed, however, that the sheath was a transformation product of the plasma membrane of the host. The chemistry of the sheath has been much discussed and some workers have carried out histochemical tests to elucidate its composition. Western (1936) found for Ustilago avenae in oats that the sheath material was not lignin, in contrast to the lignitubers described by Fellows (1928) in Ophiobolus graminis infections of wheat, but neither was it pure cellulose. He believed, however, that the composition of the sheath might differ at different points in the host; the type of sheathing material might then depend on the type of cell being penetrated and the stage of wall development. Callose may also be deposited in the sheath (see Rice, 1927); this is known for wheat infected by various fungi eg. Alternaria, Helminthosporium (Young, 1926).

Whether the amount of sheathing indicates the degree of resistance of the host to infection is not known. Western (1936) noted that sheath formation did not occur in Ustilago avenae and U. hordei infections of the less resistant varieties of oats. On the other hand, for U. nuda, Batts (1955) did not think that the production of sheaths was evidence of resistance in the host because they occurred in three susceptible varieties of wheat. Hyphae sometimes appear to be ruptured inside sheaths (Ainsworth & Sampson, 1950; Fullerton, 1970) but this may be the result of senescence of the fungus

rather than a consequence of host resistance. Nevertheless, the fact that hyphae are sometimes prevented from passing through a sheath seems to indicate resistance on the part of the host; Fellows (1928) reported that lignitubers were sometimes able to prevent the entry of hyphae of Ophiobolus graminis into a cell. In susceptible varieties of wheat Batts (1955) never saw host thickening arrest penetration of U. nuda.

5. SUMMARY

1. The galls produced by Entorrhiza species are basically similar in structure. They are confined to the roots, usually the apices. The parasite is not present in roots having a normal morphology.

2. Galls have a root cap, an active apical meristem, enlarged cortex (due to hyperplasia and hypertrophy of the normal root cortex), vascular strand(s) and a 3-5 layered exodermis. No root hairs are produced.

3. The hyphae are intercellular in the apex and then become intracellular; they are septate and often distinctively coiled. No clamp connections have been detected.

4. Teliospores are produced intracellularly on the intracellular hyphae; they are terminal. The most mature teliospores are at the base of the gall near its point of attachment to the uninfected root.

5. Sheathing material, probably of host cell wall origin, may surround hyphae and spores.

CHAPTER 3

ULTRASTRUCTURAL CYTOLOGY OF ENTORRHIZA CASPARYANA IN GALLS ON JUNCUS ARTICULATUS

1. INTRODUCTION

Very little work has so far been carried out on the ultrastructural cytology of the smut fungi. In the reviews of Hawker (1965) and Bracker (1967) no references specifically on the cytology of the smut fungi are cited. Nevertheless, one study had been published prior to this (Kukkonen & Vaissalo, 1964). These authors investigated spore formation in Anthracoidea aspera infections of Carex. They were primarily concerned with the development of the spore wall, because of its taxonomic importance; preservation of the spore cytoplasm was poor.

Fullerton (1970) made an electron microscope study of the intracellular hyphae of some members of the Ustilaginales. This is the only published work on the ultrastructural relationships between the hyphae of systemic biotrophs and the cells of their hosts, a biotroph being defined here as a parasite which derives its energy from living cells. Fullerton found that encapsulation (formation), similar to that found with haustoria-forming parasites, was a constant feature of the intracellular hyphae of the smut fungi he examined. Furthermore, sheath formation was far more extensive than had previously been reported for parasites with haustoria. Fullerton defines 'encapsulation' and 'sheath' as follows:

"encapsulation, any simple or complex structure between the plasma membranes of the host and parasite which is neither typical

host nor fungal cell wall;
sheath, that part of the host-parasite interface that resembles host cell wall, whether it forms merely a collar around the invading hyphae or completely surrounds it." The terminology has arisen mainly from work on host-haustoria interfaces and there is some controversy and confusion concerning the nomenclature for various structures. In the present study the definitions of Fullerton and Ehrlich and Ehrlich (1971) are followed rather than those of Bracker (1967). The only work, other than Fullerton's, on the ultrastructure of the hyphae of smut fungi is a detailed study of a mycelial mutant of Ustilago hordei (Stein, 1970, cited by Robb, 1972).

Laseter et al. (1968), Hess and Weber (1970) and Allen et al. (1971) studied teliospores of Tilletia caries, T. controversa and T. foetida using the technique of freeze-etching described by Moor and Mühlethaler (1963). Work carried out previously in their laboratory had shown that, although the spore walls fixed suitably, it was difficult or impossible to chemically fix the cytoplasm of dormant spores for thin sectioning; freeze-etching overcame these problems. Suitable chemical fixation was possible, however, with germinated T. caries teliospores (Allen et al., 1971). Khanna and Payak (1971) have briefly reported on the ultrastructure of the spores of three smut fungi using thin-sectioning techniques. Both cytoplasmic and wall structure were described but not illustrated, therefore the quality of cytoplasmic preservation is unknown.

The most recent ultrastructural studies of smut fungi are those of Kozar and Weijer (1971) who examined the sporidia of Ustilago hordei and Robb (1972) who observed the pregermination development of teliospores of the same species.

These workers used thin-sectioning techniques in their investigations.

Apart from the work of Kukkonen and Vaissalo (1964) on the intercellular spore formation of Anthracoidea all investigations on the teliospores of smut fungi have been confined to spores after their removal from the host tissue. In the present study the development of the intracellular smut fungus, Entorrhiza casparyana, is followed in its normal host, Juncus articulatus, from host cell penetration to spore formation and maturity.

2. MATERIALS AND METHODS

The material of Entorrhiza casparyana used in this study was collected in turf on Juncus articulatus from Stony Bay, Banks Peninsula, Canterbury, 20 February, 1971. Mature and young developing galls were then fixed according to one of the following schedules:

a) 2% aqueous $KMnO_4$ for 2 hours at 20°C with evacuation for 30 mins. at ca. 60 cm Hg vacuum pressure (Mollenhauer, 1959).

b) 3% glutaraldehyde in 0.025M phosphate buffer, pH 6.9, for 4 hours at 4°C with evacuation for 30 mins. at ca. 60 cm Hg vacuum pressure; washed in 3 changes of phosphate buffer, remaining in the last change for 3 hours.

Material fixed according to schedule b) was then post-fixed in 2% OsO_4 in 0.025 M phosphate buffer for 14 hours at 4°C, dehydrated in acetone at 10% stages and embedded in araldite polymerised at 35°C for 24 hours and then at 60°C for

another 24 hours (Fineran & Bullock, 1972).

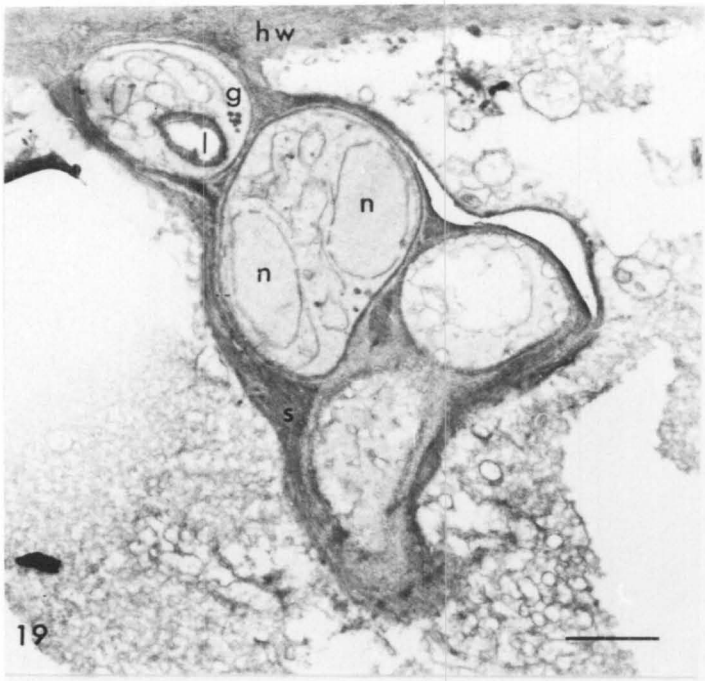
Sections were cut on an LKB ultra-microtome II using a diamond knife and mounted on 100 mesh collodion/carbon coated copper grids. They were then stained for 10 mins. in lead citrate (Reynolds, 1963) followed by 10 mins. in a saturated solution of uranyl nitrate. Sections were examined with an Hitachi HS-7 electron microscope.

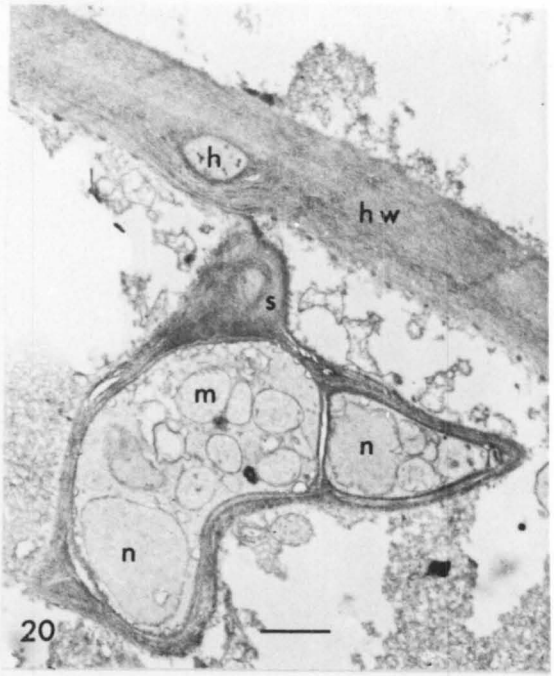
3. RESULTS

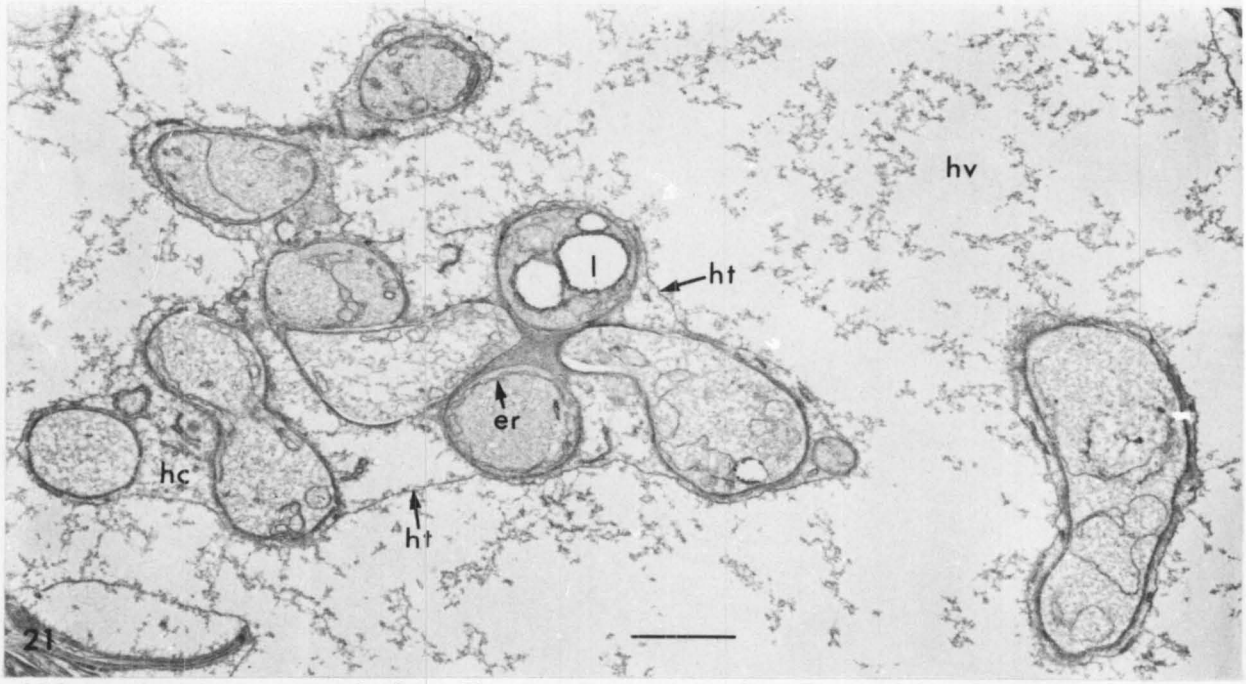
3.1. Hyphae

Hyphae are small, ca. 1.5-2.5 µm in diameter near the host cell wall (Figs. 19 & 20) and mainly 0.5-1.5 µm within the cell (Figs. 21-25). The hyphae appear to coil immediately after penetrating the host cell wall (Fig. 19) and coils are observed within the host cell either ramifying in the host cytoplasm (Fig. 22) or subtending spores (Fig. 23). These coils are distinguishable in longitudinal section because the hyphae are usually cut transversely and the sections of the coil are more or less evenly spaced. Sometimes bends in the coil are sectioned (top and bottom hyphal segment, Fig. 23).

The hyphal wall is always single-layered. The walls are thickest in hyphae close to the host cell wall (Figs. 19 & 20). Occasionally a slightly thickened wall is apparent on what is probably a spore stalk (Fig. 34). Elsewhere within the host cytoplasm the hyphae are generally thin-walled (Figs. 21,23). Septa are observed infrequently (Figs. 20,24) probably because hyphae are usually sectioned transversely.







Abbreviations used for E. casparyana in J. articulatus in Figs. 19-40:
Host: hc - host cytoplasm; hgb - host Golgi body; hm - host mitochondrion; ht - host tonoplast; hv - host vacuole; hw - host cell wall.
Fungus: er - endoplasmic reticulum; g - glycogen granules; h - hypha; l - lipid body; m - mitochondrion; n - nucleus; s - sheath; w_1 - 1st spore wall layer; w_2 - 2nd spore wall layer; w_3 - 3rd spore wall layer.
Fixation: $KMnO_4$ - fixation in potassium permanganate; Glut - OsO_4 - fixation in glutaraldehyde followed by post-fixation in osmium tetroxide.
Scale: line on prints represents 1 µm.

Fig. 19 Penetrating hypha, ensheathed by host cell wall material, showing binucleate condition, mitochondria, ER, lipid and possibly glycogen.

Fig. 20 Penetrating hypha showing sheath, septation, nuclei, mitochondria and crenulate plasmalemma.

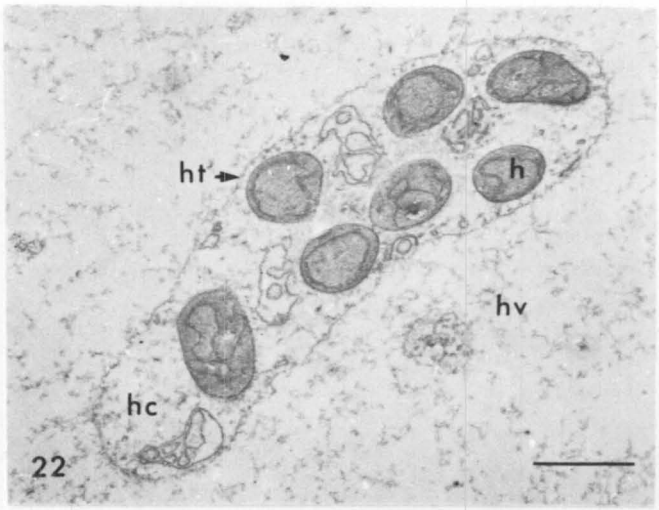
Fig. 21 Coiling hyphae surrounded by host cytoplasm demarcated from the host vacuole by the tonoplast. Note lipid bodies, and ER forming a band near the fungal wall.

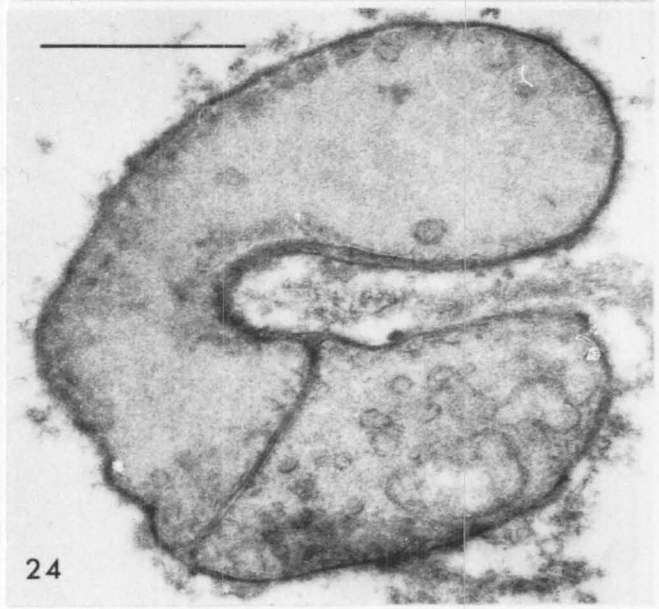
Figs. 19 - 21: $KMnO_4$.

All septa are complete but this may have been due to the plane of sectioning.

The plasmalemma is most easily observed in $KMnO_4$-fixed preparations where the protoplast pulls away slightly from the wall (Figs. 19 & 20). However, it is also apparent in glutaraldehyde-OsO_4 fixed material at higher magnifications (Fig. 23). In hyphae soon after penetration, the plasmalemma is often invaginated at intervals (Fig. 20). Some of these invaginations appear to have contents but it is difficult to be certain at such low magnifications whether these bodies are lomasomes or fixation artefacts. Invaginations of this type are not noted in the smaller hyphae ramifying throughout the cytoplasm.

Mitochondria with some cristae evident are well-developed in some hyphae (Figs. 19 & 20). They appear mainly oval (Fig. 20), and up to nearly 0.75x1.0 μm in size, although some irregular forms are apparent. Lipid bodies are present as electron transparent areas surrounded by a narrow electron-dense zone in $KMnO_4$ fixed material (Figs. 19,21). They measure up to ca. 0.65 μm in size. Small electron-dense round bodies are noted in some hyphae (Fig. 19). By analogy with similar bodies studied at higher magnification in the spores, these are almost certainly glycogen granules. Vacuoles occur in some hyphae (Fig. 23) but are not often conspicuous. In Fig. 23 one vacuole is almost round, ca. 1.5 μm in diameter, and completely fills the width of the hypha. Another vacuole is smaller and somewhat irregular. Endoplasmic reticulum (ER) is reasonably well-developed and often tends to form a band not far inside the plasmalemma (Figs. 21& 22). Ribosomes are evident in glutaraldehyde-OsO_4 fixed preparations at high





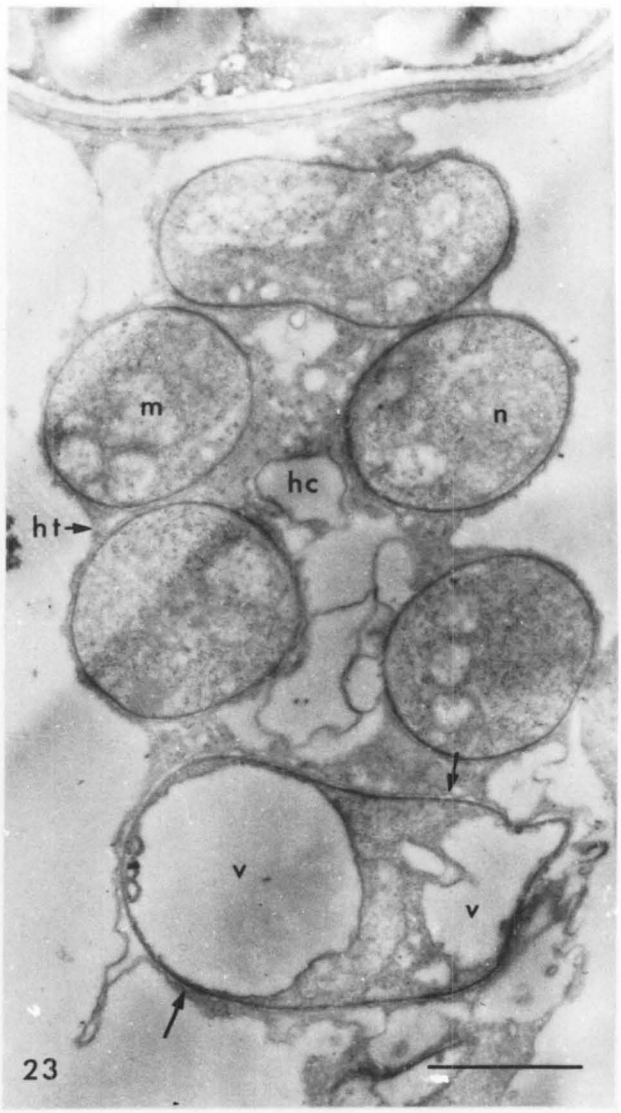


Fig. 22 Hyphal coil surrounded by host cytoplasm separated from the vacuole by the tonoplast. Note bands of ER within the hypha. $KMnO_4$.

Fig. 23 Hyphal coil, subtending a spore (top), showing ribosomes, vacuoles and indistinct nuclei and mitochondria. The coil is surrounded by host cytoplasm but separated from it by host plasmalemma (arrows). Glut. - OsO_4

Fig. 24 Hypha showing a septum. $KMnO_4$.

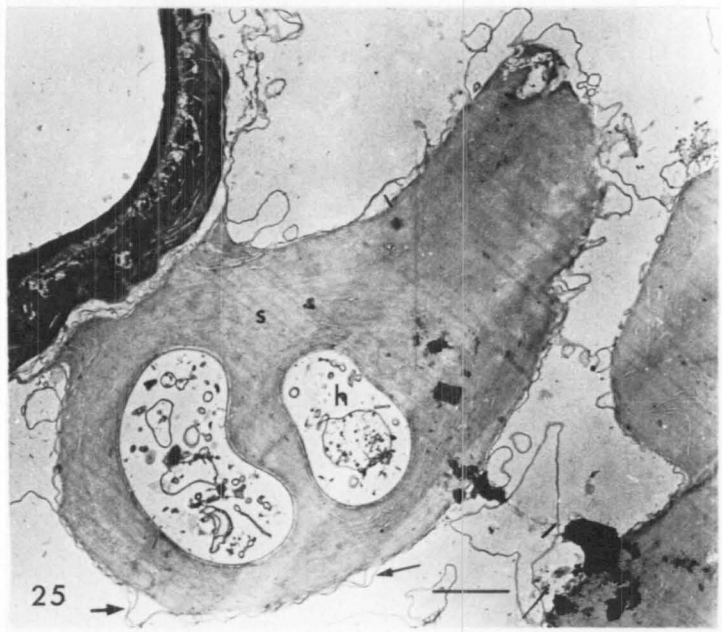


Fig. 25 Ensheathed hyphal coil surrounded by distorted host plasmalemma and tonoplast, closely adpressed. The sheath is less electron dense than the adjacent spore wall. Note degenerate contents of hypha. Glut. - OsO_4.

magnifications (Fig. 23). In this figure, ribosomes in the hyphal cytoplasm are more abundant than those in the host cytoplasm.

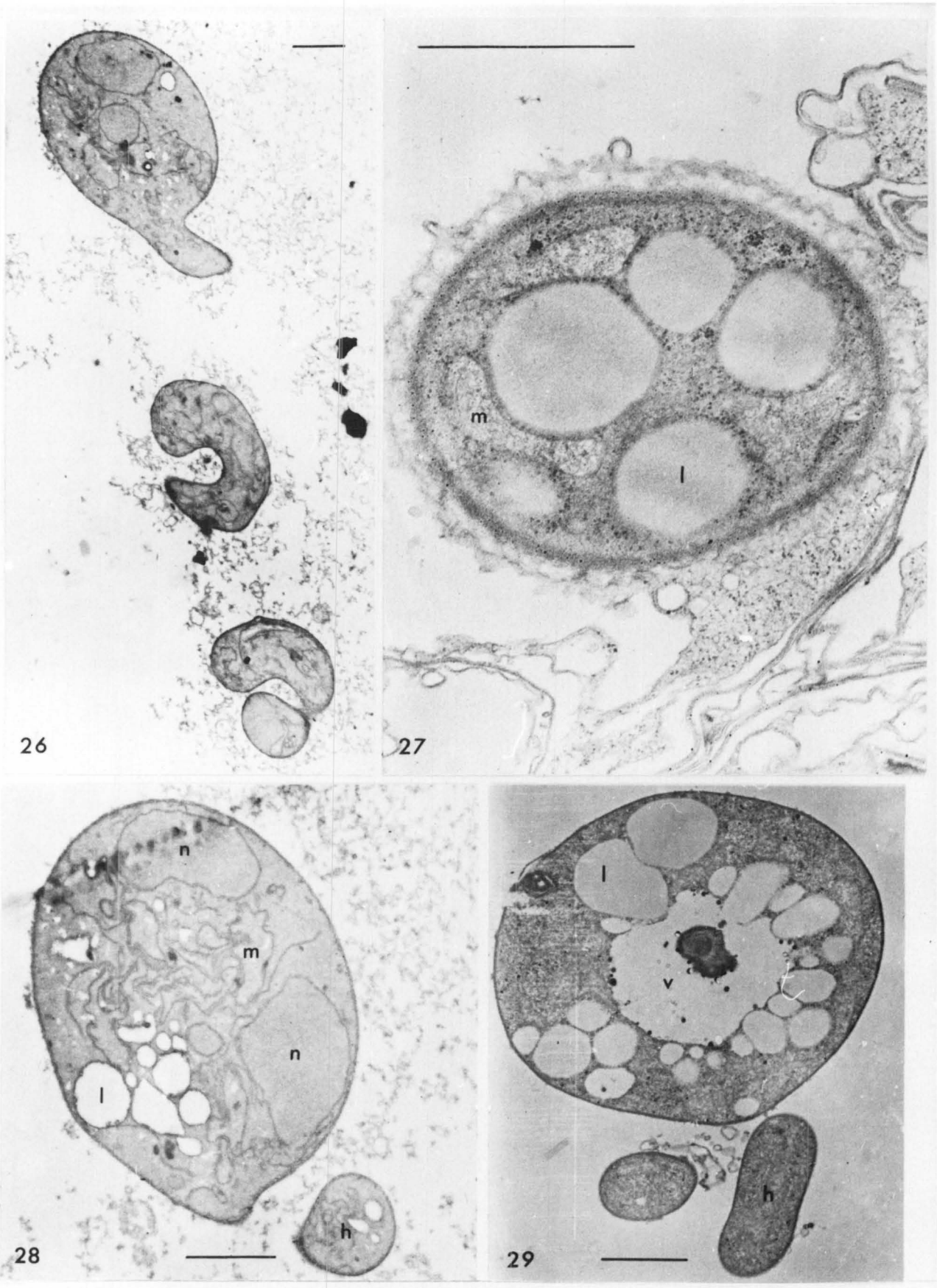
Nuclei are present within the cytoplasm (Figs. 19 & 20); some hyphae are obviously binucleate (Fig. 19). The nuclei are surrounded by a well-defined nuclear envelope containing numerous nuclear pores. Sometimes the individual membranes of the envelope are not closely apposed (Fig. 20). Nuclei are up to 1.5 x 2.0 μm in size and oval or approximately round.

Degenerated hyphae with disrupted membrane systems are often found during the later stages of infection (Fig. 25). These hyphae may or may not be ensheathed.

3.2. Spore Development

3.2.1. Spore initiation

Spores are initiated terminally on coiled hyphae. Soon after initiation the spore is still in direct continuity with the spore stalk (Fig. 26). The spore contents are very little differentiated from the contents of the hypha. Lipid bodies develop rapidly in the young spore (Figs. 27-30). They are slightly electron-dense and homogeneous in appearance with glutaraldehyde-OsO_4 fixation but after $KMnO_4$ fixation they are electron-transparent with some traces of electron-dense material, especially around their periphery. Lipid bodies are present in hyphae but to a lesser degree (Figs. 21,28). They are oval to round and up to ca. 1.0 μm in diameter during early stages of spore development when spores are less than ca. 4.5 μm in diameter (Fig. 29.). A large vacuole, 2 μm or more in diameter,



Figs. 26 - 29 Spore initiation.

Fig. 26 Terminal spore, in direct continuity with stalk, and portions of subtending hyphal coil. $KMnO_4$.

Fig. 27 Obliquely cut spore showing lipid bodies, mitochondria and ribosomes. Glut. - OsO_4.

Fig. 28 Binucleate spore showing lipid bodies, mitochondria and ER. The irregular mitochondria and crenulate plasmalemma may be fixation artefacts. $KMnO_4$.

Fig. 29 Spore with central vacuole, containing a membranous inclusion, lipid bodies, ribosomes and poorly differentiated mitochondria. Glut. - OsO_4.

may be present (Figs. 29 & 30) and may contain membranous inclusions (Fig. 29). Mitochondria are abundant. They are most easily recognized in $KMnO_4$ fixed preparations (Figs. 28, 30) where they are oval, ca. 0.35 x 0.6 µm in size (Fig. 30) or sometimes distorted (Fig. 28). Short profiles of ER are present but not conspicuous (Figs. 28, 30) and the cytoplasm is rich in ribosomal material (Figs. 27,29).

The plasmalemma can be differentiated in some $KMnO_4$ fixed preparations (Fig. 28) and with some difficulty in glutaraldehyde-OsO_4 fixed material (Fig. 29). In Fig. 28, the plasmalemma has a slightly crenulated appearance but this may be a result of fixation as the plasmalemma of the adjacent hypha is similar whereas that of the glutaraldehyde-OsO_4 fixed spore is smooth (Fig. 29).

The spores are binucleate in these early stages (Fig. 28 & possibly Fig. 26). The nuclei are up to 1.25 x 2.0 µm i.e. similar in size to those found in hyphae.

During this stage of spore development (Figs. 26-30) the spore wall is thin, single-layered and does not differ in appearance from the hyphal wall.

3.2.2. Increase in size of spore prior to development of additional wall layers

Spores described above ranged in size from approximately 2 µm in diameter (Fig. 27) to 4 µm in diameter (Fig. 29) or 3 x 5 µm (Fig. 30). Subsequently there is an increase in size of the spore without much change in spore structure (Fig. 31).

Lipid bodies continue to increase in size, up to ca. 2.0 µm in diameter (Fig. 33), and in number; mitochondria

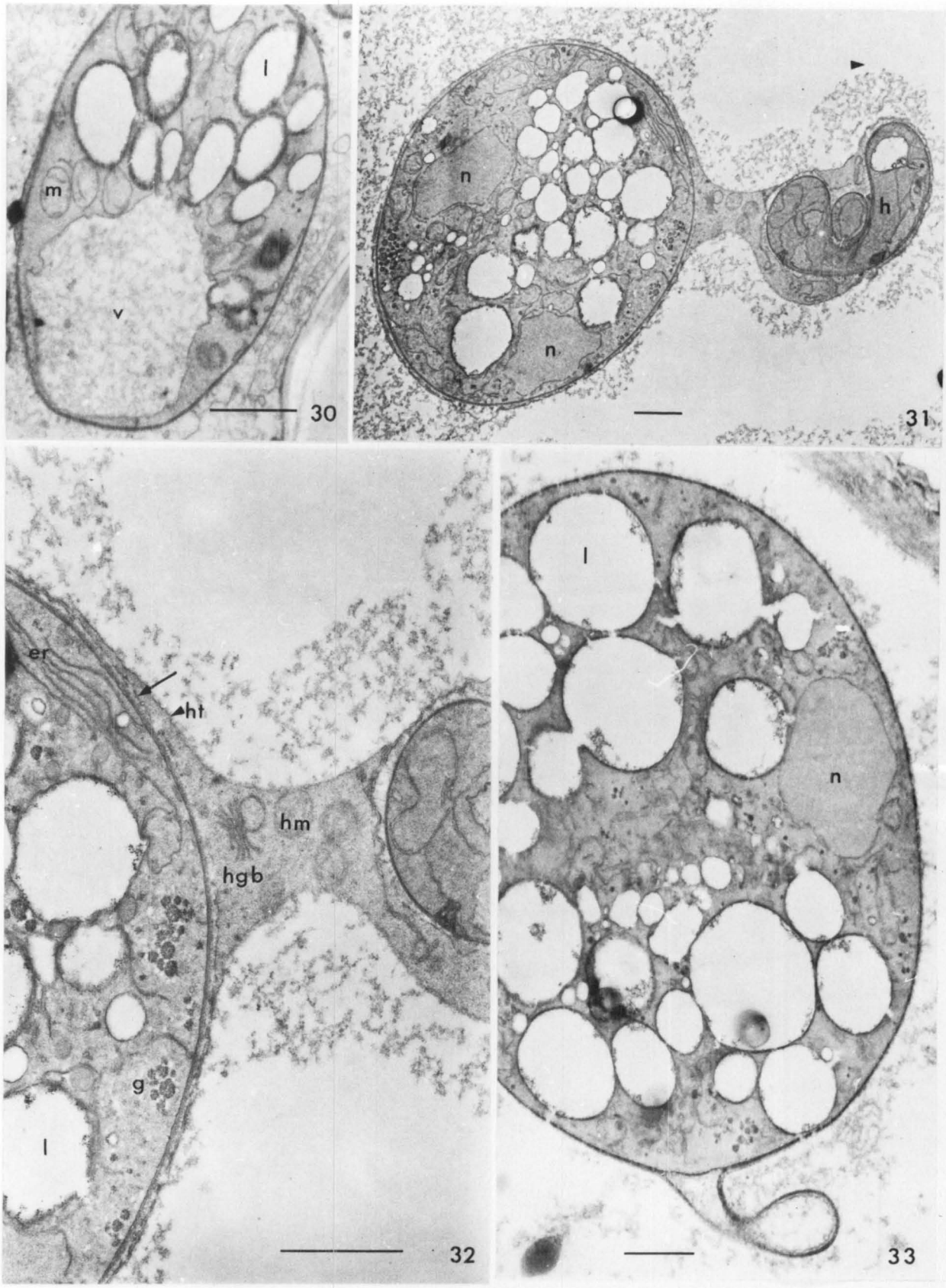


Fig. 30 Young spore with large vacuole, lipid bodies and mitochondria. $KMnO_4$.

Figs. 31 - 33 Spore development prior to development of wall layers. $KMnO_4$.

Fig. 31 Binucleate spore enveloped by host cytoplasm continuous with that surrounding the subtending hypha.

Fig. 32 Portion of Fig. 31 at higher magnification showing glycogen granules, lipid bodies and ER in spore, and Golgi body, mitochondria and ER in host cytoplasm. Host plasmalemma (arrow) and tonoplast are closely adpressed to the spore.

Fig. 33 Spore cut off from stalk by a septum. (cf. Fig. 26)

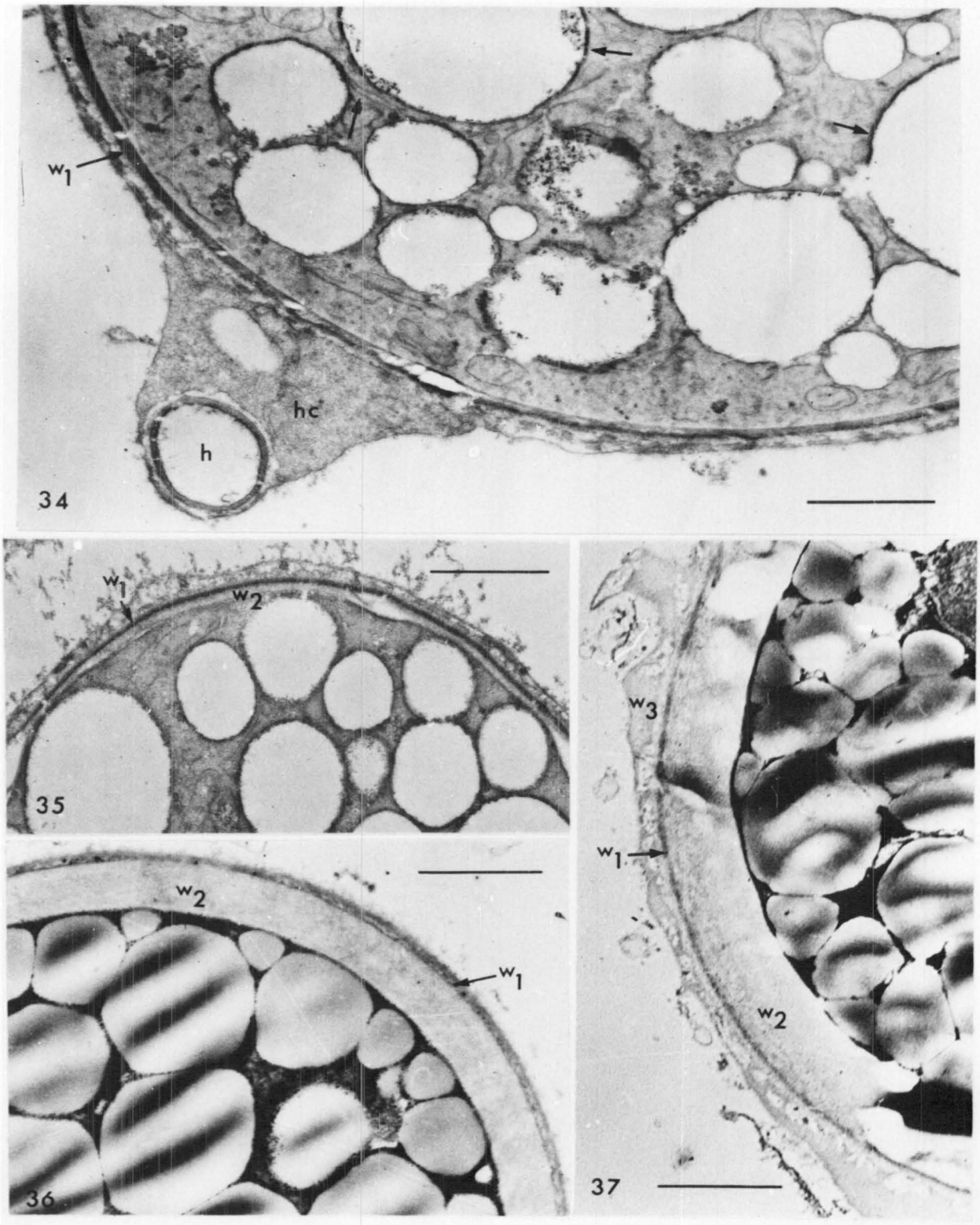
remain conspicuous and the spore is still binucleate (Fig. 31). Some long profiles of ER are evident and show a close association with the boundaries of many lipid bodies (Fig. 34). Glycogen granules, which were not detected in the earlier stages are present (Figs. 31-34). They occur as roughly isodiametric β particles which aggregate into distinctive rosette configurations (α particles) (see Bracker, 1967).

In most spores the spore wall is now approximately twice as thick as the hyphal wall although apparently similar structurally (Figs. 32,34). A considerable increase in size can be seen by comparing these two micrographs both taken at a similar magnification. A septum is now apparent cutting off the spore stalk (Fig. 33).

3.2.3. Development of additional wall layers

In spores as small as 7.5 µm in diameter a second, less electron-dense wall layer is initiated on the inside of the first wall layer (Fig. 35). In this example the first wall layer has developed less than in some spores possessing only one wall layer (cf. Fig. 34) indicating that the sequence and degree of development of the wall layers may differ from one spore to another. In spores of 8 µm diameter (eg. Fig. 36) the second spore wall layer has increased considerably in thickness to ca. 0.45 µm. The first wall layer is also more developed in this example than in younger spores.

After the expansion of the second wall layer, the third wall layer begins to form on the outside of the spore (Fig. 37). It is irregular in its surface topography and internal structure. Immediately external to the first wall layer, the third wall layer is less electron-dense and somewhat diffuse in



Figs. 34 - 37 Development of spore wall.

Fig. 34 Spore showing increase in thickness of first wall layer (cf. Fig. 32). Note ensheathing of lipid bodies by ER (arrows). $KMnO_4$.

Fig. 35 Development of second wall layer inside the first. $KMnO_4$.

Fig. 36 Increase in thickness of second wall layer; lipid bodies pack the cell. Glut. - OsO_4.

Fig. 37 Beginning of third wall layer, outside the first wall layer. Note its diffuse, irregular nature. Glut. - OsO_4.

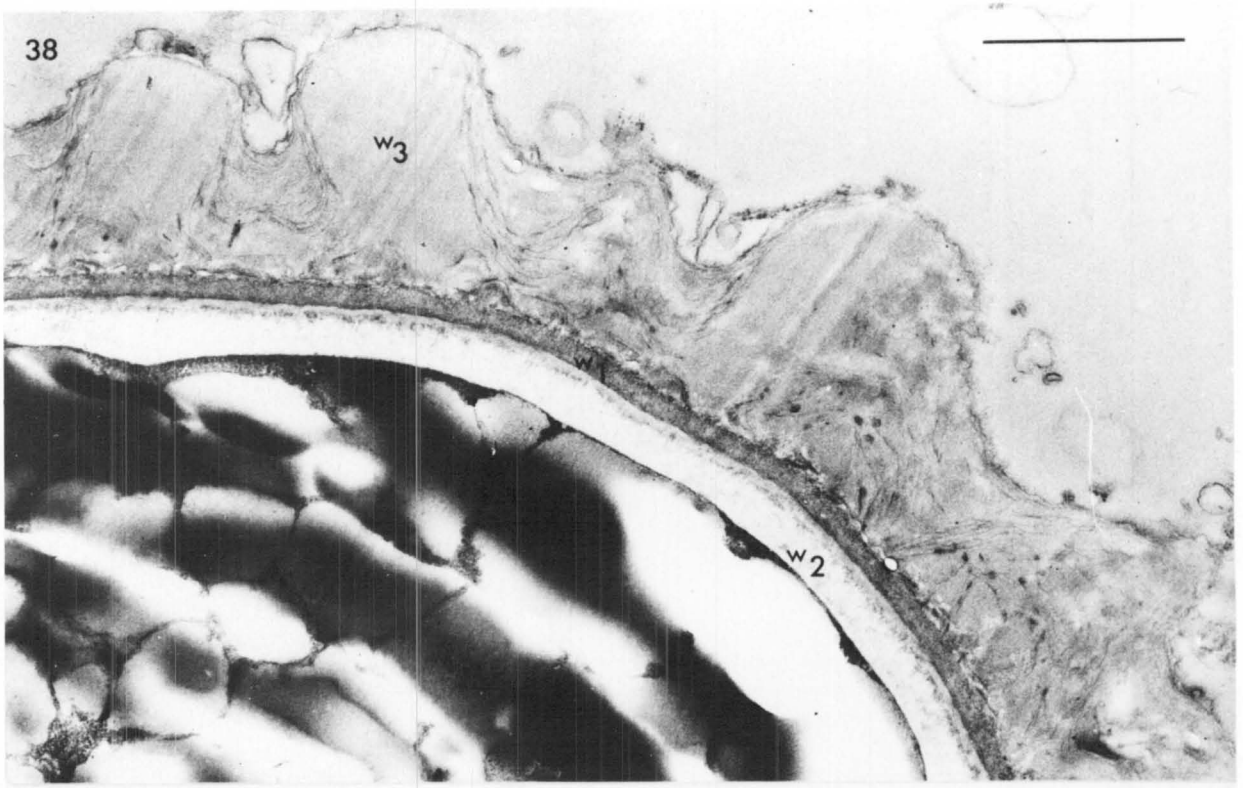
appearance. As the spore matures there is a pronounced development of the third wall layer producing the pattern of sculpturing characteristic of the species (Figs.38 & 39). In Fig. 38 the third wall layer is 1.5 µm thick where the warts occur. There is some internal organisation in this layer with the diffuse area mentioned earlier remaining and becoming differentiated to various degrees. In addition fibrillar contours often outline the warts and concentrate in the hollows between them. In some spores (Fig. 39) these contours are evident as electron-transparent areas.

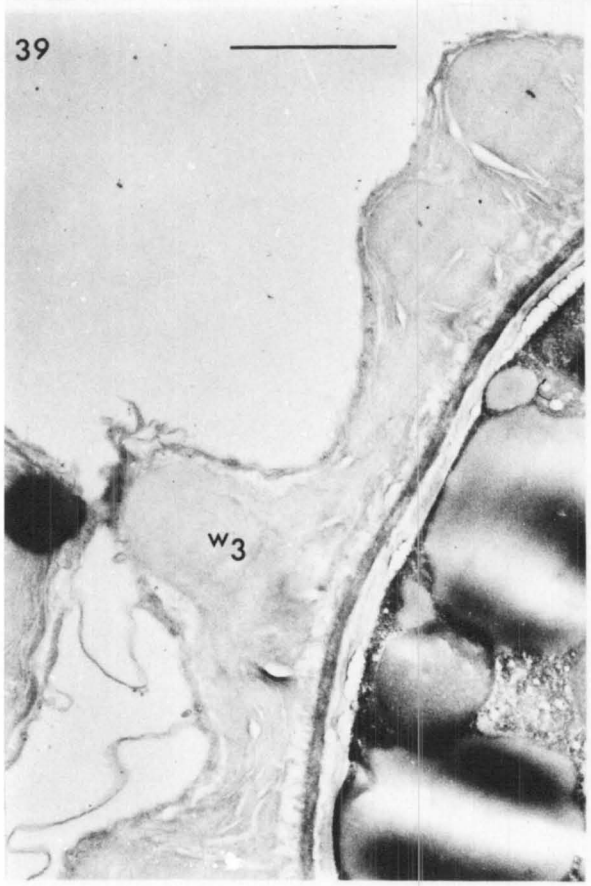
In Fig. 38 the first wall layer has also increased considerably in thickness to a little more than 0.1 µm and the second wall layer is distinctly differentiated into a narrow outer zone and a broad inner zone. The outer zone is more electron dense than the inner. This differentiation of a second zone within the second wall layer appears to occur about the same time as the development of the third wall layer.

In these mature or nearly mature spores lipid bodies pack the cytoplasm and tend to obscure other inclusions and organelles; the chatter during sectioning is indicative of the density of the lipid. In many spores with well-developed walls the contents are completely lost probably due to poor fixation and the difficulty of sectioning the dense lipid.

3.3. The Host - Parasite Interface

Penetrating hyphae are surrounded by an extension of host cell wall material (Figs. 19 & 20). Whether this invariably occurs is not known. This layer of host cell wall





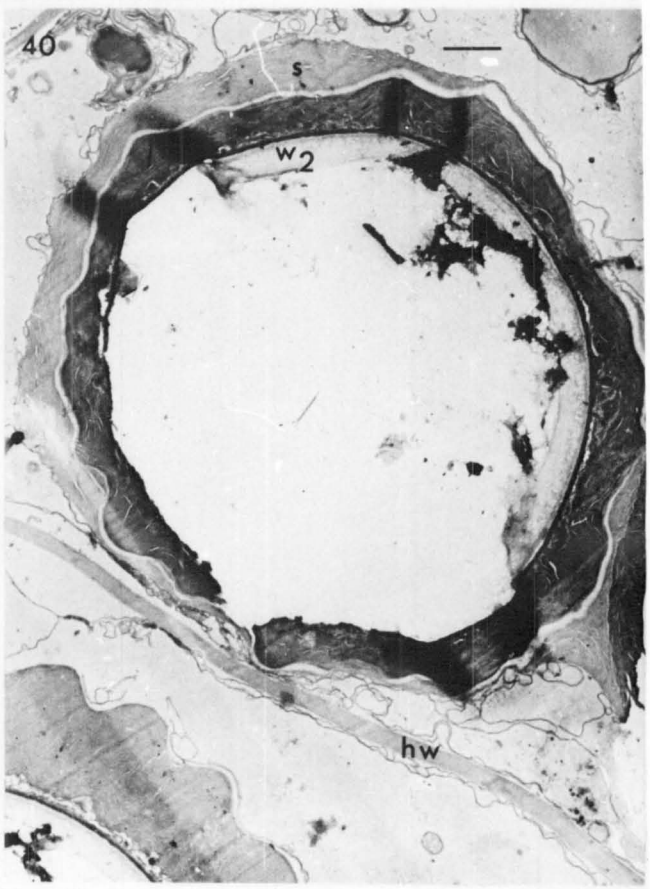


Fig. 38 Mature spore wall showing warty third wall layer, first layer and second layer. Note fibrillar contours within third layer and differentiation of second layer into two zones.

Fig. 39 Mature spore wall showing the electron transparent nature of contours (cf. Fig. 38) and well developed diffuse zone on the inside of the third wall layer.

Fig. 40 Mature spore ensheathed by material probably of host cell wall origin. Contents of spore and some of the inner wall layer are lost in preparation. Note disrupted host membranes surrounding the spore.

Figs. 38 - 40: Glut. - OsO_4.

material is referred to as a sheath, conforming with the terminology of Fullerton (1970) and Ehrlich and Ehrlich (1971). The host plasmalemma is poorly preserved but is sometimes apparent surrounding this sheath (Fig. 19).

Within the host cell most hyphae are not ensheathed (Figs. 21-23) but are individually surrounded by the host plasmalemma which is detectable at higher magnifications (Figs. 23, 32, 34). The hyphal system or coil is surrounded by a small pocket of host cytoplasm delimited from the host vacuole by the tonoplast (Figs. 21-23). At the extremities of the hyphal coil the host tonoplast and plasmalemma may be noted closely adpressed to the hypha (Fig. 23).

The same intimate relationship of host and parasite is found for young spores with only the first wall layer (Figs. 31, 32, 34) and for spores showing the early development of the second wall layer. Each spore is closely surrounded by host plasmalemma. Usually the host cytoplasm is concentrated at the base of the spore in the region of the spore stalk (Figs. 31 & 32). The host cytoplasm is separated from the host vacuole by the tonoplast. In the area enveloping most of the spore, which is usually largely devoid of cytoplasm, the tonoplast is closely apposed to the plasmalemma.

The host cytoplasm associated with hyphae and young spores may sometimes be slightly disrupted (Figs. 21-23) but is often intact (Figs. 31 & 32). Golgi bodies are present Figs. 32 & possibly 21 & 22), profiles of regular ER (Figs. 21, 31, 32) and some membranous material, probably ER, of a very irregular nature (Fig. 22). Mitochondria are present (Figs. 21, 31, 32) and ribosomes (Fig. 23).

As spores develop, the host cell appears to be disrupted

and the intimate relationship between host and parasite appears to be lost. Disrupted host membranes often surround mature spores (Fig. 40).

Sometimes during the later stages of development of the fungus in the host, hyphae and mature or nearly mature spores are ensheathed by material apparently of host cell wall origin (Figs. 25, 40). These sheaths are similar in appearance to those associated with penetrating hyphae but are more highly developed. They may be as thick as the wall layer of the spore (Fig. 40) or up to 5 μm in diameter surrounding hyphae (Fig. 25). This sheathing material contains electron-transparent channel-like areas similar to those found in the third spore wall layer but differs generally in appearance from spore wall material in its electron density (Fig. 40). Sheaths are surrounded by the host plasmalemma with the host tonoplast closely adpressed (Fig. 25). The membranes are distorted, however, because of the advanced stage of disruption of the host cell.

Hyphae enveloped by such sheaths may sometimes be in an advanced stage of degeneration (Fig. 25) or sometimes apparently still active.

The sheaths described above, in contrast to those surrounding penetrating hyphae, are often separated from the hyphae or spores which they envelope by a narrow zone representing extracellular space (Figs. 25, 40). However, this may be an artefact of fixation.

4. DISCUSSION

4.1. Hyphae

The hyphae in this intracellular parasite have ultrastructural features in common with those of previously described fungi (Hawker, 1965; Bracker, 1967). They are basically eukaryotic in their organisation and lack plastids. Glycogen and lipid are the main storage products. In common with the majority of higher fungi, Golgi bodies have not been detected.

There is no evidence that the protoplast ultrastructure of the hyphae of obligate or near-obligate parasitic fungi is different from that of facultative parasites or saprophytes (Bracker, 1967). For this reason the most useful information that can be obtained from a study of this nature is on cytological details of the life history of the organism, its development and activity within the host and relationship with the host cell.

Hyphal ultrastructure in the smut fungi has received little attention. According to Bracker (1967) and various text-books on mycology (eg. Webster, 1970) the smut fungi, like the rust fungi, are distinct from other basidiomycetes in their septal pore structure. However, there appear to be no publications of original work in support of this statement. Fullerton (1970) has investigated the intracellular hyphae of some smut fungi with the electron microscope but he was primarily concerned with the host-parasite interface (see 4.3) and did not discuss the protoplasmic structure of the hyphae. His micrographs, in general, show little cytoplasmic detail.

The electron microscope is particularly useful for elucidating details of the hyphal phase of smut fungi because the hyphae are often so small that cytological details are difficult to determine unequivocally with the light microscope. Prior to the widespread use of the EM in biology, Fischer and Holton (1957) stated that there was

> "very little information on the mycelial phase (of smut fungi), presence or absence of haustoria and the general relationship of the host and pathogen."

With E. casparyana a number of features of the life history have been confirmed with the EM. The most important of these is the binucleate state of the parasitic phase. Hyphae entering the host cell are binucleate and produce binucleate spores terminally. The suggestion by Ferdinandsen and Winge (1914) that hyphal fusion preceded spore formation in Entorrhiza has been discounted. Hyphal coiling is, however, a characteristic feature of infection and makes problems such as these difficult to resolve with the light microscope.

Hyphae which have recently penetrated the cell (Figs. 19 & 20) and those involved in spore formation (Fig. 23) appear to have a relatively high level of metabolic activity. This is indicated by the abundant mitochondria in these cells. Another feature of many hyphae is the almost continuous band of ER immediately inside the fungal cell wall and plasmalemma. The position of the ER in these cells is probably of some functional importance.

4.2. Spore Development

Although there have been numerous ultrastructural investigations of mature or dormant spores these have been largely confined to spores of saprophytic fungi or to spores

of parasitic fungi after their release from the host. There are very few studies on spore development; the only one involving a smut fungus appears to be that of Kukkonen and Vaissalo (1964). These authors investigated Anthracoidea aspera which forms its spores intercellularly. In contrast, the present study follows the development of intracellular spores. The formation of intracellular spores in a biotrophic system (i.e. involving living organisms) is an unusual occurrence and does not appear to have been previously investigated with the electron microscope.

Of the investigations which have been carried out on the ultrastructure of fungal spores, relatively few have involved spores with thick walls. This is because the protoplasts are "notoriously difficult to fix for electron microscopy" (Bracker, 1967) except after they have germinated (Allen et al., 1971). The penetration of chemical fixatives and embedding materials may be assisted by breaking (Ekundayo, 1966) or cracking the spores (Lowry & Sussman, 1968). However, a more successful technique for examining the ultrastructure of spores with thick walls appears to be freeze-etching (Moor & Mühlethaler, 1963). Several workers have used this technique to study the ultrastructure of the spores of smut fungi (Laseter et al., 1968; Hess & Weber, 1970; Allen et al., 1971). Chemical fixation followed by thin sectioning has generally been used successfully only for developmental studies of spore walls (Kukkonen & Vaissalo, 1964) or for germinating spores where the permeability of the spore wall probably allows penetration of the fixative (Robb, 1972); the preservation of the cytoplasm is variable for dormant or

mature spores.

Chemical fixation has been suitable in the present work for studying spore development during the early stages. However, as the spore matures and the spore wall thickens it becomes increasingly difficult to discover spores where the contents have been adequately preserved. Because of inadequate preservation and the difficulty in sectioning the dense accumulation of lipid, the entire protoplast may be lost. However, the development of spore wall layers can still be followed because these fix satisfactorily (see also Allen et al., 1971).

During spore initiation and early development in E. casparyana there are a large number of mitochondria present indicating a high level of metabolic activity. The ER is also reasonably well developed. At this stage spore metabolism is probably directed mainly towards general expansion of the protoplast and formation of the wall. By the time the second wall layer is well developed and the third wall layer is about to form, mitochondria and ER can no longer be detected but this may be due to inadequate preservation of spore contents or to the massive build-up of lipid which tends to obscure other features of the protoplast.

Lipid is present and conspicuous from the earliest stages of spore development. Although the concentration of lipid bodies varies during the early expansion of the spore they become the dominant component of the cytoplasm as the spore matures, eventually almost completely filling the spore. Lipid is the major reserve product of the mature spore being a potential source of energy rich materials for the production of membranes during the germination process. During the

development of lipid bodies in the spore there is an important, close association with the ER; in some cases ER almost completely envelopes a lipid body. Exactly what functional importance this has is not known but the ER may be involved in the build-up of lipid material.

Another reserve product, the polysaccharide glycogen, is also present in some spores in its characteristic rosette configuration but it is never conspicuous.

At some early stages of spore development, probably during active cytoplasmic expansion, a large vacuole is often apparent. Vacuoles are not observed once the second and third wall layers begin to form.

Ultrastructural studies of the developing spore confirm the binucleate nature of the young teliospore. This indicates that the development of the spore is analogous to that of other smut fungi. It has not been possible to determine at what stage nuclear fusion occurs because of the density of lipid during later stages of development. Nuclear fusion probably occurs as the spore matures as in most smut fungi (Ainsworth, 1971). Kukkonen and Vaissalo (1964) noted for Anthracoidea aspera that karyogamy occurred when the spore reached about half its final size.

Although there are few studies of ultrastructural changes in the cytoplasm during the development of thick-walled spores (eg. Manocha & Shaw, 1967; Ehrlich & Ehrlich, 1969; Mosse, 1970a) it is generally known from light microscope studies that the cytoplasmic content of the spores gradually diminishes and is replaced by storage material, often lipid. The mature spores of E. casparyana are not unlike those

described for Tilletia caries by Allen et al. (1971). In dormant spores of T. caries there were no organelles that could be positively identified as mitochondria, endoplasmic reticulum was sparse but lipid was abundant. Nuclei, presumably diploid, were present. In contrast, mitochondria were present in the smut spores examined by Kukkonen and Vaissalo (1964) and Khanna and Payak (1971); Khanna and Payak also described vacuoles. These spores may, however, have been at a different stage of development; vacuoles are not present in most resting or dormant spores (see Robb, 1972).

There are a number of studies which contribute information on spore wall structure and development (Kukkonen & Vaissalo, 1964; Williams & Ledingham, 1964; Manocha & Shaw, 1967; Skucas, 1967; Laseter et al., 1968; Ehrlich & Ehrlich, 1969; Mosse, 1970a, b; Allen et al., 1971; Khanna & Payak, 1971; Littlefield & Bracker, 1971; Henderson et al., 1972). Because of the taxonomic importance of spore ornamentation, a knowledge of the development of the ornamentation may be of considerable value in distinguishing species which appear similar with both the light microscope and scanning electron microscope. A knowledge of spore wall structure together with information on its chemical composition may also explain problems of dormancy and survival of fungal spores.

In E. casparyana three distinct wall layers are elaborated. The first layer is the most electron-dense; the second layer, formed to the inside of this and eventually differentiating into two zones, the most electron-transparent; the third wall layer, conferring on the spore the ornamentation typical of the species, develops on the outside of the two

previously formed layers. This sequence of development is difficult to explain and necessitates considerable transport of materials through the two inner wall layers during the formation of the third layer. However, a similar kind of transport does occur through pollen walls but in the reverse direction (Rowley & Flynn, 1971).

Previous studies of thick-walled spores show that there are usually several distinct wall layers. Graham (1960) used various reagents to dissolve or digest portions of the teliospore walls, in conjunction with histochemical methods, to study spores of Tilletia controversa. He demonstrated that the walls of these spores could be differentiated into three distinct layers excluding the sheath; a two-layered endospore largely chitin and hemicelluloses, and a reticulate exospore containing pectic materials, hemicelluloses, proteins, melanin pigments and lipoids. The endospore layers were separated from the exospore by a lipoid band. Earlier investigations (Meiners, 1954) distinguished only a single endospore layer.

Khanna and Payak (1968) have also investigated teliospore morphology with the light microscope. In their work with three species of Neovossia they were also able to distinguish three separate layers excluding the sheath. However, they used a different terminology to describe the layers and did not note a lipoid band.

More recently a number of ultrastructural studies have been carried out. Hess and Weber (1970) have confirmed Graham's (1960) observation that the spore wall of T. controversa is basically three-layered; partition layers were also present. These authors and Allen et al. (1971) described

three distinct wall layers and a partition layer for teliospores of T. caries. This is in contrast to Laseter et al. (1968) who described only two wall layers and a partition layer in T. caries and T. foetida. Khanna and Payak (1971) differentiate only two wall layers in Ustilago nuda and Sphacelotheca reiliana but this may only be a difference in interpretation because they describe the spore ornamentation separately as outgrowths on the surface.

Among other groups of fungi, Skucas (1967) has described a multi-layered wall for the phycomycete Allomyces, Williams and Ledingham (1964) noted three distinct layers in Puccinia graminis f. sp. tritici and Littlefield and Bracker (1971) observed three layers in frozen-etched uredospores of the rust, Melampsora lini, with the spines being distinct structures partially buried in the outermost wall layer. These layers were not readily distinguished in thin section because they stained similarly (see also Manocha & Shaw, 1967). Probably, the most complex spore wall described so far is that of the resting spore of an Endogone sp. (Mosse, 1970b). Six distinct wall layers were observed, one of which sometimes consisted of up to six definite bands. In contrast to E. casparyana, all wall layers in the Endogone spores were formed on the inside of the original spore wall which was continuous with the subtending hypha. This original, outermost wall layer was eventually sloughed off.

With so few studies published so far, it is difficult to predict how far ultrastructural studies of spore wall development can be used to indicate taxonomic relationships. Within the genus Entorrhiza different species show divergent sequences of development and completely different structural

patterns for the spore wall; this has been noted from preliminary observations on E. scirpicola and E. caricicola. Comparable studies in other genera do not appear to have been carried out except at the light microscope level. Khanna and Payak (1968) found for three species of Neovossia quite a close similarity in the disposition of the wall layers.

4.3. Host-Parasite Interface

The ultrastructure of host-parasite interfaces has been comprehensively reviewed by Ehrlich and Ehrlich (1971) and Bushnell (1972) and briefly by Hawker (1965) and Bracker (1967). Most of the work has been done on the Uredinales and Erysiphales. Very little information is available on non-haustoria forming fungi. Akai and co-workers have observed the ultrastructural relationships of host and pathogen in rice diseases caused by Pyricularia oryzae, Helminthosporium oryzae and Cochliobolus miyabeanus (see Ehrlich & Ehrlich, 1971). Hess (1969) studied a Pyrenochaeta infection of onion and Kazama and Fuller (1970) investigated a Pythium infection of Porphyra. However, these diseases were not ones where there was a prolonged intimate contact between host and fungus. Both Hess and Kazama and Fuller reported rapid death of host cells sometimes considerably in advance of the invading organism.

Very few reports have been published on non-haustoria forming fungi that are intimately associated with the living cells of their hosts. The majority of studies have been concerned with the orchid symbiosis (Dörr & Kollman, 1969; Hadley et al., 1971; Nieuwdorp, 1972) but there is one report

on the intracellular hyphae of pathogenic smut fungi (Fullerton, 1970). Ehrlich and Ehrlich (1971 unpublished data) have also made observations on Ustilago maydis in Zea mays seedlings.

It has been shown for highly specialised host-parasite relationships, whether symbiotic or pathogenic, that the fungal component although penetrating the cell wall does not penetrate the host plasmalemma. Instead, the host plasmalemma increases in size and invaginates to surround the developing hypha or haustorium. This is the simplest structural manifestation of the host response to invasion in an intimate host-parasite association and occurs in the orchid mycorrhizas already mentioned. There appears to be only one exception to this so far, the chytrid Olpidium brassicae (Lesemann & Fuchs, 1970) where the fungus is in direct contact with the host cytoplasm. The fungal thallus is surrounded by a single plasmalemma thought to be the fungal plasmalemma.

In addition to the host plasmalemma, the fungus is usually enveloped by an encapsulation, possibly of host and fungus origin, which intervenes between the cell wall of the fungus and the plasmalemma of the host cell. A sheath, probably of host cell wall origin, is also present in some infections either as a collar adjacent to the host cell wall or less frequently completely surrounding the fungus.

In E. casparyana infections of Juncus articulatus the penetrating hyphae are surrounded by a sheath of host cell wall origin. This has variously been referred to as a callosity, lignituber or papilla in light microscope investigations (see Chapt. 2). The sheath lies immediately adjacent to the fungal cell wall i.e. there is no encapsulation zone intervening between fungal wall and sheath. Surrounding the sheath is

the host plasmalemma which has invaginated as the fungus develops. This situation appears to be similar to that described by Fullerton (1970) for a number of smut fungi. However, Fullerton describes progressive deposition of host cell wall material in earlier formed encapsulations around hyphae.

Haustoria-forming parasites differ from the smut fungi described by Fullerton in that sheath formation is usually restricted to areas adjacent to the host cell wall. In the Erysiphales and Peronosporales the sheath (or collar) is probably elaborated by the host cell wall but it may have a different organisation (see Webster, 1970; Ehrlich & Ehrlich, 1971).

Very little is known about the factors governing sheath formation (Ehrlich & Ehrlich, 1971); sometimes they appear to be part of the normal penetration process, at other times they are obviously a defensive reaction on the part of the host. Bushnell (1972) believes that they are probably a nonspecific response to wounding which a successful parasite has to overcome. In the endotrophic mycorrhizas of various orchids, Nieuwdorp (1972) noted that cellulose layers were rarely found around young active hyphae but that a cellulose slime layer continuous with the original plant cell wall enveloped hyphae as the fungus became older and the protoplast disappeared. Sometimes this layer was secondarily thickened with suberin. He envisaged the development of these wall layers as part of the digestion process of the fungus by the host.

In E. casparyana infections sheathing by material apparently of host cell wall origin is noted around hyphae, hyphal coils

and around spores during later stages of infection. Hyphae within such sheaths are sometimes disintegrating so that this may be a defense reaction on the part of the host. On the other hand, degeneration of the protoplast may just be part of the normal ageing process of the hypha. The sheathing material associated with penetrating hyphae is structurally slightly different in appearance from that enveloping hyphae during later stages of development of the fungus; it may be that, functionally, it is also slightly different.

Sheathing material around spores resembles that around older hyphae. It is usually less electron dense than the outer spore wall. Because it overlays the ornamentation of the spore and is presumably a permanent feature of the spore after its dispersal, it may provide a basic source of error or confusion in taxonomic studies.

During most stages of invasion in E. casparyana infections, hyphae and spores are surrounded only by invaginations of the host plasmalemma. No encapsulation is present and the host plasmalemma is closely apposed to the fungal cell wall. This close contact is eventually lost as the spores mature. A similar situation occurs in orchid symbiosis (Dörr & Kollman, 1969; Hadley et al., 1971; Nieuwdorp, 1972). Here, too, the host plasmalemma follows the contours of the fungal endophyte. Hadley et al. showed that, where spaces occurred between the fungal cell wall and the host plasmalemma, these were probably artefacts of fixation because they did not occur in frozen-etched material.

There are very few pathogenic fungi where there is no intervening zone between the host plasmalemma and the fungus. An exception appears to be Plasmodiophora brassicae, the club-

root fungus, where a modified plasmalemma is closely adpressed to the plasmodial membrane (Williams & McNabola, 1967, 1970).

Thus there appear to be various kinds of contact between fungus and host in highly specialised host-parasite systems, almost exclusively characterised by the inability of the fungus to invade the host plasmalemma. In most cases, whether involving pathogenic or symbiotic fungi, transport between fungus and host must occur across the fungus plasmalemma, fungus cell wall and host plasmalemma. A close study of the membranes involved will show whether they are specially adapted for their particular role in the association. With E. casparyana there must be a two-way transport of materials, the fungus obtaining nutrients from the host and then secreting by-products of metabolism in addition to the growth-promoting substances responsible for growth of the gall.

5. SUMMARY

1. The parasitic hyphae have ultrastructural features in common with other fungi; they are basically eukaryotic and lack a Golgi apparatus.

2. Parasitic hyphae and developing spores are binucleate (dikaryophase) and Entorrhiza is therefore comparable to most other smut fungi.

3. The E.M. confirms light microscope observations that hyphal coils are widespread in the host cell.

4. Developing spores are metabolically active; a large number of mitochondria are present and the ER is reasonably well developed.

5. During later stages of spore development there is a massive accumulation of lipid, and mitochondria and ER are no longer detected.

6. There are 3 distinct wall layers in mature spores of E. casparyana. The middle one of these is the first formed and appears to be an extension of the hyphal wall; a second electron-transparent layer develops on the inside and a third layer, conferring on the spore the ornamentation typical of the species, develops on the outside. E. casparyana thus resembles other thick-walled resting spores in having a multilayered wall.

7. The host-parasite interface in E. casparyana infections is ultrastructurally simple. During most stages of invasion hyphae and spores are surrounded only by invaginations of the host plasmalemma. No encapsulation is present and the host plasmalemma is closely apposed to the fungal cell wall. This close contact is lost as the spores mature. The intimate host-parasite contact and lack of disruption of host cell contents during most of the development of the fungus indicates a highly specialised host-parasite relationship.

8. The EM confirms light microscope observations on the presence of a sheath apparently of host cell wall origin surrounding hyphae, hyphal coils and older spores. The sheath lies immediately adjacent to the fungal cell wall and is surrounded by the host plasmalemma. Sheath formation may be part of the normal infection process or a defense reaction of the host.

9. Sheathing material around spores overlays the characteristic ornamentation of the spore; as it is presumably a permanent feature of the spore after its dispersal it may provide confusion in taxonomic studies.

CHAPTER 4

INFECTION STUDIES WITH *ENTORRHIZA CASPARYANA*

1. INTRODUCTION

Very few smut fungi infect subterranean portions of plants, particularly the roots, and there are correspondingly few accounts in the literature of inoculation experiments involving these fungi. Preliminary experiments have, however, been reported by Mitra (1928) and Mundkur (1938) for *Urocystis coralloides* on the roots of *Brassica* spp. Weber (1884) had no success with infection studies involving *Entorrhiza* on *Juncus bufonius* although he used soils in which the plants normally grew and varying degrees of moisture. No other infection studies with *Entorrhiza* have been reported.

The work presented here was carried out chiefly to determine the effect of infection on the growth of the host plant. As there had been no previous reports involving successful inoculation of *Entorrhiza*, the first objective was to obtain infected plants. In an attempt to ensure infection, conditions similar to those operating in nature were employed. Soil water from an infected site was used as inoculum and plants were grown in both well-drained and waterlogged soil.

Because the experiment was carried out in asterile conditions the effects on growth were more likely to resemble those occurring in nature. Infection studies employing pure cultures in sterile soil may fulfil Koch's postulates but they provide an artificial situation because they remove one important variable from the host-parasite complex; this is the interaction with other soil micro-organisms. These

micro-organisms may produce substances stimulatory or antagonistic to germination, growth or infection so that the picture obtained from a sterile environment may not depict what occurs in nature. This is particularly important when considering root-infecting organisms because each organism in the soil is in balance with the total soil population and especially, in the root zone, with the rhizosphere microflora (see Saksena, 1969). With air-borne diseases interactions between organisms on the surface of the aerial parts of plants must also be considered but these interactions are not so complex as those occurring in the soil because fewer organisms are involved in the more specialised phyllosphere environment.

The experiment was of an exploratory nature and it was expected that only relatively major effects on growth would be detected. The results from such an experiment would then form the basis for future analyses on the growth of the host plant.

During the course of the experiment observations on the development of infection were carried out.

2. MATERIALS AND METHODS

Plants of Juncus articulatus growing in wet river silt at a single site on the Waimakariri riverbed were collected on 15 Dec. 1971. Several clones were sampled to provide sufficient material for the experiment; these clones were adjacent and their margins were not well-defined. Plants examined earlier from this site had shown no sign of infection.

The plants were washed free of silt and divided into

vegetative rooting portions approximately 12-15 cm. in height and bearing one main shoot. At the same time, these plants were confirmed to be free of galls. 9 cm plastic pots were filled with potting soil which had been electrically pasteurised at a temperature of 82.5°C about 6 months previously. The soil was well-watered and one rooting portion of Juncus articulatus was established in each of 100 pots. The pots were set up randomly in 2 blocks of 50 on a bench in a glasshouse.

One block of 50 plants was inoculated, the second block remained uninoculated as a control. The inoculum was a soil-water suspension obtained from the margin of the water where heavily infected plants of Juncus articulatus were growing at the Groynes, at the source of the south branch of the Waimakariri River. The suspension was obtained by allowing water to flow into hollows and mix with soil under uprooted Juncus plants. Spores of Entorrhiza were present in this suspension. Inoculation was effected by pouring ca. 20 ml of the suspension on to the soil surface in each of the 50 pots. This was repeated with freshly collected soil-water suspensions on two subsequent occasions in an attempt to ensure infection. These suspensions were applied 1 week and 4 weeks, respectively, after the initial inoculation. To minimise the chance of cross-infection the inoculated pots were separated on the bench from the uninoculated controls by a distance of approximately 2 metres and a small break in the bench-top sufficient to allow free water to drain away.

The 50 inoculated and 50 control pots were further divided into 2 blocks of 25 to be subjected to different soil drainage treatments (Fig. 41). One block of 25 pots was

placed directly on the glass-house bench so that water drained away freely; the second block of 25 pots was placed in sterilised metal trays which were filled with water to a depth of about 5 cm or two-thirds the pot height. This water was overflowed regularly during routine glasshouse watering. This treatment was an attempt to simulate the type of habitat which Juncus articulatus often occupies. All pots were watered uniformly with tap water (artesian).

Two inoculated plants, one from each water treatment, were examined closely for signs of infection a) $3\frac{1}{2}$ weeks after soil inoculation and b) 7 weeks after inoculation.

During the period 21 March to 10 April, 1972 (i.e. 3 to $3\frac{1}{2}$ months after inoculation), after it had been established that infection had taken place, 10 plants from each set of 25 were randomly selected for analysis. The plants were analysed as follows. Shoots (including rhizomes), roots and galls (where present) were separated for dry weight determinations. The dry weight was determined after 3 days at ca 40°C followed by 24 hours at 105°C. Shoot number and gall number were also recorded. The root and gall fractions were separated from the loam soil by processes of wet sieving and flotation. Material was collected on a 1 mm sieve and subsequently on a 595 micron sieve. The smallest galls appeared to be retained on the fine sieve partly due to surface tension. The distribution of galls in the rooting zone was also determined. In the pots where there had been free drainage of water, the top 3.5 cm of soil was analysed separately from the bottom 3.5 cm ; in those pots which had been standing in water an attempt was made to relate the distribution of galls to the water level by separately analysing the portions of the root

Fig. 41 Control plants immediately before first dry weight analysis. Note experimental layout: plants on left in well-drained soil, plants on right in waterlogged soil.

system above and below that level.

Of the remaining plants, 10 from each set were randomly selected for repotting into 15 cm pots on 17 March. These plants were to be available for analysis at a later date.

3. RESULTS

Standard deviations were calculated for all means and the significance of differences between the means was assessed by applying t tests (Sokal & Rohlf, 1969).

3.1. Efficacy of Soil Water from an Infected Site as Inoculum

The results are summarised in Table 4.1.

TABLE 4.1. Efficacy of soil water as an inoculum.

	Well-drained soil		Waterlogged soil	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Percentage of plants infected	50	90	30	100
†Wt. of galls per plant (mgms)	3.16 (0 - 44)	68.45** (0 - 1154)	1.14 (0 - 1.5)	271.2*** (54 - 671)
†No. of galls per plant	4.92 (0 - 160)	107** (0 - 2546)	1.32 (0 - 3)	336.9*** (48 - 1420)

10 plants per treatment
† means based on logarithmic transformation of data (Sokal & Rohlf, 1969)
** $p < 0.05$
*** $p < 0.01$

A comparatively large percentage (40%) of the controls became infected. However, the percentage of plants which became infected in inoculated soil was considerably higher (95%). A more detailed comparison of the controls with inoculated pots, based on the mean weight of galls and the mean number of galls per plant, showed that the degree of infection in inoculated soil was, in fact, significantly higher than in uninoculated soil under both conditions of soil drainage tested. The range of infection of plants in a well-drained soil is illustrated in Fig. 42.

These results therefore show clearly that the soil - water suspension used as an inoculum was effective in bringing about infection of Juncus articulatus.

3.2. The Effect of Soil Drainage on Infection

3.2.1. The effect of soil drainage on the degree of infection.

The results are summarised in Table 4.2.

TABLE 4.2. Effect of drainage on degree of infection

	Well-drained soil	Waterlogged soil
†No. of galls per plant	107 (0 - 2546)	336.9 (48 - 1420)
†Wt. of galls per plant (mgms)	68.45 (0 - 1154)	271.2 (54 - 671)
††Wt. of galls as % of total plant wt.	1.54 (0 - 9.61)	2.57 (0.52 - 5.71)
††Wt. of galls as % of root system wt.	3.51 (0 - 18.46)	7.00 (2.06 - 18.27)

10 plants per treatment
† means based on logarithmic transformation of data
†† means based on arcsine transformation of data (Sokal & Rohlf, 1969).

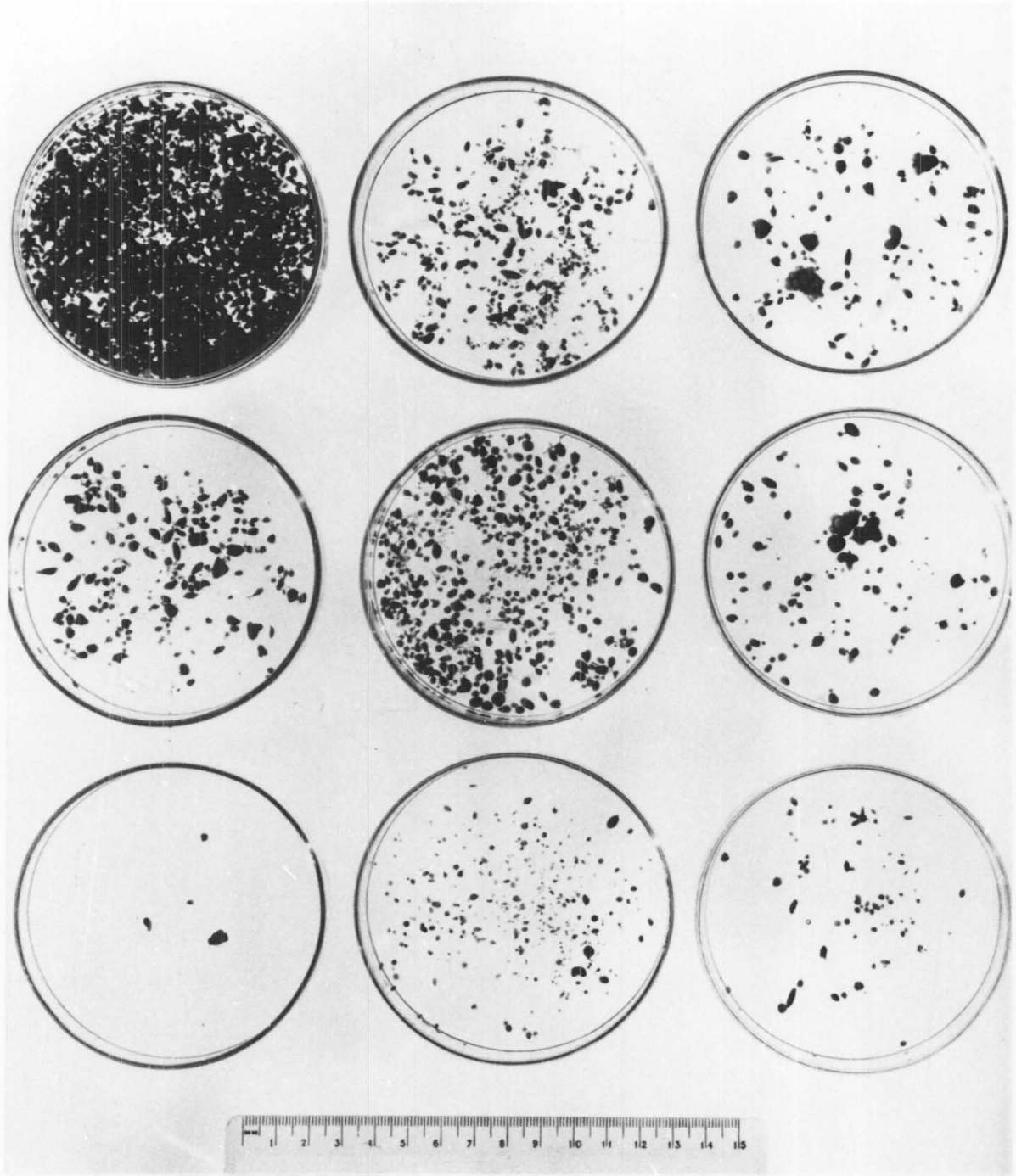


Fig. 42 Range of infection of plants in well-drained soil 3 - $3\frac{1}{2}$ months after soil inoculation: one petri dish per plant (10th plant uninfected).

From the table it is evident that the mean infection was consistently higher in water-logged soil than in well-drained soil. However, in all cases, the differences in the degree of infection between water-logged and well-drained soil were not statistically significant ($p > 0.1$).

3.2.2. The effect of soil drainage on the distribution of infection.

The results are summarised in Table 4.3.

TABLE 4.3. Effect of drainage on distribution of infection

	Well-drained soil		Waterlogged soil	
	Top 3.5 cm	Bottom 3.5 cm	Above water-level (Top 2 cm)	Below water-level (Bottom 5 cm)
†No. of galls per plant	33.66 (0 - 1282)	73.09 (0 - 1264)	317 (40 - 1388)	15.36*** (2 - 49)
†Wt. of galls per plant (mgms)	Not separately assessed		245.40 (42 - 630)	16.23*** (0.1 - 112)

10 plants per treatment

† means based on logarithmic transformation of data

*** $p < 0.01$

In well-drained soil there was a greater concentration of galls in the basal half of the pot. However, the difference in numbers between the top and bottom halves of the pot was not statistically significant ($p > 0.1$).

In contrast, in waterlogged soil the concentration of galls (in terms of both number and weight) above the water level in the top 2 cm of the pot was very significantly higher

than the concentration below water level (Fig. 43). It was also observed that within the top 2 cm the galls were further concentrated in a narrow layer extending no more than 0.5 - 1.0 cm below the soil surface. Many galls in fact protruded above the level of the soil (Fig. 44). This did not occur in well-drained soil. Galls in the bottom 5 cm of soil were almost entirely distributed around the margins of the pot. They appeared to be morphologically similar in size and shape to those appearing above the water level.

3.3. The Effect of Infection on Host Growth

The results are summarised in Table 4.4.

From the table it can be seen that infection increased the total dry weight of the plant under both environmental conditions tested. However, this increase was not statistically significant ($p > 0.1$). The increase reflected an increase in the dry weight of both the shoot and the root system. However, again these increases were not statistically significant. Figs. 45 and 46 illustrate plants at the time of analysis.

The ratio of shoot to root system was not altered by infection and therefore it appeared that the increased growth resulting in the production of galls might have been counteracted by a decrease in root elongation or production of new roots. However, in this experiment this hypothesis cannot be proven because although the ratio of shoot : root (excluding galls) was higher in infected plants under both environmental conditions, the differences in both were not statistically significant.

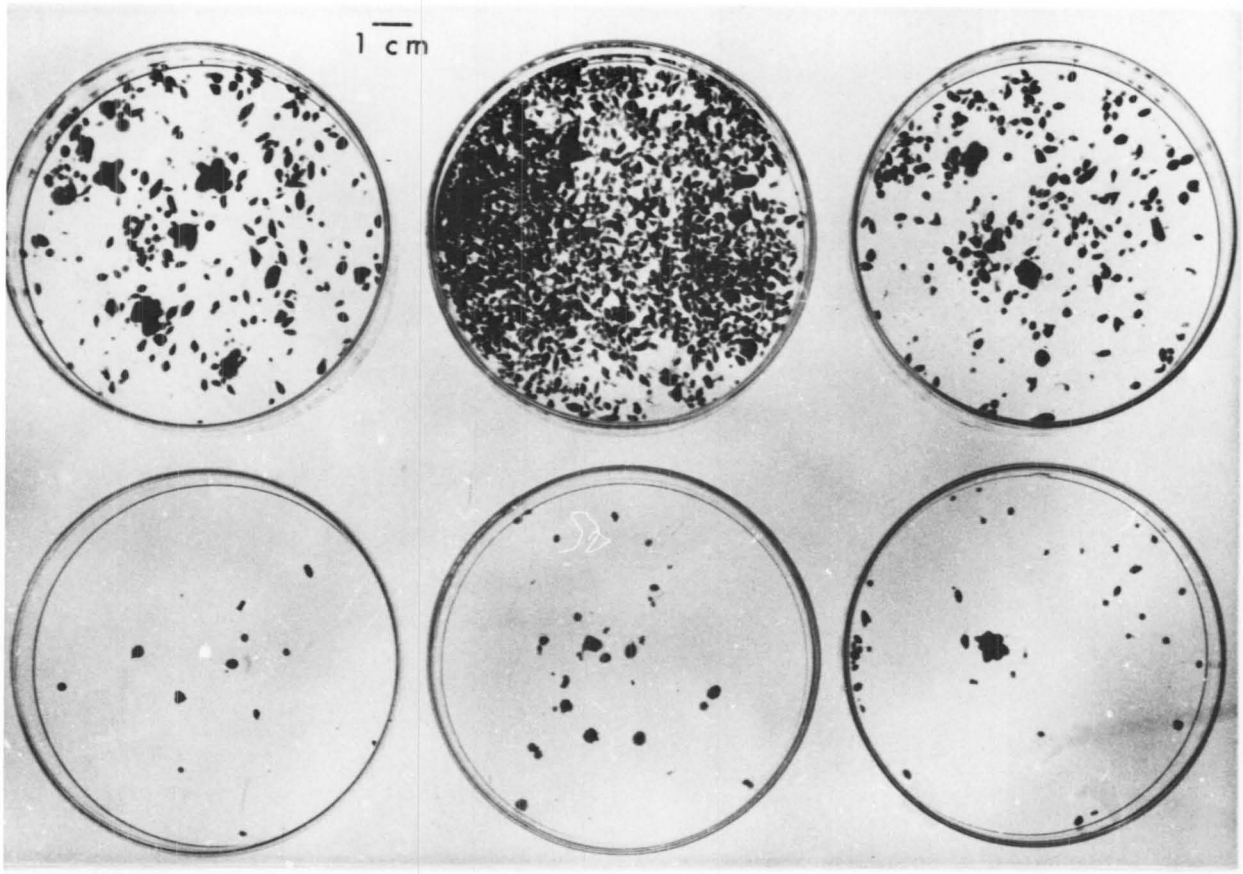


Fig. 43 Infection of 3 plants in waterlogged soil; each vertical petri dish pair shows galls from one plant. Petri dishes in the top row show the number of galls above water level; petri dishes in the bottom row show the number of galls below water level.

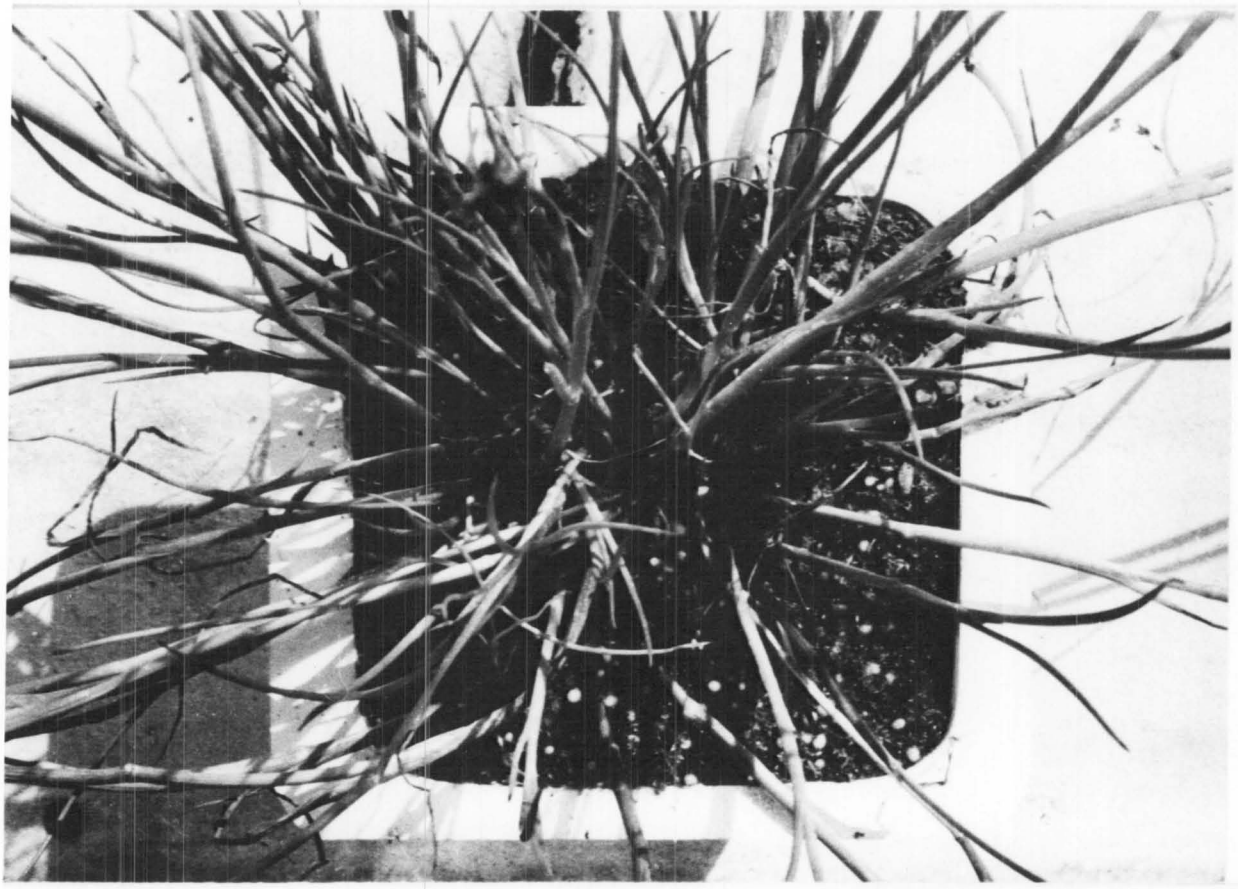


Fig. 44 Plant growing in inoculated, waterlogged soil for 3 - $3\frac{1}{2}$ months; note galls at surface of soil.

TABLE 4.4. Effect of infection on host growth (3-3½ months after soil inoculation).

	Plants in well-drained soil		Plants in waterlogged soil	
	Healthy (mean of 5 plants)	Infected (mean of 9 plants)	Healthy (mean of 7 plants)	Infected (mean of 10 plants
Total dry wt.(gms)	7.59 ± 1.51	8.58 ± 2.46	12.13 ± 2.63	13.71 ± 2.77
Shoot dry wt. (gms)	4.48 ± 1.05	4.86 ± 1.04	8.12 ± 1.71	9.00 ± 1.49
Root system dry wt. (gms)	3.12 ± 1.35	3.72 ± 1.61	4.01 ± 1.07	4.71 ± 1.81
Root dry wt.(exc.galls)	3.12 ± 1.35	3.48 ± 1.38	4.01 ± 1.07	4.35 ± 1.73
Shoot:root system ratio	1.44 ± 0.27	1.46 ± 0.41	2.07 ± 0.36	2.06 ± 0.66
Shoot:root (exc.galls) ratio	1.44 ± 0.27	1.53 ± 0.40	2.07 ± 0.36	2.27 ± 0.64
Shoot no.	69.0 ± 17.5	64.3 ± 24.3	55.0 ± 10.1	71.8 ± 28.4

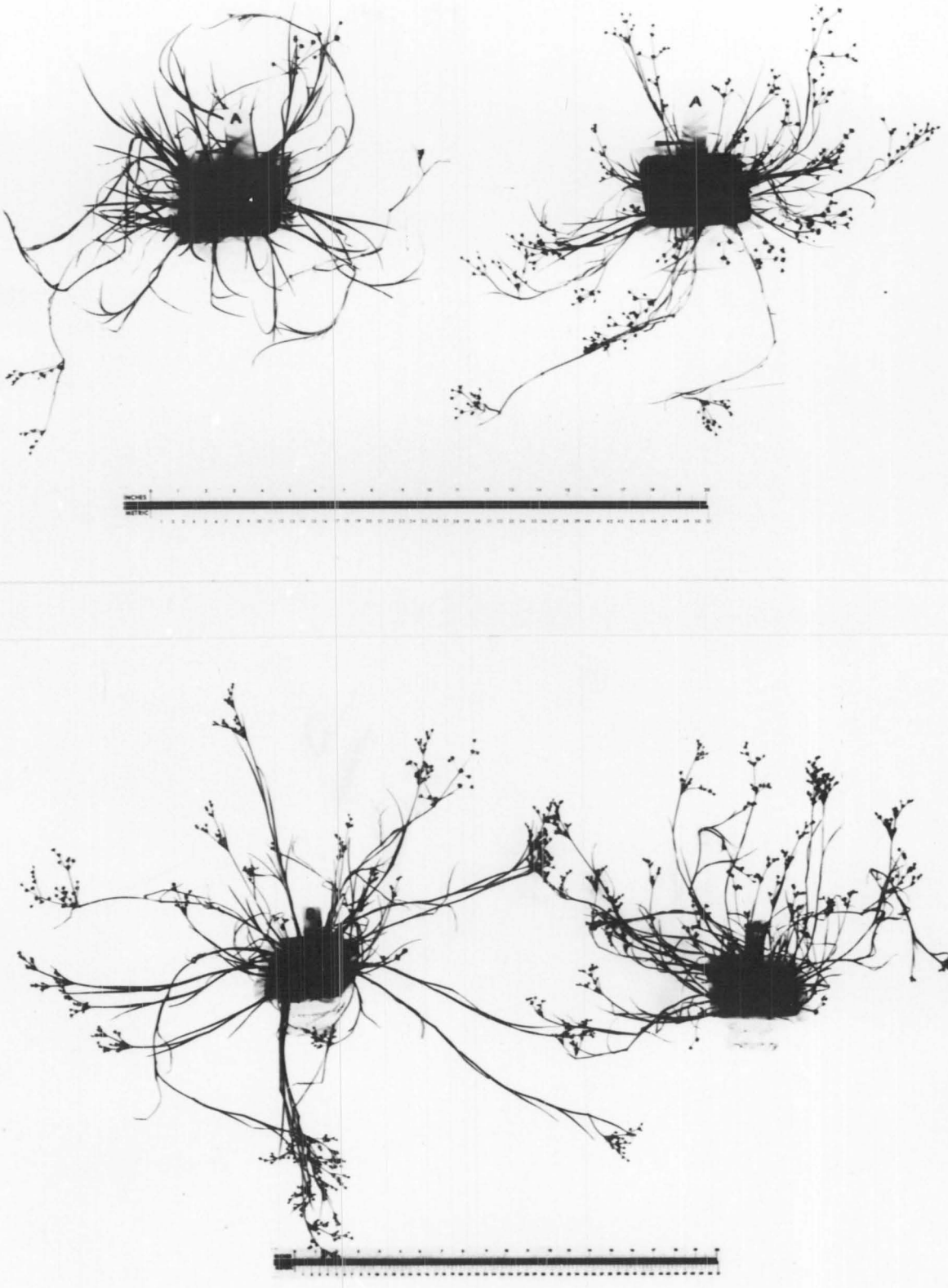
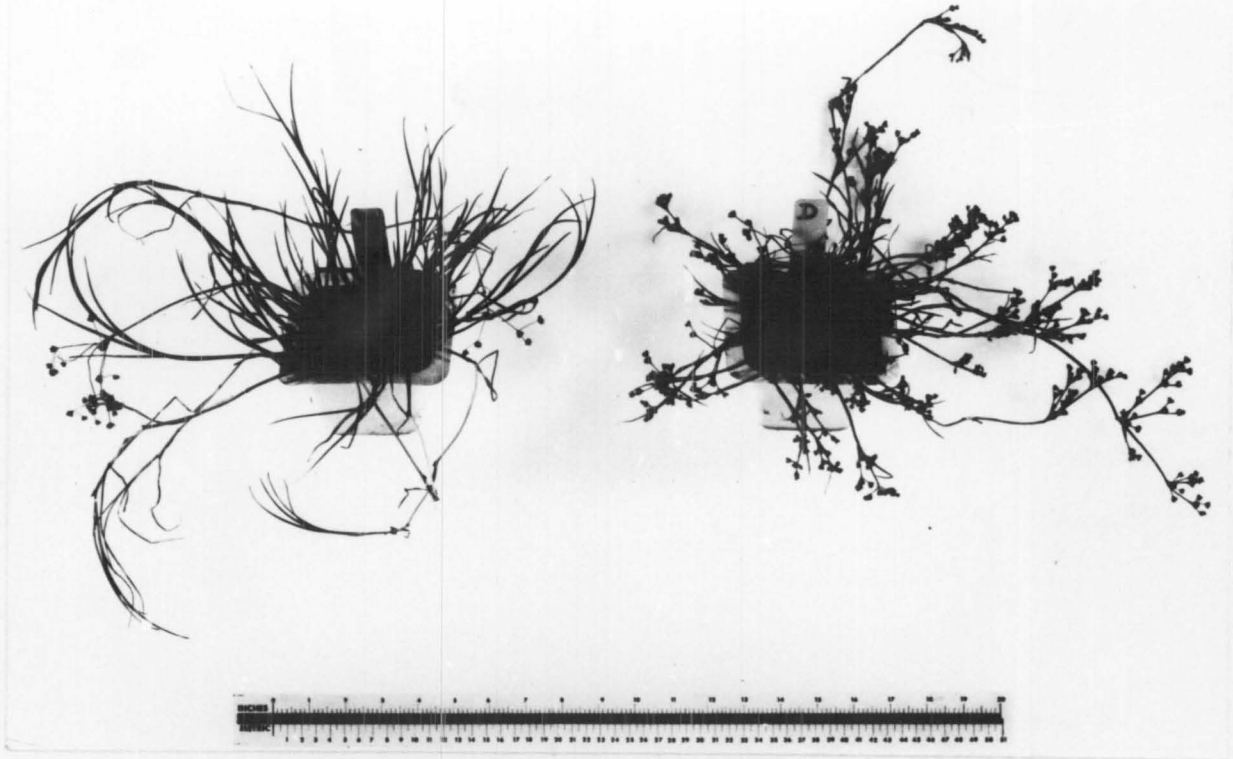


Fig. 45 Size of control plants at the time of the first dry weight analysis:
A - well-drained soil
B - waterlogged soil



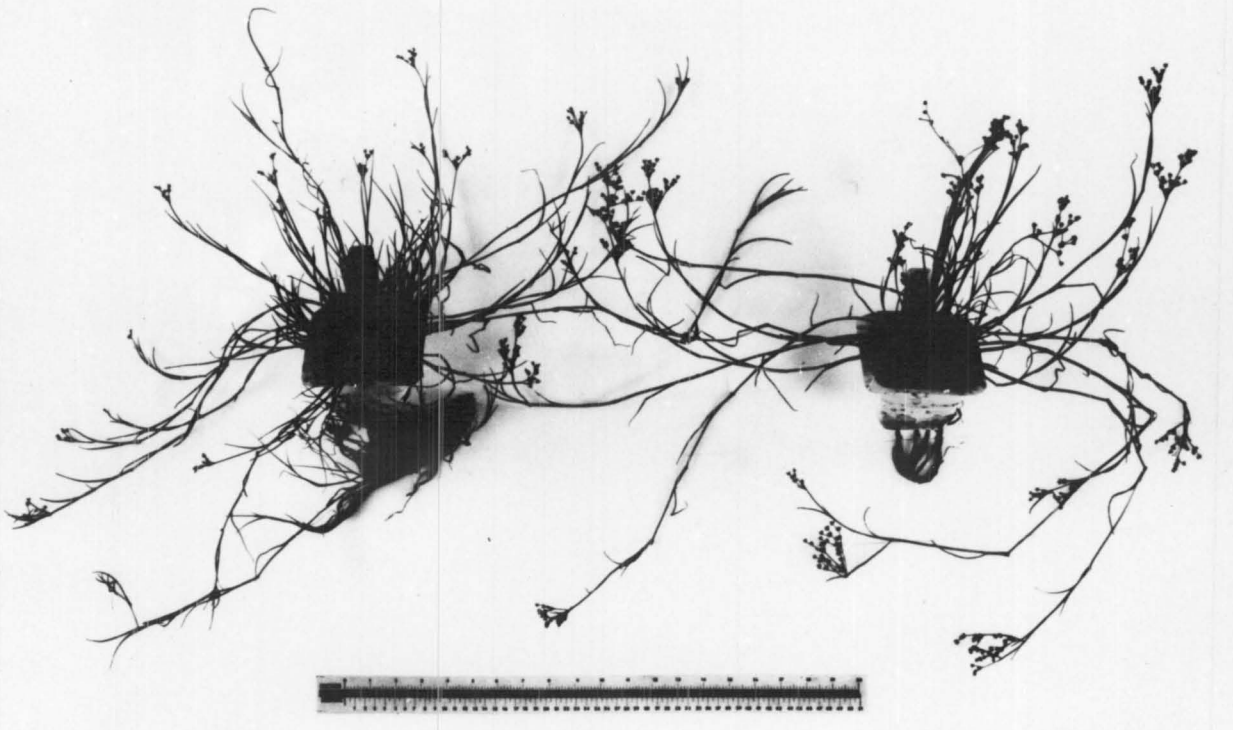


Fig. 46 Size of plants in inoculated soil at the time of the first dry weight analysis:
D - well-drained soil
C - waterlogged soil

The number of shoots produced was not significantly different in infected and healthy plants.

3.4. Development of Infection

During the course of this experiment some general observations on the development of the infection were made.

After $3\frac{1}{2}$ weeks and 7 weeks from the time of setting up the experiment, two inoculated pots, one from each drainage treatment, were washed out and carefully examined to discover if plants had become infected. No galls were evident and a microscopic examination of approximately 10 root tips per pot indicated no sign of infection; it would, however, be extremely difficult to detect infection, on this scale, prior to swelling. Occasional spores of Entorrhiza casparyana were noted in the soil.

In plants growing in water-logged soil there were no obvious signs of galls at the soil surface $8\frac{1}{2}$-9 weeks after inoculation. However, in these pots, galls were evident above the surface of the soil $10\frac{1}{2}$ weeks after inoculation. These observations and the prevalence of young galls when plants were analysed after 13-15 weeks indicates that the majority of galls probably formed about 8-10 weeks after soil inoculation.

At the time of the first dry weight analysis, the maximum amount of infection recorded per plant was 2546 galls weighing 1.2 grams (Fig. 42). This represented 18.5% of the dry weight of the root system and 9.6% of the total dry weight of the plant. The plant had been growing in a well-drained soil.

After a further 5 months, two inoculated plants from each drainage treatment were selected for a dry weight analysis

of the shoot, root and gall fractions. It was not possible to examine a greater number of plants because of the difficulties of sampling (see 4.3 addendum). The maximum amount of infection recorded per plant was 4267 galls weighing 14.3 grams (Fig. 47). This represented 54.9% of the dry weight of the root system and 29.7% of the total dry weight of the plant. The plant had been growing in a waterlogged soil.

The greater number of galls recorded in this second analysis may indicate an increase in gall number during the 5 months since the first analysis. However, it is also possible that the number of galls might reflect differences in spore densities of the original inocula. Nevertheless, there is a real increase in the weight of galls. This is shown by the fact that at the first analysis 2546 galls weighed 1.2 grams whereas at the second analysis 4267 galls weighed almost twelve times this amount, 14.3 grams.

The increase in the weight of galls after 5 months did not appear to be at the expense of either the uninfected root mass or the growth of the shoot; it appeared to be a localised increase which increased the total dry weight of the plant proportionally. However, because of the limited number of plants sampled (eight including controls) and the variability in plant weights at this stage, the results cannot be assessed critically.

At the time of the second dry weight analysis the plants had been growing in inoculated soil for approximately 8 months. Many of the larger galls, in contrast to those examined after 3 months, had begun to disintegrate (Fig. 48). It therefore seems probable that there might be some increase in the number

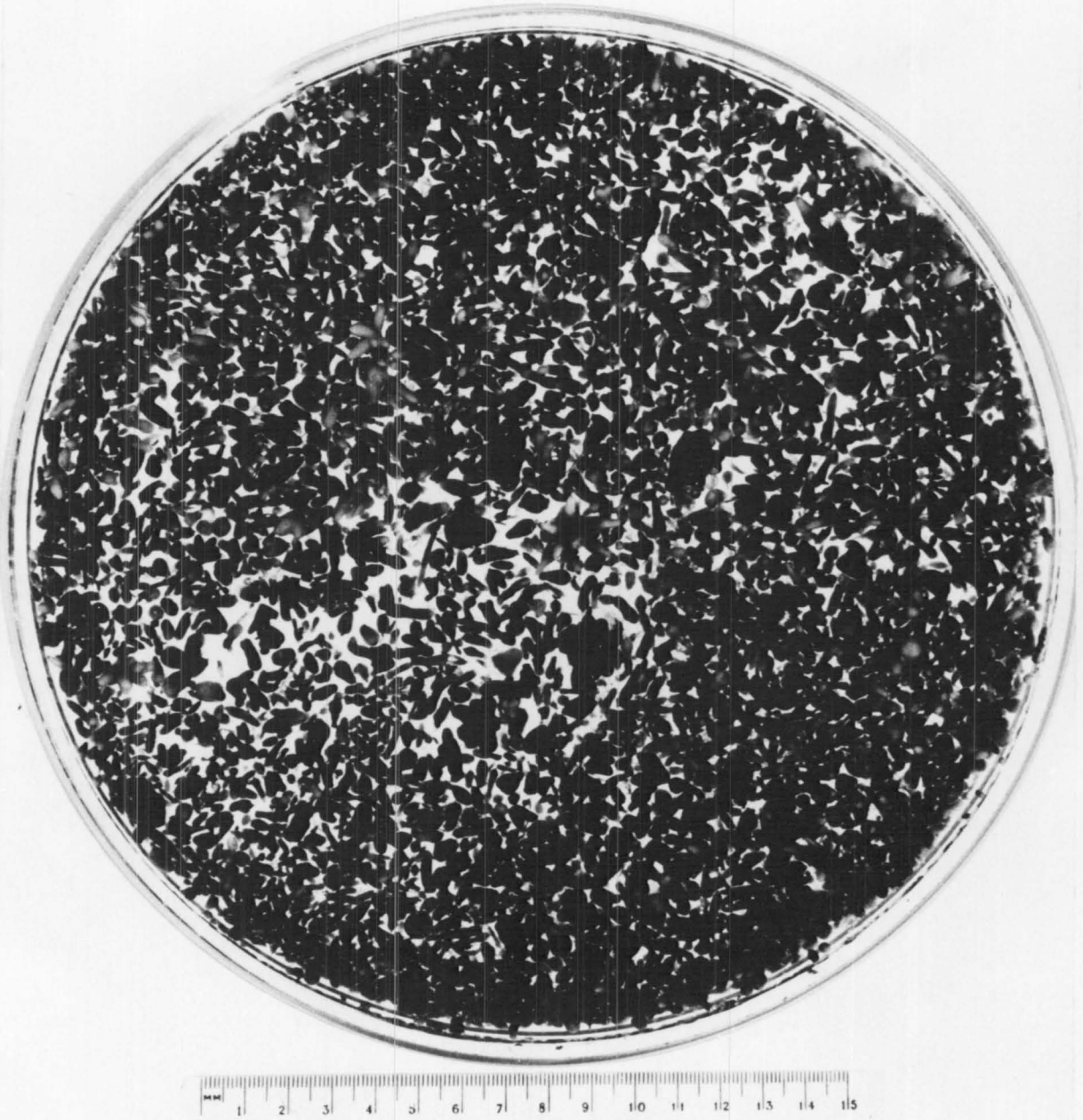


Fig. 47 Largest number of galls recorded from one plant $8\frac{1}{2}$ months after soil inoculation. The plant had been growing in waterlogged soil.

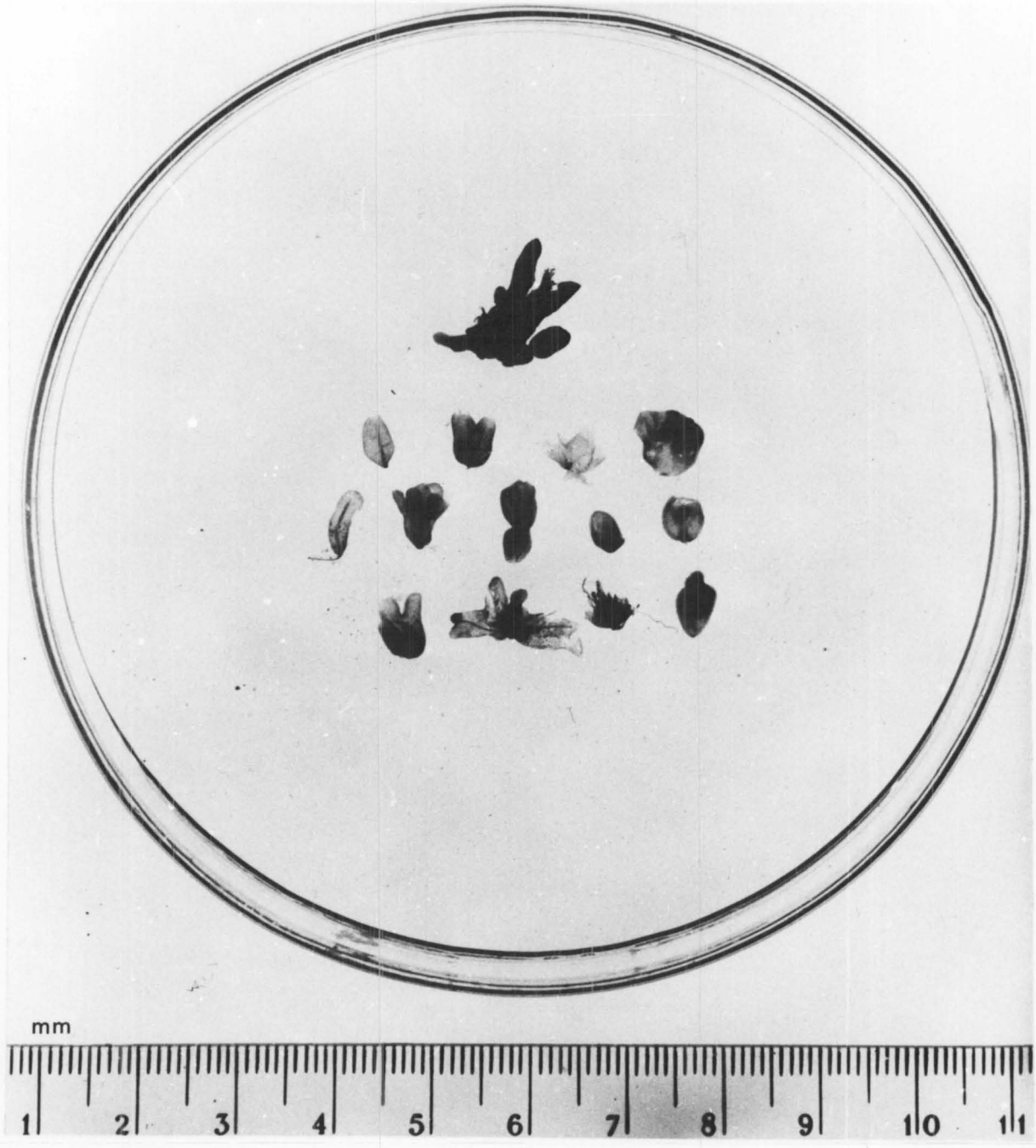


Fig. 48 Disintegrating galls present on plants $8\frac{1}{2}$ months after soil inoculation. Note that in many galls only the vascular strand and outer layers of the gall remain.

of galls as a result of a secondary cycle of infection. Some of the increase is probably also due to different lengths of dormancy of spores in the original inoculum because at the time of the first analysis there was a complete range in the size and stage of development of galls despite the fact that disintegrating galls were not observed.

4. DISCUSSION

4.1. Efficacy of Soil Water from an Infected Site as Inoculum

The inoculum contained spores of Entorrhiza casparyana similar to those found in galls of infected plants of Juncus articulatus in nature and to those found in galls of infected plants in this experiment. However, no attempt was made to free these spores from the suspension and use a pure surface sterilised spore inoculum. Consequently, various other micro-organisms were introduced into the pots in the suspension. Therefore, although it can be deduced that Entorrhiza casparyana is able to successfully infect Juncus articulatus under experimental conditions and this is always associated with typical gall formation, it is not possible to state whether this would occur in pure culture. This experiment does not therefore prove conclusively that E. casparyana is the only agent of infection and gall formation.

This experiment has shown that spores present in soil and particularly in soil water will effectively bring about infection producing in one instance at least 2456 galls per plant within 3-3½ months after inoculation. Running water

is probably in fact one of the main agents of spore dispersal accounting for the widespread infection of plants alongside ditches, streams and lakes and in seepage areas. Furthermore, the experiment has shown that Entorrhiza will infect established plants. Many smut fungi infect only at the seedling stage of growth eg. Urocystis cepulae and Tilletia caries. U. cepulae attacks onion seedlings only during the first 2-3 weeks after germination; after this the cotyledon becomes resistant and provides a barrier to infection (Wheeler, 1969). For T. caries, Sartoris (1924) reported that "infection takes place at the time of seed germination and diminishes rapidly after the 6th day".

The lack of plant infection in one inoculated pot and the infection of several plants in uninoculated pots requires an explanation. The absence of infection of one plant in well-drained soil was probably due to a low spore density in that pot. It seems unlikely that spores would be completely absent after 3 successive inoculations but it is possible that only a low number of spores was present. Although the soil water suspensions were obtained from a heavily infected area they were not pooled and pots were inoculated from separately collected suspensions in which spore densities were not individually checked. The spore density was therefore unknown for each inoculum. A certain minimum spore density may be necessary to ensure infection. A high "inoculum potential" for the infective agent is often required for successful infection in soil - borne diseases (Macfarlane & Last, 1959; Saksena, 1969). Fischer and Holton (1957) stress that the spore load at the infection court is a significant factor in the incidence of smut diseases. In some cases the inoculum potential may

be achieved by synergism between germinating spores eg. Tilletia caries (Heald, 1921) but this is unlikely to be the explanation for spores which are dispersed singly. In such cases a high inoculum density is probably necessary because few spores reach the infection site under suitable environmental conditions; those that do should be capable of independent infection (Garrett, 1970). In the present experiment the wide range in degree of infection (expressed in terms of the number of galls per plant) probably directly reflects the variation in spore densities from one pot to the next.

Infection of control plants was unexpectedly high, especially for plants growing in well-drained soil. There are a number of possible explanations. Spores of Entorrhiza casparyana may have been attached to roots of Juncus plants used in this experiment. Although the plants were not infected, spores may have been present in the soil but infection suppressed because of some environmental factor. The silty soil in which the plants were growing may have been unfavourable for spore germination. Secondly, there may have been a low spore density in the loam potting soil used in the experiment. This soil had been pasteurised but may have either contained spores which resisted the pasteurisation process or become contaminated during the period before its use. Spores may also have been present in the tap water used for watering the pots. The extent of spores in natural environments is not known. Finally, contamination from inoculated pots must be considered. It seems unlikely that this would have been responsible for the infection of controls. Not only were uninoculated pots separated from inoculated pots by a distance

of 2 metres but there was also a break in the bench top through which any free water possibly containing spores could drain. Furthermore, this would not explain contamination of pots standing in sterilised metal trays. However, cross-infection as a result of water splash, wind or insect transmission (spores soil - borne on the bodies or present in the gut), although unlikely, cannot be completely discounted.

4.2. The Effect of Soil Drainage on Infection

Very little can be concluded from the present experiment regarding the effect of soil drainage on the degree of infection. The tendency for there to be a higher level of infection in water-logged soil may be a real feature of Entorrhiza casparyana - Juncus articulatus infections but the variability in the plant material and in the spore densities of the inocula means that no decision can be reached in this respect.

Various authors in the past have attempted to correlate the degree of infection with soil moisture. Lagerheim (1888) believed that Entorrhiza infections of Juncus articulatus developed best in sandy soils that were not too wet. He did not find infection in plants growing in silty or very wet soils. Liro (1938) commented for Entorrhiza infections of Juncus alpinus that the galls were smaller and less branched in moister localities to the extent that galls present on plants growing in water were scarcely branched at all. Results from the present experiment do not support this observation.

Many collections of Entorrhiza have been made from areas where there was abundant free water eg. Entorrhiza on J.articulatus (Schwartz, 1910) near springs in a marsh, E. scirpicola

in springs in a rather sandy area (Correns, 1897), E. raunkiaeriana in submerged roots of Scirpus fluitans in a dune-lake and E. caricicola on Carex limosa in a bog (Ferdinandsen & Winge, 1914). However, in the absence of direct comparisons with the degree of infection in plants of the same species growing in drier areas, these latter observations reveal very little about the effect of soil moisture on infection. There is probably a greater chance of finding infection in wetter areas because these are the preferred habitats of the majority of the host species.

The observations from the present experiment on the effects of soil drainage on the distribution of infection are more conclusive. Where drainage was impeded numbers of galls above the water level were significantly higher than those below water level. Where drainage was unimpeded galls were relatively evenly distributed throughout the soil.

How does soil water affect the distribution of infection? There are several possibilities. The effects may be directly on the spore, directly on the host or a combined effect on the host-parasite complex. Water may affect the spore either by its influence on germination or by physical effects on spore movement. Many fungal spores will not germinate when submerged due to the low oxygen levels. However, some galls appear below water level even at the base of the pot so that insufficient aeration cannot be the controlling factor here. Moreover, separate observations on factors affecting the germination of Entorrhiza casparyana spores (see Chapt. 5) show that germination of submerged spores occurs regularly. This corresponds with the general observation that plants with submerged roots will often become infected in nature. Spores also require

adequate moisture (either free water or a high relative humidity) for germination. Soil moisture appears to have been sufficient for germination not only where drainage was impeded but also where it was unimpeded because in these pots galls developed throughout the soil and numbers reached a maximum.

The distribution of infection within a pot is most likely to have been dependent on the movement of water through the soil carrying spores with it. It must be recalled that the inoculum was applied to the surface of the soil. In pots having free drainage of water, galls were distributed throughout the soil with a tendency for greater numbers in the basal half of the pot (although this was not statistically significant). Galls were often concentrated around drainage holes and in the root mat at the bases of pots. This distribution would appear to be a direct consequence of the movement of water through the soil.

In pots where the soil was constantly water-logged there would be almost no downward movement of water. In fact, the movement of water would be largely upwards as a result of evaporation from the surface layers of the soil. For this reason, there would be very little dispersion of spores even in the soil above water level. The few galls present below water level were largely around the margins of pots where spore movement may have occurred more readily. The distribution of infection might also have been influenced indirectly by the effect of water level on the development of the root system. However, no signs of altered root growth under water-logged conditions were observed. Alternatively, the water level may have influenced the growth of galls. However, there seems little evidence for this.

Previously, very few observations have been made concerning the distribution of infection in the soil or on the root system. Lagerheim (1888) noted for Entorrhiza on J. articulatus that galls were present only in the upper layers of the soil and Schwartz (1910) stated that in some cases he found galls just beneath the soil under a depth of about 1 inch of water. It would appear that galls develop equally well submerged or above the water level and that the major controlling factors are those governing spore distribution. This is borne out by an observation from the present experiment. Many plants growing in water-logged soil produced rhizomes which had rooted in the surrounding water. Except for one isolated instance, galls were conspicuously absent on these roots. This was almost certainly due to the low spore density in the large volume of water in the tray in which the plants were standing.

Although the effect of the nutrient status of the soil on infection was not studied in the present experiment some authors have attributed a high degree of infection to the presence of abundant nutrients in the soil. Schwartz (1910) believed that a rich alluvial soil containing iron salts favoured infection and Brefeld (1912) thought that heavy infection of Juncus bufonius was due to the influence of nutrients from manure applied to the soil. He postulated that the nutrients affected the growth of a saprophytic phase in the soil.

4.3. The Effect of Infection on Host Growth

This experiment shows that infection has no profound effect on growth on a dry weight basis under the conditions tested. It is conceivable, however, that minor effects on

growth do occur and that these have not been evident in the present experiment. Effects on growth may be more pronounced when the host plant is subject to stress or is at the seedling stage of growth. Furthermore, the degree of infection was relatively low at the time the plants were analysed and it is possible that, with further development of the galls , plants would show a more marked response to infection.

These results are not unexpected in view of field observations and the observations of all previous workers that infected plants cannot be detected by their above-ground appearance. There has been no cause to believe from past reports that the parasite is harmful to the growth of the host and Schwartz (1910) even thought Entorrhiza infections might stimulate the growth of Juncus articulatus. However, the effects of some diseases are often not readily recognised, as with moderately retarded growth that appears normal (Horsfall & Dimond, 1959). There have been occasions when workers have believed erroneously that crops have not been affected by disease (eg. powdery mildew of clover, wheat leaf rust); nevertheless, accurate measurements showed 25-35% losses in yield (Chester, 1959). Such effects are even less apparent if the population is regularly infected, and with Juncus articulatus this could often be so. Therefore, although the host-parasite relationship between Entorrhiza casparyana and J. articulatus appears well-balanced, with little adverse effect to the host, it is only by having accurate measurements on the effect of the disease on the plant, preferably in relation to the degree of infection present, that field observations can be critically assessed.

One might expect, by analogy with other smut fungi or root-infecting fungi, some effect on growth. In systemically

infected plants growth is generally inhibited. Even when the symptoms of the disease are largely masked eg. during vegetative growth of cereals infected with smut fungi, a statistically significant inhibition of growth of the host plant is found to occur eg. wheat infected by Ustilago nuda (Mather & Hansing, 1960; Gaunt & Manners, 1971). Often there is pronounced stunting as with Tilletia controversa, dwarf bunt of wheat. Dwarfing results not only from systemic infections but from localised infections comparable to those caused by Entorrhiza. Urocystis coralloides, which forms galls on the roots of Brassica spp., reduced the height of mustard (B. campestris) plants in naturally infected soil by almost 45% (Mundkur, 1938). Furthermore, seed yield was reduced by just over 40% and flowering occurred 5 days earlier in infected plants. However, despite these effects the disease is not easily recognised in the field. Urocystis coralloides appears to be the only smut fungus strictly comparable to Entorrhiza in its ecology i.e. it is a localised, gall-forming, soil-borne, root-infecting fungus. Numerous galls, which break off easily, are present on the fine roots and larger galls occur on the main roots. However, U. coralloides differs from Entorrhiza in that the galls are considerably larger and the tissues more disrupted. In such localised infections the effect on growth may be the result of diffusion of enzymes or toxic metabolites throughout the host, or deprivation by the parasite of metabolites necessary for development of the host. In both cases the effect on growth is likely to be less pronounced than in systemic infections. Localised infection may also interfere with plant growth mechanically eg. by blocking or disrupting the stelar tissues.

This may occur in U. coralloides infections because, although the vascular system is not invaded (Mundkur, 1938), it may become distorted by growth of the surrounding tissues (Mitra, 1928).

Localised infections differ considerably from systemic infections in that the degree of infection is dependent on the number and position of infection sites. If there are only a small number of infection sites and, furthermore, these are situated at the apices of an extensive root system, as in Entorrhiza infections of Juncus articulatus, the loss would be expected to be much less than a large number of infection sites on a leaf, eg. Ustilago striiformis, reducing the photosynthetic area, or one or two root galls on plants with a tap root system eg. U. coralloides or Plasmodiophora brassicae on crucifers. In club-root disease, caused by P. brassicae, infected roots fail to absorb nutrients, are often unable to transport food and either seedlings die before they reach maturity or plants are severely stunted.

Entorrhiza infections do not usually invade the xylem and the stele does not appear to be distorted by the enlargement of the surrounding cortex; moreover, the position of the galls, largely at the apices of roots, precludes them from mechanically disrupting the flow of water and nutrients from the soil to the aerial parts of the plant. There is still an extensive root hair region for absorption behind the galls; furthermore, a large amount of absorption probably occurs directly through the cells at the root surface. The root meristem continues to function, although perhaps at a reduced rate or abnormally, and as there is no significant difference in the root dry weights (either including or excluding the galls) of

infected and uninfected plants in the present experiment, there is no reason to expect much alteration in the absorptive capacity of the root system. This situation might have changed, however, if the experiment had been continued and the galls allowed to develop further. If the galls developed at the expense of normal roots and the shoot:root ratio (including galls) remained the same in both infected and uninfected plants, the effect of infection on the growth of the plant would depend on whether absorption occurred through the surface of the gall and, if so, whether the surface area of the root system for absorption was reduced by the altered morphology.

Alternatively, Entorrhiza infections might affect growth by elaborating toxins or producing enzymes which would interfere with the normal metabolism of the plant. If this were the case, one might expect significant effects on growth with only small amounts of infection; however, this did not occur in the present experiment.

In several diseases where the effects on growth have been analysed, the infected areas increased in dry weight at the expense of other plant parts (see Gaunt & Manners, 1971). However, the extent to which fungal material accounted for the localised increase in dry weight was usually undetermined. In Entorrhiza infections, the localised nature of the infection allows a reasonable estimate to be made of the fungal material; this can be regarded as being proportional to the dry weight of galls and allows an indirect assessment of the degree of infection.

Finally it should be mentioned that disease does not always result in a dry weight decrease; dry weight increases

may be produced by infection. An example is the "bakanae" disease of rice where gibberellins cause an overgrowth effect.

In a highly evolved host - parasite relationship (and morphologically and cytologically Entorrhiza appears to have such a relationship) there is also no reason why the increase in fungal mass should not be exactly counterbalanced by a decrease in plant mass. If no toxic metabolites are produced or no mechanical disruption occurs the host plant may function normally, merely redirecting some of its reserves to the production of mycelium and spores.

In this experiment a soil inoculum, or more precisely a soil-water inoculum, has been used to bring about infection. For this reason, the effects on growth may be more like those occurring in nature. Mitra (1928) and Mundkur (1938) found for U. coralloides that effects on growth were not so great when macerated galls rather than a soil inoculum were used in infection studies. Furthermore, Mitra was unable to obtain infection in soils which he had artificially inoculated with spores. However, this may have been because the inoculum was not large enough (Mundkur, 1938). Leach et al.(1946) have also noted for Ustilago striiformis that soil inoculation, with fresh teliospores from the field or culture, was more successful than seedling infection by various means. Moreover, the field source of inoculum was more successful than the cultured source.

Addendum.

An attempt was made to analyse plants for the effect of the fungus on growth after a further 5 months. However, this part of the experiment had to be discontinued because of a

number of problems. Firstly, plants were so large that it was no longer practicable to separate shoot, root and gall fractions for dry weight analysis; the degree of infection was so great that the task of separating the galls alone was almost impossible. Secondly, the soil did not rinse easily from the root system; in this respect sand or a sand:soil mixture would have been easier to manage.

One method of overcoming these problems might have been to sample only a portion of the root mass by taking a weighed fraction of the soil. This, however, also proved unsuitable; because of the rhizomatous nature of the host plants, the root system was often very unevenly distributed in the pots. Furthermore, the localised nature of the infection also meant that it was sometimes very unevenly distributed. It therefore seemed apparent that, if this method was used, the standard deviations would be so large as to negate possible differences in the growth of plants resulting from infection. Lieth (1968) has noted that the accuracy of measurements on root mass is low and this is accentuated if estimates of the root mass have to be made by sampling a weighed fraction of the total weight of soil.

4.4. The Development of Infection

The manner in which Entorrhiza infects its hosts is not known. In most smut fungi the dikaryophase is established before infection. In Tilletia caries, the sporidia conjugate, either while still attached to the promycelium or when detached, and from the fusion bridge a secondary sporidium (or conidium) is formed. It is this spore which is infective. For his early experiments on the infection of wheat by Tilletia caries,

Sartoris (1924) used pots with removable sides and placed the seeds on the surface of the soil against these sides. The seedlings could then be removed, relatively free of soil, and examined with the low power of a microscope. Such a technique would be useful in elucidating early stages of infection of Entorrhiza.

Glass-sided containers masked in some way to exclude light would be suitable for observing the development of the galls. However, with both these techniques it is necessary to remember that the growth of roots along a soil/pot interface may be different from that occurring in undisturbed soil in natural situations (Newbould, 1968).

In the present experiment galls probably developed in most pots 8-10 weeks after soil inoculation. With young seedlings infection may develop earlier. In some pots, Juncus articulatus seeds brought in with the soil-water inoculum, germinated and became infected at a very early stage of development. Many of these seedlings produced galls containing spores although they possessed only one or two roots.

The time taken from infection to spore production is normally regarded as the incubation period for the fungus (Fischer & Holton, 1957). For systemic inflorescence smut fungi this period is extremely long but for some smut fungi it may be quite short. Blizzard (1926) found, for Urocystis cepulae infections of onion, that pustules first appeared 9-14 days after soil was inoculated with mycelia produced in culture; complete spore formation was evident in 3-4 weeks. Leach et al. (1946) found for Ustilago striiformis that the incubation period using a soil inoculum could be as short as 36 days; however, it was usually longer. If a syringe was used to inoculate the growing points of shoots the incubation

period was sometimes as short as 13 days.

5. SUMMARY

1. Spores of Entorrhiza casparyana present in soil are capable of infecting established plants of Juncus articulatus under experimental conditions.

2. The degree of infection in well-drained and water-logged soil is not significantly different.

3. In well-drained soil there is no significant difference in the numbers of galls in the bottom and in the top halves of a pot. Spores which were inoculated on to the surface of the soil are probably carried through the soil by the downward movement of water.

4. In water-logged soil the concentration of galls above the water level and particularly in the top 0.5-1.0 cm of the soil is very significantly higher than the concentration below water level. In this case there is no downward movement of water and spores remain in the upper layers of the soil where the inoculum was applied.

5. There is no effect on the growth of the host plant on a dry weight basis after 3 months under the conditions tested. The number of shoots produced is not significantly different in infected and healthy plants. This supports field observations that infected plants cannot be detected by their above-ground appearance.

6. Galls probably formed mainly 8-10 weeks after soil inoculation.

7. 3-3½ months after soil inoculation the maximum amount of infection recorded per plant was 2546 galls weighing 1.2 grams. This represented 18.5% of the dry weight of the root system and 9.6% of the total dry weight of the plant.

8. 8 months after soil inoculation the maximum amount of infection recorded per plant was 4267 galls weighing 14.3 grams. This represented 54.9% of the dry weight of the root system and 29.7% of the total dry weight of the plant. This increase in weight did not appear to be at the expense of shoot or root growth; it appeared to be a localised increase which increased the total dry weight of the plant proportionally.

9. Some disintegration of galls was evident 8 months after inoculation whereas this was not observed after 3 months.

CHAPTER 5

TELIOSPORE GERMINATION IN ENTORRHIZA CASPARYANA

GENERAL INTRODUCTION

In the Ustilaginales the manner in which the teliospores germinate is the basic character for distinguishing the two families, Ustilaginaceae and Tilletiaceae. These families are separated mainly on the septation of the promycelium and the position of the sporidia (basidiospores). In the Ustilaginaceae the teliospore germinates to form a septate promycelium characteristically with four cells each containing a single haploid nucleus resulting from meiosis of the diploid teliospore nucleus. Within each of the cells of the promycelium the nucleus divides and passes into a sporidium budded off laterally from the cell eg. Ustilago avenae. In the Tilletiaceae, eg. Tilletia caries, the teliospore germinates to form a promycelium which is usually non-septate. The diploid nucleus undergoes meiosis and then usually one or more mitotic divisions occur so that 8-16 haploid nuclei are formed. The sporidia, corresponding in number to the nuclei produced, arise in a whorl terminally on the promycelium.

Some species may be difficult to classify under this system. These include species which germinate directly without producing either a promycelium or sporidia. As long ago as 1924 Cunningham suggested that another family could be established to include these species. Furthermore, the pattern of germination in some species which do produce promycelia and sporidia, eg. Ustilago longissima, may overlap aspects of germination in both the Tilletiaceae and the Ustilaginaceae. For

this reason some workers have suggested that the distinctions between these two families are of doubtful significance. However, as Duran and Safeeulla (1968) suggest, extensive germination studies are required before well-informed decisions on the number of families in the order can be made; there remain many species which have been inadequately studied.

Decisions regarding the taxonomic affinities of species within the Ustilaginales are complicated by the fact that the teliospores may not germinate readily. However, Duran and Safeeulla (1968) and Duran (1972) have shown that it may not be as difficult to germinate the teliospores of most smut fungi as was once supposed. Their attempts to germinate some species were, nevertheless, unsuccessful.

It is therefore evident that a knowledge of the factors controlling spore germination is important from the taxonomic point of view. This knowledge is also extremely important biologically because it enables further studies on the life history of the species and opens the way to a variety of experimental investigations on the fungus itself and the host-parasite complex.

Spore germination in the genus Entorrhiza has been recorded by Weber (1884), Schwartz (1910), Brefeld (1912) and Thirumalachar and Whitehead (1968). The only detailed account with illustrations is that of Weber (1884). These records are mainly of germination in Entorrhiza aschersoniana from Juncus bufonius although it appears that Brefeld also observed germination of Entorrhiza cypericola from Cyperus flavescens. No detailed observations on the germination of Entorrhiza caspary-ana from Juncus articulatus have been previously recorded; nevertheless, this may have been the host-parasite complex for which

Schwartz (1910) noted the germination of one spore. His account was ambiguous, however, and the exact identity of the parasite cannot be determined.

Weber observed germination only after galls had overwintered outdoors, in moist sand, in conditions as close as possible to those normally experienced by infected plants. Spores remaining in galls (i.e. in situ) germinated abundantly in either water or moist sand at 10°C but spores isolated from galls rarely germinated. He obtained germination as early as February (late winter) but in field experiments germination did not occur before May.

The spores germinated to produce one to four promycelia, usually three. The promycelia were usually unbranched, aseptate (although a single septum was occasionally evident in older germ tubes) and bore sickle-shaped, sometimes distinctively coiled, aseptate sporidia singly at their tips or just below (i.e. laterally).

Schwartz attempted to germinate spores that had overwintered, using water and various nutrient solutions and temperatures from 10° - 25°C, but he was largely unsuccessful. The one spore which germinated produced three small germ tubes but no sporidia.

Brefeld, using both water and nutrient solutions, was unsuccessful in obtaining germination from freshly collected material and also from spores that had overwintered for one year. Eventually, after two to three years he obtained germination, firstly from the younger lightly pigmented spores and later from the older dark brown spores. On germination a highly branched septate mycelium was produced bearing long pointed conidia in basipetal succession from sterigmata.

This was similar to the germination of Acrostalagmus (syn. Verticillium) and on this basis Brefeld doubted that Entorrhiza was a smut fungus.

Thirumalachar and Whitehead (1968) described germination of fresh teliospores for the first time. They fixed teased-out spores to slides by alternate wetting and drying of the spore mount and then inverted the slides over water in moisture chambers following the method of Thirumalachar and Narasimhan (1953). Only a few spores germinated after incubation for more than 6 days. The spores produced slender septate germ tubes bearing two to three falcate sporidia at the apex.

Ferdinandsen and Winge (1914) attempted to obtain germination from spores of Entorrhiza raunkiaeriana overwintered at a low room temperature but they were unsuccessful.

In view of these sometimes conflicting reports further information on germination in Entorrhiza is desirable. Weber's (1884) original observations have not always been accurately reported, especially with respect to the septation of promycelia and sporidia (eg. Massee, 1891; Liro, 1938; Ainsworth & Sampson, 1950; Savulescu, 1957). As the septation of the promycelium is one of the basic characters distinguishing the Ustilaginaceae from the Tilletiaceae, the nature of the promycelium in Entorrhiza must be closely studied. In addition further evidence to confirm the taxonomic status of this genus is necessary.

The work carried out during the present study is treated under three main headings:

A) Factors affecting germination

B) The morphology and cytology of germination

C) Ultrastructural changes associated with germination.

Germination is defined here as the visible appearance of a germ tube.

A. FACTORS AFFECTING GERMINATION

1. INTRODUCTION

It is clear from the general introduction above that teliospores of Entorrhiza do not germinate readily. Many smut fungi have spores which do not germinate immediately they are mature. Such spores have a period of after-ripening or dormancy under natural conditions but this can be reduced by various treatments. On the other hand, species of Entyloma and Doassansia, which resemble Entorrhiza in that spores are held rather firmly in the host tissue, have spores which germinate at maturity in situ.

Duran and Safeeulla (1968) suggested as a result of their experiments on a large number of smut fungi that the spores of most species in which germination has not been previously attempted or demonstrated may in fact be readily germinable. Where spores did not germinate immediately, they found that chilling was the most successful method of breaking dormancy. In contrast, they found that compounds reported to stimulate or trigger spore germination in other fungi and slime moulds were generally ineffective.

In the present work there were two main objectives:

a) to find whether teliospores germinated at maturity in situ under natural conditions
b) to induce germination of teliospores under laboratory conditions.

2. MATERIALS AND METHODS

Galls collected routinely for taxonomic investigations were examined for *in situ* germination of teliospores. The spores were teased out of the galls and examined either in water or in lactophenol cotton blue (LPCB).

For laboratory experiments on germination, galls containing teliospores of *Entorrhiza casparyana* were obtained from *Juncus articulatus* from the Groynes at the source of the south branch of the Waimakariri river. Attempts to germinate the spores largely followed the methods described by Duran and Safeeulla (1968). The procedures used in individual experiments are outlined in tables 5.1, 5.2 and 5.3. Details of these methods are described below:

1) Inoculum

Galls for germination studies were selected according to whether they were

a) mature i.e. firm, dark brown, with well-developed teliospores

b) old and decaying, i.e. with outer wall partly ruptured and cortex disintegrating.

Galls were either used whole (for *in situ* germination), sectioned under aseptic conditions or used for the preparation of spore suspensions. The spore suspensions were obtained directly from the galls if the spore concentration was sufficiently high and surface sterilisation unnecessary or by centrifugation of the contents if surface sterilisation of the spores was required and numbers were low.

2) Surface sterilisation of galls and spores.

Three different chemical surface sterilants were used :

TABLE 5.1 EXPERIMENTS ON THE GERMINATION OF FRESH TELIOSPORES

Source of material	Nature of inoculum	Surface sterilisation	Conditions of culture: Medium	Conditions of culture: Temp.	Observations
Exp. 1.		Galls:			
Mature galls 1. 4.71	Thin section of gall	a/ sterile H_2O b/ 10% Janola c/ 70% alcohol	Sterile H_2O	Ambient ca. 20°C	No germination after 7 days
Exp. 2.		Galls:			
Mature galls 19. 4.71 (ruptured wall in some)	Thin section of gall	a/ sterile H_2O b/ 10% Janola c/ 70% alcohol	Sterile H_2O	Ambient ca. 20°C	No germination after 7 days
Exp. 3.		Galls:			
Mature galls 21. 4.71	Thin section of gall	a/ sterile H_2O b/ 10% Janola	Sterile H_2O	a/ 4°C b/ 15°C	No germination after 7 days
Exp. 4.		Galls and spores:			
Mature galls 27. 5.71	Spore suspension	a/ sterile H_2O b/ 10% Janola	a/ MEA b/ PDA c/ PSA	a/ 10°C b/ 20°C	No germination after 6 days
Exp. 5.		Galls:			
Mature galls 15. 6.71	Spore suspension	a/ sterile H_2O b/ 10% Janola	a/ sterile H_2O b/ H_2O agar c/ MEA d/ PDA	a/ 10°C b/ 20°C	No germination after 14 days
Exp. 6.		Galls:			
Old galls 15.12.71 (no germination detected micro-scopically)	Spore suspension	10% Janola Spores: a/ none (control) b/ 10% Janola	a/ sterile H_2O b/ coverslip cultures: i. H_2O agar ii. MEA iii. PDA iv. PSA	Ambient ca. 25°C	No germination after 7 days

TABLE 5.2 EFFECT OF SOIL MICROORGANISMS AND ROOT EXUDATES ON GERMINATION

Source of material	Nature of inoculum	Surface sterilisation	Conditions of culture: Medium	Conditions of culture: Temp.	Observations
Exp. 1.					
Mature gall left in soil for 4 days after collection 29.11.71	Whole gall	None	On filter paper on soil from beneath susceptible J. articulatus	Ambient 20°C	No germination after 3 days
Exp. 2.					
Mature galls stored at 7°C for 4 days in water 29.11.71	Whole gall	None	a/ in water b/ on soil from beneath susceptible J. articulatus c/ on soil and roots from beneath susceptible J. articulatus d/ on filter paper on soil from beneath susceptible J.articulatus e/ on filter paper around germinating seeds of J. articulatus	Ambient ca. 20-23°C	No germination after 3 days - but would have been difficult to detect under conditions used
Exp. 3.					
Mature galls produced under glasshouse conditions since December, 1971 17. 7.72	Spore suspension	a/ sterile H_2O b/ 10% Janola	I Around surface sterile 6 day seedlings of J. articulatus on Hoaglands + streptomycin agar on: a/ coverslip cultures (H_2O agar) b/ cellophane cultures II Coverslip cultures on H_2O agar (control)	20-23°C varying between continuous light, continuous dark, and alternating light and dark	No germination after 7 days

TABLE 5.3 EXPERIMENTS ON THE GERMINATION OF STORED TELIOSPORES

Source of material	Nature of inoculum	Surface sterilisation	Conditions of storage: Medium	Conditions of storage: Temp.	Observations
Exp. 1.					
Mature galls (some firm, some partly broken down) 17. 6.71	Whole gall	a/ sterile H_2O b/ 10% Janola for 2 mins	Sterile soil extract solution	ca. 7°C	1/ 1 tube examined after 12 weeks - no germination. 2/ 3 tubes examined after 13 months - ABUNDANT GERMINATION in one tube. 3/ all tubes (18) examined after 15 months a/ sterile H_2O - germination in 14 of 20 galls (5 of 7 tubes) b/ 10% Janola - germination in 20 of 21 galls (8 of 9 tubes)
Exp. 2.					
Mature galls (some firm, some partly broken down) 16.12.71	Whole gall	Sterile H_2O	Unsterilised tap H_2O	a/ 7°C b/ ca. 20°C	a/ ABUNDANT GERMINATION observed after 7 months b/ no germination after 7 months
Exp. 3.					
Galls $8\frac{1}{2}$ months old or less from plants cultivated in the glasshouse Galls: 1/ immature (white or slightly pigmented at base) 2/ mature 3/ decaying	Whole gall	a/ sterile H_2O for 1, 2 and 3 b/ 10% Janola for 2 mins for 1 and 2	a/ sterile H_2O for 1, 2 and 3 b/ sterile soil extract soln. for 1, 2 and 3	a/ 7°C b/ 20°C	No germination after 7 months

a) 10% Janola (a commercial disinfectant containing 3.5% available chlorine) for 2 minutes

b) 70% alcohol for 1 minute (galls only)

c) 1% copper sulphate ($CuSO_4$) solution for 24 hours (spores only)
- this is widely used as a mild surface sterilant for the spores of smut fungi (Ainsworth & Sampson, 1950).

1-2 drops of a wetting agent were added to each of these solutions to increase the effectiveness of the surface sterilisation procedure. After treatment with the surface sterilant the material was rinsed in 2-3 changes of sterile water.

10% Janola for 2 minutes was the method used routinely throughout these experiments.

As the sensitivity of the spores to chemical surface sterilisation was not known, for most experiments controls were set up in which galls were rinsed for 1-2 minutes in each of 3-5 changes of sterile water.

3) Surface sterilisation of Juncus articulatus seeds:

Several preliminary experiments indicated that 10% Janola (plus wetting agent) for a period of 30 minutes was effective in surface sterilising most seeds. The seeds were plated out on Hoagland's nutrient agar and only those which germinated and remained sterile were used in the experiments. This method of surface sterilisation did not noticeably reduce the percentage of germination.

4) Media

A number of agar media were used in an attempt to germinate the spores:

a) tap water agar (H_2O) - Booth (1971)

b) 2% malt extract agar (MEA) - Oxoid

c) potato dextrose agar (PDA) - Oxoid

d) potato sucrose agar (PSA) - Booth's (1971) recipe was followed, substituting sucrose for dextrose.

Dilute soil extract solution was used as a liquid nutrient medium for spore germination. It was prepared as follows:

300 gms of garden soil were left in 1 litre of tap water in a warm place for 24 hours. The supernatant was then decanted off, filtered and sterilised by autoclaving at 15 lbs pressure for 20 minutes.

Hoagland's nutrient agar was used for seed germination of Juncus articulatus. It was prepared by making up a one-fifth strength solution of Hoagland's complete medium (Machlis & Torrey, 1956) with 0.5% agar.

Streptomycin was sometimes added to media to inhibit the growth of bacteria. It was added to 100 ml amounts of medium that had been autoclaved and cooled to ca. 45°C so as to give a final concentration of 40 units per ml. (1 gm = 1 million units).

5) Culture techniques

Material for spore germination was sometimes plated out directly on agar. However, this made microscopic observations difficult. Two alternative procedures were employed to overcome this:

a) cellophane cultures

Cellophane squares were boiled in distilled water for 10 minutes to partially sterilise them and remove plasticisers. They were then placed directly on an agar medium and spore material for germination was placed on the cellophane. These cellophane cultures could then be examined directly by mounting in lactophenol cotton blue on a microscope slide.

b) coverslip cultures

This very useful technique, first described by Taschdjian (1954), is outlined by Booth (1971).

To test the effect of root exudates and the soil microflora on spore germination several experiments were set up. In the first experiments (Table 5.2, exps. 1 & 2) an attempt was made to simulate natural conditions. Whole galls were placed, either directly or on moistened filter paper, on the surface of unsterilised soil freshly obtained from an area where susceptible plants of Juncus articulatus were growing. The second group of experiments was designed specifically to test the effect of root exudates on spore germination (Table 5.2, exps. 2e, 3). Spores for germination were placed either on filter paper, cellophane or coverslip cultures close to germinating seeds of Juncus articulatus under aseptic conditions. Some spores were immediately adjacent to the root hairs of the potential host.

3. RESULTS

Germination was recorded in situ in galls on Juncus articulatus from the following 6 localities in Canterbury: Pigeon Bay - Port Levy Rd., Sept. 1970; Groynes, Nov. 1971; Blowing Pt. Bridge, Dec. 1971; Smiths Creek, Dec. 1971; Erewhon, Dec. 1971; Lake Heron, Dec. 1971. Further details of these collections are given in Chapter 7.

Germinating teliospores were found only in some of the galls which had considerably decayed. In these galls the cortical tissue had disintegrated especially at the base of the gall leaving the vascular tissue as isolated strands; the

outer uninfected layers of the gall were either partly broken down or ruptured. These galls were soft, the spores were readily released and bacteria, fungi, protozoa and nematodes had invaded the decaying tissue.

In laboratory experiments no germination of fresh teliospores was obtained although various conditions were tested. These included a range of incubation temperatures from 4° - 25°C, a variety of culture media, different forms of inoculum, and spores of various ages collected in different seasons (Table 5.1, exps. 1-6). There was also no germination when fresh teliospores either *in situ* or as a spore suspension were placed on soil from beneath *Juncus articulatus* seedlings or on Hoagland's nutrient agar adjacent to 6 day old seedlings (Table 5.2, exps. 1-3). All results were negative regardless of whether chemically surface sterilised inocula or inocula rinsed only in sterile water were used. Throughout these experiments the light conditions varied from alternating light and dark when plates were incubated in the laboratory to intermittent light when plates were placed in temperature controlled incubators.

In some material which was held at 7°C spores were induced to germinate. Spore germination and sporidial production were detected after 7 months in sterile water and after 13 months in soil extract solution (Table 5.3, exps. 1 & 2). Germination occurred *in situ* in both chemically surface sterilised and unsterilised galls. At what stage germination occurred is difficult to determine because neither source of material was examined at regular intervals. There was however no germination after 12 weeks in one tube of soil extract solution. Comparable material did not germinate when fresh (Table 5.1, exps. 5 & 6) or when held at room temperature.

Spores obtained from plants grown in the glasshouse for $8\frac{1}{2}$ months could not, however, be induced to germinate when held at $7^{o}C$ for 7months (Table 5.3, exp. 3). This was irrespective of the stage of development of the galls, whether or not they were surface sterilised and the medium in which they were stored.

Although germination was successful in some collections incubated at a low temperature, not all spores in this material showed germination. Some of these spores were less mature and were present in young galls; however, others appeared mature (the brown pigmentation of the spore wall was reasonably well developed) and occurred in older galls.

4. DISCUSSION

The occurrence of germination in decaying galls may be a result of the inactivation of germination inhibitors which may have been present in the living cells of the host. Alternatively, it could be postulated that substances stimulatory to germination are produced or released during the decay process. However, there is little evidence to suggest which, if either, of these hypotheses might be true. The release of a substance during decay which stimulated germination would explain Weber's (1884) observation that germination was abundant <u>in situ</u> but infrequent when the spores were isolated from the gall. However, it does not explain why spores can infect plants when added to soil in suspension (see Chapt. 4) unless it is presumed that the majority of spores are still associated with decaying fragments of galls. Alternatively, completely different factors may be involved when spores germinate in the vicinity

of host plants. In such cases, germination may be affected by specific host exudates or by the complex interactions of microorganisms and metabolites in the rhizosphere.

Whether germination occurred in any particular gall would also depend on whether there were spores in that gall which had reached maturity. The fact that teliospore germination was noted only between September and December (spring and early summer) after the galls had overwintered may also indicate that the spores required an after-ripening period. However, at this stage there is insufficient evidence to allow too much emphasis to be placed on this observation. Further work is required to find whether there is a seasonal variation in teliospore germination in nature or whether germination occurs throughout the year and depends primarily on the attainment of a certain stage of development by the spores.

Teliospore germination has not previously been recorded *in situ* in freshly collected galls for the genus *Entorrhiza*. However, the germination of mature spores *in situ* is not unlike the situation occurring in both *Entyloma* and *Doassansia* which also have spores enclosed in the host tissue. In most other smut fungi spores do not germinate until after they are released from the sorus.

All attempts to germinate fresh teliospores under laboratory conditions were negative. Altering various environmental factors appeared to have no effect. Lower temperatures are usually the most likely to favour germination of smut fungi from temperate collections (Meiners & Waldher, 1959; Duran & Safeeulla, 1968). Smut fungi not germinating in water may require the addition of various nutrients; in these experiments either nutrient media were used containing dextrose, maltose

or sucrose as a carbon source, or soil extract, which is known to stimulate germination in certain circumstances (Meiners & Waldher, 1959).

Because of the host specificity shown by Entorrhiza species and the known stimulatory activity of host exudates on spore germination in some fungi (Schroth & Hildebrand, 1964) the effect of root exudates from surface sterilised young seedlings of Juncus articulatus growing on Hoagland's agar was tested. No effect on germination was obtained. The spores of some other smut fungi do, however, germinate in response to host exudates. Noble (1924) reported that spores of Urocystis tritici received a general stimulus for germination not only from the host (wheat) but also from many non-hosts. More recently, Saxena and Khan (1971) have shown that host diffusates affect the germination of spores of Ustilago scitaminea.

Although the light factor was not considered in detail there was no reason to believe that light would have been inhibitory or insufficient in these experiments. Meiners and Waldher (1959) found for 9 species of Tilletia that the amount of light provided by occasionally opening an incubator door is usually sufficient for germination; increasing the amount of light may however increase the rate of germination or the percentage of germination. On the other hand very few species germinated in complete darkness.

In view of the fact that none of the environmental factors tested induced spore germination it was concluded that the Entorrhiza spores probably exhibited constitutive or endogenous dormancy (Sussman, 1965, 1966). However, it is possible that not all factors favourable for germination were present in any one experiment.

Constitutive dormancy is a true dormancy conferred on the spore by its innate properties; this is in contrast to exogenous dormancy which is environmentally induced. Spores which are endogenously dormant require an after-ripening period, after they mature, before they will germinate. The after-ripening period may be of quite long duration in nature but may be reduced under laboratory conditions by various types of treatment. This activation may be brought about in one of three main ways: by temperature treatment, either high temperature or low temperature, light treatment or treatment with complex chemicals. All three types of treatment outlined above have been found to be effective for smut fungi but the most widely used method is chilling (Sussman, 1965; Duran & Safeeulla, 1968). This is effective, for example, for Tilletia caries: 3°C for 16 days (Gassner & Niemann, 1954 cited by Sussman, 1965), 4°C for more than 3 months (Holton, 1943) or 5°C for 5-10 weeks (Lowther, 1948).

Entorrhiza therefore appears to resemble many other smut fungi in it response to chilling. However chilling does not induce germination in all collections; spores from infected plants grown in the glasshouse failed to germinate after being subjected to treatments similar to those which were successful for collections from the field. This situation has frequently been reported in the literature; the requirements for germination noted above for T. caries indicate a range of response for different collections. The variation in results may be attributable to the source of the material, the environmental conditions under which the infection developed, the season in which the spores were collected, the age of the spores and

possibly many other factors. Once Entorrhiza spores have been activated they are able to germinate at the same temperature; in many fungi the conditions for activation and germination are different.

The requirement for chilling may be related to the natural environment. Duran and Safeeulla (1968) noted that for North American smut fungi northern collections often required chilling in contrast to southern collections. Entorrhiza so far as is known (apart from one record from Algeria) and its Cyperaceous and Juncaceous hosts are largely temperate genera. During the winter, soil temperatures are extremely low in many regions where Entorrhiza has been recorded (Central Europe and Scandinavia) and the ground may be under snow for several months of the year. The ability of spores to be activated and germinate while submerged in water or soil extract solution may also be related to their natural environment; most fungal spores do not germinate while submerged possibly because of the high oxygen demands at germination.

Thirumalachar and Whitehead (1968) are the only authors to report germination of fresh teliospores. In their experiments it is possible that the spores were activated, incidentally, by the alternate wetting and drying required to adhere the spores to glass slides. Alternate wetting and drying has been used successfully in breaking the dormancy of rust spores. It is also possible that the spores were activated by the potassium permanganate ($KMnO_4$) they used as a mild surface sterilant as $KMnO_4$ has been reported to stimulate germination in other smut fungi (Gassner & Niemann, 1955a cited by Sussman, 1965). In the present experiments hypochlorite (Janola) was used routinely as a surface sterilant partly because in some cases it had been

recorded as being stimulatory to germination. Das (1948), for example, found for Tilletia holci that a 10% solution of bleaching powder (hypochlorite) stimulated germination of the spores.

Potassium permanganate and hypochlorite salts are just two of many compounds, including chemicals of known composition and complex substances occurring naturally in the environment of the spore, which have been used to break dormancy in fungal spores. Although Sussman (1965) lists a large number of instances where smut fungi have been induced to germinate by using various chemicals, Duran and Safeeulla (1968) found that chemical stimulants were largely ineffective. Host exudates (Noble, 1924; Saxena & Khan, 1971) and soil extract (Meiners & Waldher, 1959) may, however, be stimulatory.

The maturity of the spores used in germination studies is a prerequisite for successful germination. In Entorrhiza the time of maturity is difficult to establish. Normally nuclear fusion and development of the spore wall and pigmentation are accepted as the criteria for maturity. Whether nuclear fusion has occurred cannot be decided readily, even with the electron microscope (E.M.) because cytological details are normally obscured by the heavy spore ornamentation and the high lipid content. However, the E.M. does show that spores having incompletely developed walls are sufficiently mature cytologically for germination (see C. 3). If it is accepted that spores which have been released from the sorus are mature this also is a difficult criterion to apply here. Unlike the spores of most smut species, those of Entorrhiza are firmly held in the tissue until their passive release on decay of the gall. Whether this decay occurs at regular times or randomly

and whether the galls persist for more than one season is not known.

It would seem from the experiments so far that only those spores which are present in galls which have begun to break down are capable of being activated. Nevertheless, E.M. studies show pregermination nuclear division occurring in spores still enclosed by the host cell wall; the host cell contents had, however, degenerated. Further work is necessary to elucidate this aspect.

Although Entorrhiza, like most smut fungi, appears to show an endogenous type of dormancy, in some smut fungi dormancy may be environmentally controlled i.e. exogenous. Gassner and Niemann (1955b cited by Sussman, 1965) found, for example, that unsterilised soil had an inhibitory effect on spores of Tilletia secalis but that this could be dissipated by autoclaving the soil; the soil then actually stimulated germination. In this instance dormancy was conferred on the spore by soil fungistasis. Chemicals were able to reverse the inhibitory factor in the soil (Niemann, 1957 cited by Sussman, 1965).

What confers dormancy on Entorrhiza is not known. The permeability of the spore wall may be involved. Brefeld's (1912) observations that lightly pigmented spores germinated more readily than darkly pigmented spores is circumstantial evidence that the more highly developed spore wall may confer greater impermeability. Thick walls are often associated with impermeability and in some spores enzymatic digestion or cracking of the wall may facilitate germination (Sussman, 1966).

The fact that Entorrhiza spores germinate in situ and in spore suspensions in high concentrations does not suggest

that self-inhibitors are released by these spores. Nevertheless, this may be a factor in some smut fungi. Noble (1924), Holton (1943) and Lowther (1948) specify that the spores of smut fungi must be pre-soaked before maximal germination can be obtained. It may be that endogenous inhibitors are leached from the spores during soaking in the same way that Allen (1955) has shown dissipation of endogenous inhibitors from wheat rust uredospores floated on water. Endogenous inhibitors are present in the spores of Tilletia caries. In this species, germination percentages are correlated with spore concentration (Riehm 1923, cited by Sussman, 1965) and trimethylamine has been isolated from the spores and shown to inhibit germination in low concentrations (Ettel & Halbsguth, 1963, cited by Sussman, 1966). These workers suggested that this substance is the endogenous inhibitor of the spores of smut fungi.

The present work on factors affecting spore germination in Entorrhiza has of necessity been exploratory in nature. The observations have arisen mainly from attempts to obtain germinating spores for taxonomic purposes. Undoubtedly the factors governing spore germination are complex and the effect of any one factor may vary depending on the other conditions present. In this study, spores collected from the field have been successfully induced to germinate after a period of chilling whereas those from glasshouse plants infected from the same source failed to respond to the same treatment. In future experiments, material from the field should be collected at regular intervals to discover whether seasonal or environmental conditions influence germination in situ or the response of the spores to chilling. In addition, further experiments should be carried out using sori and spores of known maximum age, as

the maturity of the spores and whether they have been produced during the current season's growth may also be factors controlling their ability to germinate.

B. THE MORPHOLOGY AND CYTOLOGY OF GERMINATION

1. INTRODUCTION

The following account is a detailed description of the morphology and cytology of teliospore germination in Entorrhiza casparyana as observed by light microscopy. Teliospore germination has not previously been described in this species.

2. MATERIALS AND METHODS

Germinating teliospores were observed in situ during routine observations of galls for taxonomic purposes; germination was detected in material studied in the laboratory usually 2-3 days after collection. The collections examined were of galls on roots of Juncus articulatus from 6 localities in Canterbury (see A. 3).

Material on Juncus articulatus from the Groynes, which had been stored at 7°C in soil extract solution for several months (see Table 5.3, exp. 1), was also studied.

The teliospores were teased from galls and mounted either in water or in lactophenol cotton blue.

In addition, spore suspensions from galls shown microscopically to contain germinating spores and sporidia were set up on coverslip cultures (see A. 2) and the effect of various solid media noted. The galls were from 3 of the 6 collections noted above i.e. Blowing Pt. Bridge, Smiths Creek and Erewhon.

Germinating spores were examined and photographed with a Leitz Orthoplan microscope fitted with an automatic camera attachment.

Spores were germinated on the following media:

a) tap water agar

b) malt extract agar

c) potato sucrose agar (for details see A. 2)

Neither the galls nor the spores were chemically surface sterilised.

The plates were incubated at room temperature (ca. 20°C) in an attempt to approximate the conditions under which the spores from the same collections had originally germinated *in situ*.

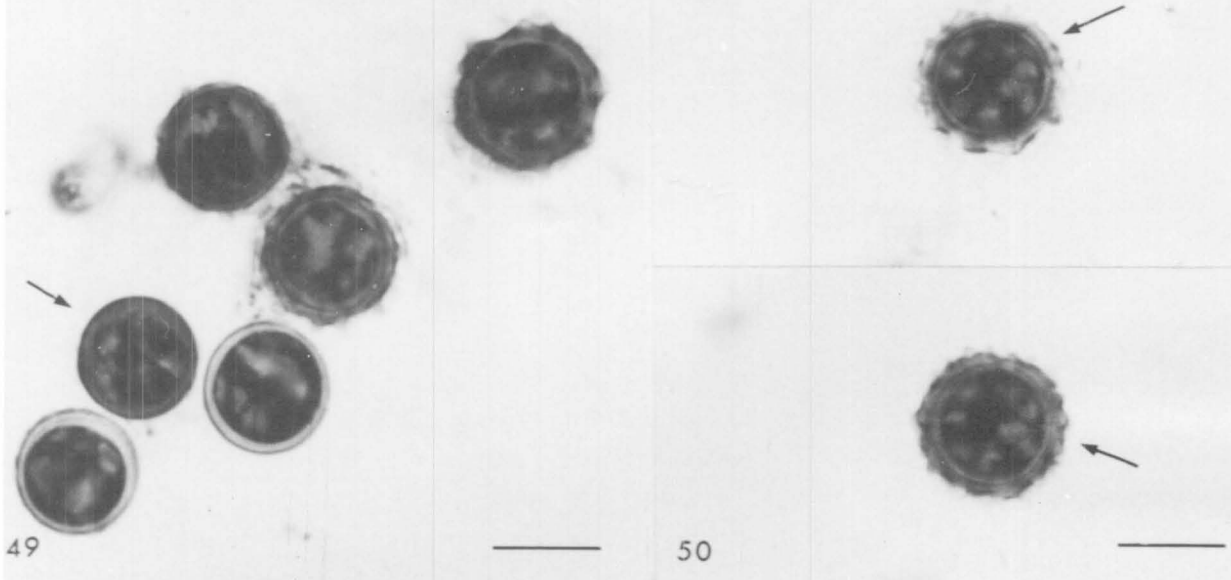
3. RESULTS

3.1. Germination *in situ*

Germination followed the same pattern in all material examined although there were differences in the stage of development that had been reached in the various samples.

Spores believed to be undergoing pregermination nuclear division (karyokinesis) were observed in preparations showing germinating spores (Fig. 49). The contents of these spores had deeply stained blue "organelles" in lactophenol cotton blue and these were interpreted as nuclei. A large number of nuclei were present; in Fig. 49 up to 5 can be differentiated in one spore. Sometimes nuclei could not be distinguished but staining indicated considerable cytoplasmic activity and possibly strands of chromatin.

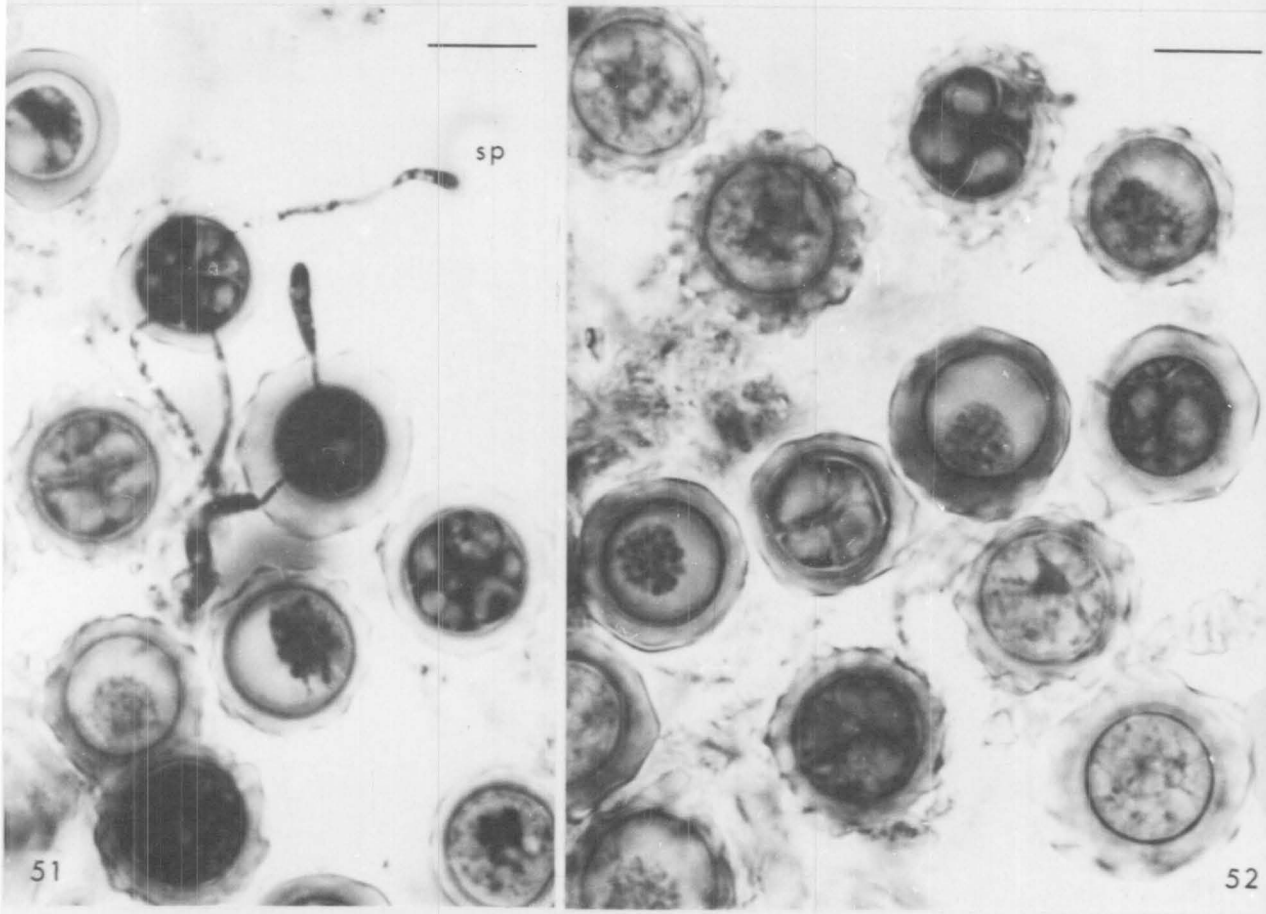
Figs. 49 - 73 Spore germination in Entorrhiza casparyana.
Scale: line represents 10 µm except where otherwise indicated.



Figs. 49 & 50 Nuclear activity and cytoplasmic division in spores germinating in situ: Erewhon collection.

Fig. 49 One spore (arrow) has up to 5 distinct nuclei.

Fig. 50 Two cleavage lines (arrows) in one spore at different foci.



Figs. 51 & 52 Cytoplasmic division in spores germinating in situ: Smiths Creek collection.

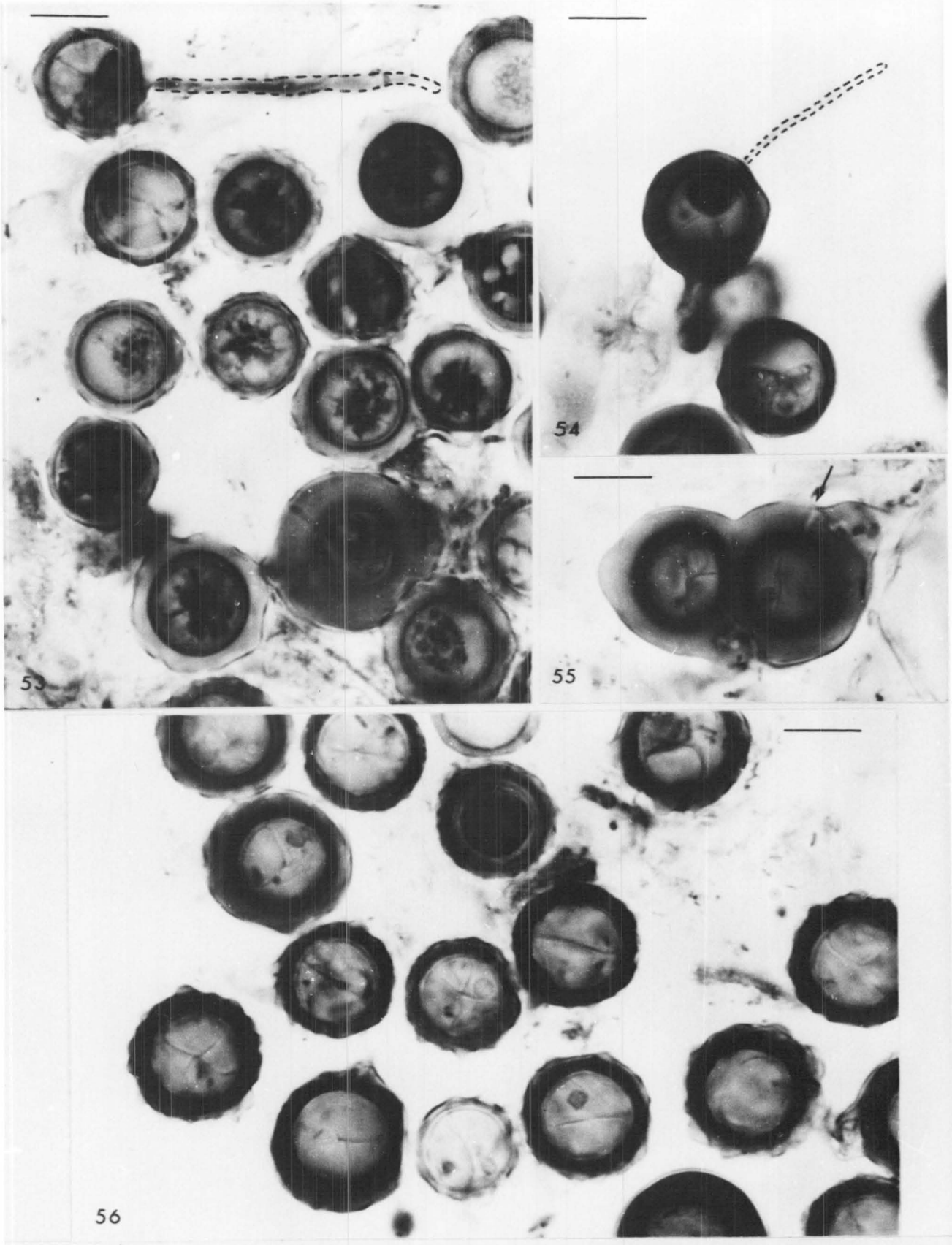
Fig. 51 Emergence of promycelia, one with a single sporidium (sp).

Fig. 52 Note sectors rounding off in one spore.

A second pregermination phase which followed nuclear division was cytoplasmic division (cytokinesis) during which the cell contents separated into 2, 3 or 4 sectors (Figs. 50, 53). At this stage definite nuclei could not be distinguished but the spores had deeply stained contents. The walls formed during cytokinesis did not appear to have any particular orientation to the spore stalk; the first formed wall appeared both transversely, parallel and at various angles to the stalk. Cytokinesis was not always visible in germinating spores; it may have been absent or possibly obscured by the thick spore wall.

In several cleaved spores germ tubes were emerging (Figs. 51, 53, 54); each germ tube arose from one cytoplasmic sector. There seemed to be a tendency in some spores for these sectors to round off (Figs. 52, 54). In a number of germinating spores some sectors not associated with germ tubes appeared to have lost their contents (Figs. 53 & 54); these sectors were presumed to have already germinated. Many spores showing conspicuous cleavage lines had probably completed the germination process (Figs. 55 & 56); in Fig. 55 a channel in the spore wall indicates the position where a germ tube has emerged and then possibly degenerated. These spores appeared to be empty or to have only fragments of cytoplasm.

Germinating spores were either thin-walled (Fig. 49) or thick-walled (Fig. 51). They produced either 1, 2, 3 or 4 promycelia, usually 30-60 µm long and 1.5-2.5 µm wide (Figs. 57-59). The promycelia were invariably aseptate, regularly vacuolated except at the tip and almost invariably unbranched; occasionally, however, branches occurred. The promycelia were constricted as they passed through the spore wall to 0.5 µm or



Figs. 53 - 56 Spores germinating <u>in situ</u>: Smiths Creek collection.

Fig. 53 Spore (at top) with emerging promycelium; 2 sectors appear empty.

Fig. 54 Emergence of promycelium from an 'active' cytoplasmic sector.

Fig. 55 Post-germination stage in a double spore showing position of promycelial emergence (arrow).

Fig. 56 Germinated spores showing cleavage lines and little evidence of contents.

less (Fig. 51). The positions of promycelial emergence were not evident as definite germ pores on ungerminated spores and appeared to have a random orientation with respect to the spore stalk.

Sporidia were observed terminally on the promycelium or laterally, just below the tip (Figs. 57-61); more often they were already detached (Fig. 62). Up to 4 sporidia were produced on each promycelium (Figs. 58-61). The sporidia were long, slightly tapered towards each end, aseptate, and frequently either curved or looped into various distinctive forms (Figs. 57-62). This made their real length very difficult to measure; nevertheless, their size appeared to vary between 12-35 μm x 1.0-1.8 μm, usually ca. 20-25 μm x 1.5 μm. When sporidia were not distinctively curved and produced singly, they were often difficult to distinguish from the promycelium (Fig. 57). However, a septum separating off the sporidium could sometimes be detected at high magnifications and the sporidium could often be identified by the fact that it tapered towards the ends. For some reason, promycelia bearing sporidia had a diameter slightly smaller than that of other promycelia (these may still have been developing) and closer to that of the sporidia. The sporidia were vacuolated when mature.

Sporidial conjugation or further development was not observed.

3.2. Germination on Agar Media

Germination was noted after 3 days for some spores on all media. The same pattern of germination occurred throughout but as contamination was less on tap water agar most of the observations were carried out on that medium.

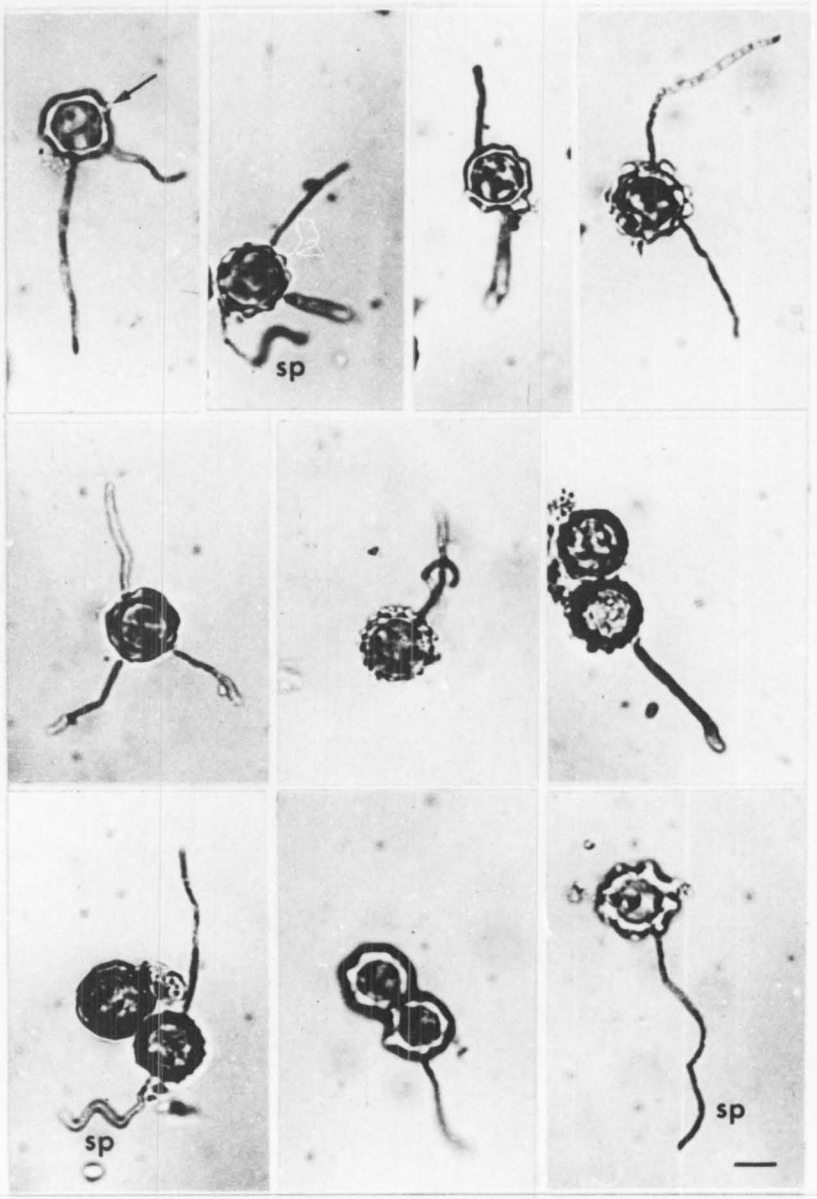
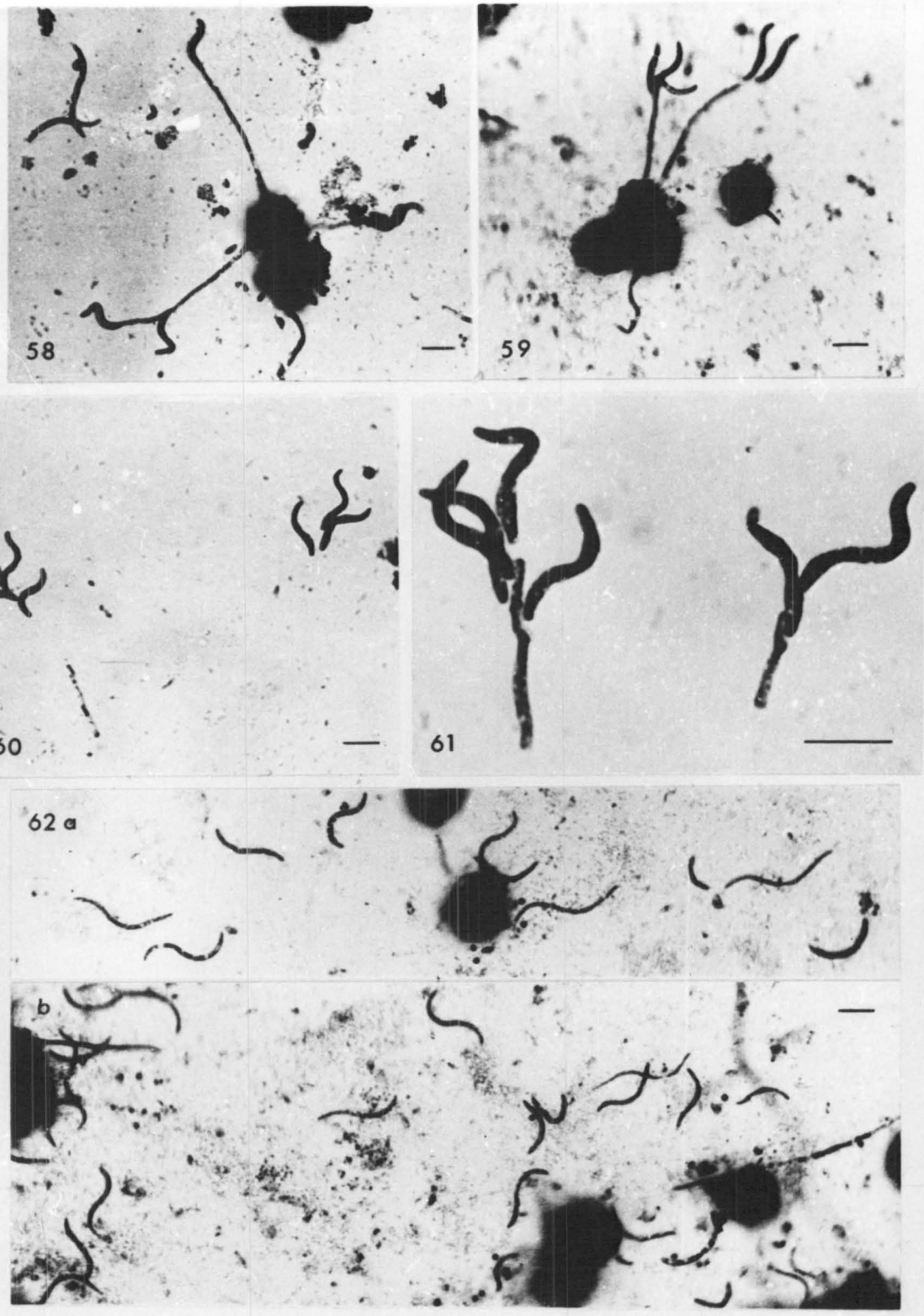


Fig. 57 Spore germination in situ showing range in numbers of promycelia produced; cytoplasmic cleavage (arrow) and sporidia (sp) evident for some spores: Pigeon Bay - Port Levy collection.



Figs. 58 - 62 Spore germination *in situ* after several months at 7°C.

Fig. 58 Spore with 3 promycelia each with up to 2 sporidia.

Fig. 59 Two promycelia emerging from one spore, one with 3 sporidia.

Fig. 60 Sporidia remaining in clusters after lysis of the promycelia.

Fig. 61 Sporidia at higher magnification; note lack of septation and both terminal and sub-terminal points of attachment to the promycelium. Four sporidia are present on one promycelium.

Fig. 62 a, b Detached sporidia showing range in form and size.

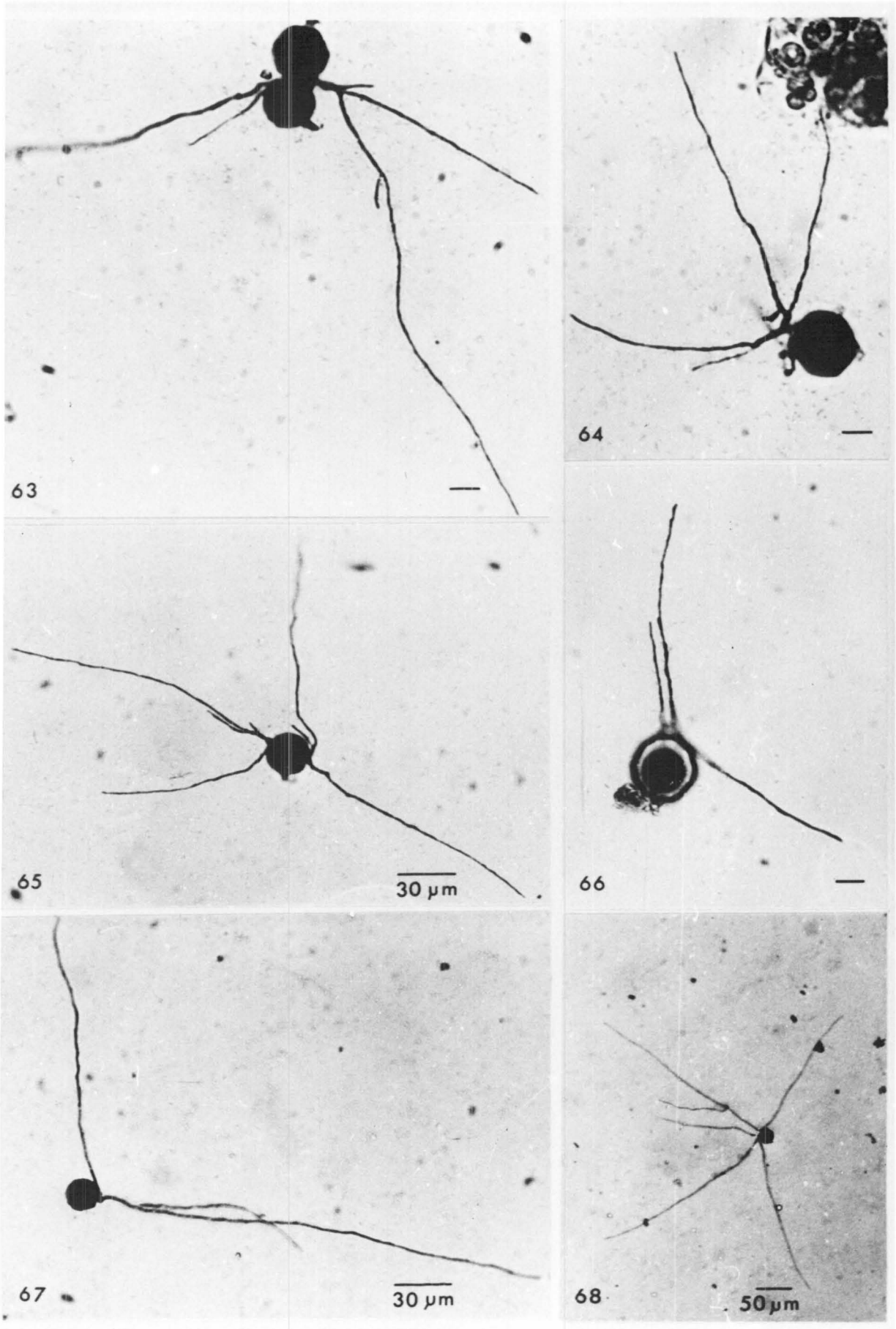
Germination was conspicuously different from that occurring *in situ*. The germ tubes, instead of having a determinate length, grew indefinitely forming septate, branched hyphae (Figs. 63-69). Septa were evenly spaced (Fig. 70) and branches were directed both forward and backward (Fig. 69). No sporidia were produced. Cytoplasmic division was evident in some spores (Fig. 71).

The number of germ tubes formed varied from 1 to 4. However, in several spores a larger number of hyphae radiating from the spore was evident (Figs. 68 & 69). In these spores and many others the germ tubes branched almost immediately after emerging from the spore wall (Figs. 71-73); at the point of branching the germ tube often formed a small vesicle (Figs. 72 & 73).

The hyphae arising from germinating spores were up to 3.0 µm wide and after 3 days had reached a maximum length of ca. 250 µm. After 6 days hyphae up to 700 µm in length were recorded. Further observations on the growth of these hyphae were prevented because of excessive contamination.

4. DISCUSSION

The method of germination of *E. casparyana* teliospores *in situ* closely follows the pattern described in detail by Weber (1884) for *E. aschersoniana*. The length of the sporidia in *E. casparyana* (usually 20-25 µm) is greater, however, than that described by Weber for *E. aschersoniana* (up to 12-15 µm in his figures). This may be a specific difference. The present study also shows that sporidia occur not only singly on the promycelium as Weber described and in 2's and 3's as



Figs. 63 - 68 Spore germination after 3 days on tap water agar to show range in form and length of hyphae. Note branching and lack of sporidia.

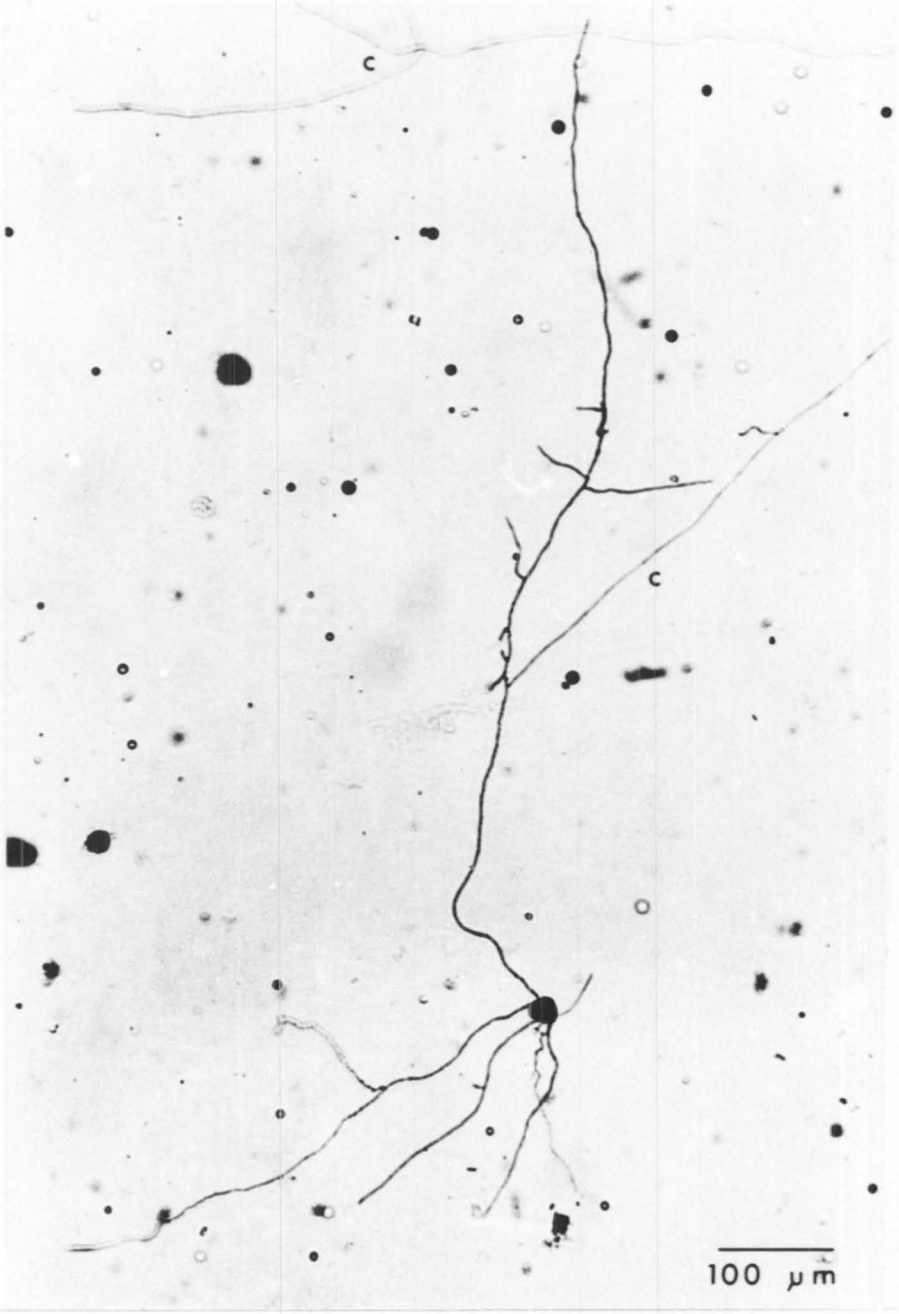
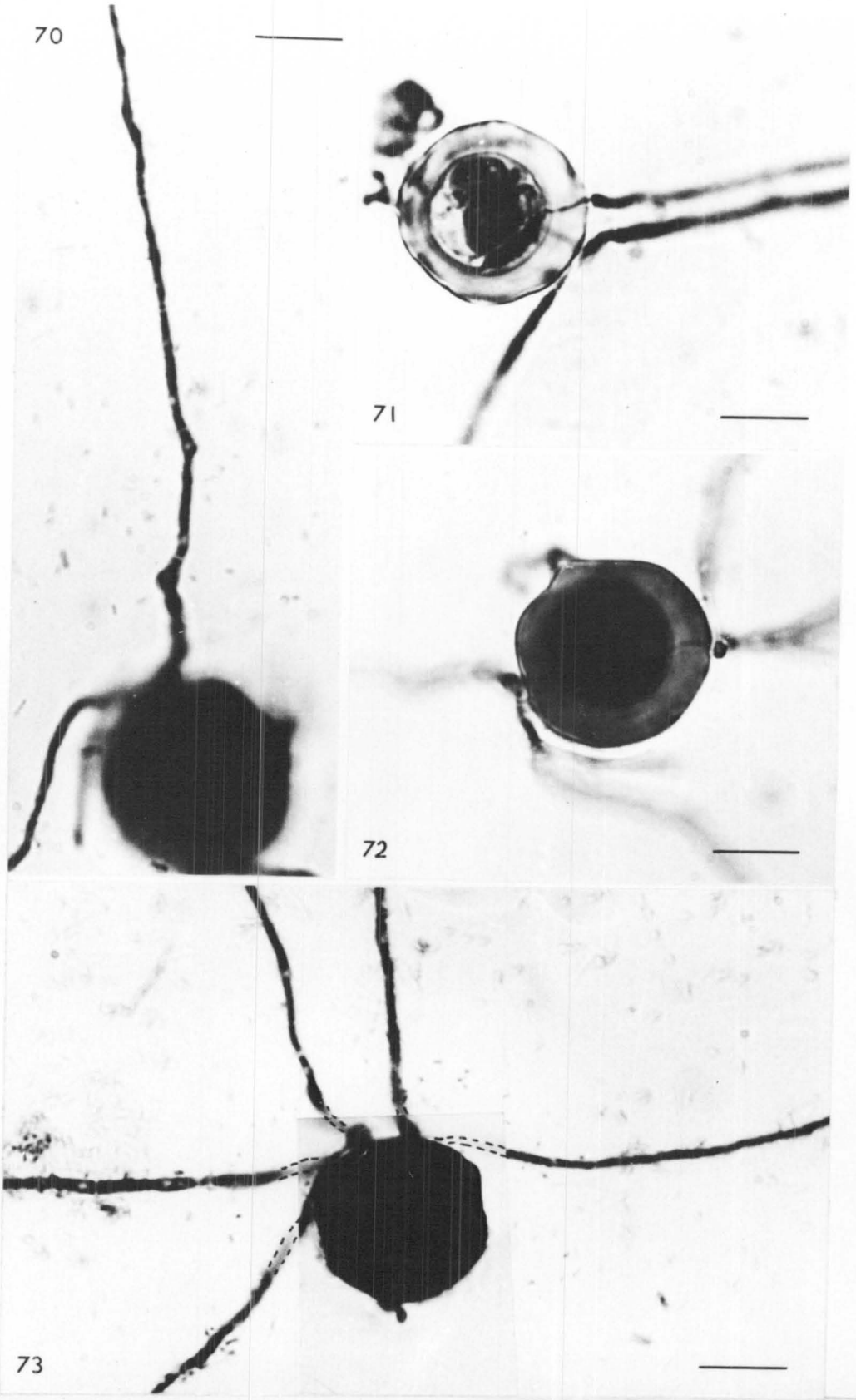


Fig. 69 Germinating spore after 6 days on tap water agar showing length of hyphae. c = contaminant hyphae.



Figs. 70 - 73 Higher magnification of some spores from Figs. 63 - 68.

Fig. 70 Shows regular septation of germ tube.

Fig. 71 Note cytoplasmic division and constriction of the germ tubes as they pass through the spore wall.

Figs. 72 & 73 Small vesicles are present at the point where germ tubes emerge, and branching occurs from these points

described by Thirumalachar and Whitehead (1968) but also in 4's. As the sporidia are readily detached from the promycelium it is difficult to know whether the number of sporidia formed per promycelium varies between 1 and 4 or whether the lower numbers are a result of some sporidia becoming detached.

Weber noted occasional septation in the promycelium but this was almost certainly due to ageing and retraction of contents. Thirumalachar and Whitehead recorded that promycelia were septate but gave no detailed descriptions of their observations. In the present study promycelia have invariably been aseptate supporting Weber's observations for the majority of spores. His results have, however, been misinterpreted by many reviewers who record the promycelium as being septate.

The cytoplasmic division of the spore contents described here for E. casparyana has not previously been reported. It is easily missed with light microscopy and so it may well have been present in germinating spores of E. aschersoniana but not detected.

The remarkable similarity in the pattern of germination for the two species described in detail so far (E. aschersoniana and E. casparyana) indicates that this mode of germination may be distinctive for the genus. On this basis there appears to be little reason for following the decision of Thirumalachar and Whitehead (1968) to include Entorrhiza under Melanotaenium, a genus where spore germination although poorly known is completely different.

The mode of germination also confirms that Entorrhiza is best placed in the family Tilletiaceae of the Ustilaginales. The genus has the basic characteristics of the Tilletiaceae i.e. teliospores which produce aseptate promycelia with a

whorl of terminal sporidia. The partitioning of the cytoplasm into four and the production of up to four promycelia, however, appears to be unique among the smut fungi. The only member of the Tilletiaceae listed by Ainsworth and Sampson (1950) as having more than one promycelium is Entyloma calendulae where "in rare cases two promycelia developed from one chlamydospore". However, Urocystis occulta is also recorded as having 2-3 germ tubes, sporidia occurring on the stronger promycelium (Kühn, 1858 cited by Blizzard, 1926). Also, some species of Ustilago may have more than one promycelium but this is an unusual occurrence; Leach et al. (1946) have reported that certain physiological forms of U. striiformis may have several promycelia or germ tubes.

Entorrhiza resembles most other smut fungi in lacking definite germ pores.

The pattern of germination obtained for E. casparyana on solid media was entirely different from that occurring in situ under both field and laboratory conditions. Promycelia and sporidia were not formed; in their place germ tubes gave rise to highly branched, septate hyphae. In this respect germination was not unlike that described by Brefeld (1912) for Entorrhiza in liquid media. Brefeld, however, also described the production of conidia.

From the limited information available it is not possible to deduce what culture conditions were responsible for altering the form of germination. The two different patterns of germination were obtained with spores from the same gall indicating that the environment plays a major role in determining the form of germination. Direct germination by the production of septate highly branched hyphae has not been observed under any

other conditions.

In the present study the form of germination would appear to have been determined by the solid nature of the medium. However, this does not explain Brefeld's observations; Brefeld used liquid media throughout his experiments. The nutrient status of the media does not seem to be implicated; in this work and in Brefeld's studies both water and nutrient media have been used in spore germination experiments and no differences in the morphology of germination have been recorded. Finally, it is possible that spores germinating after their removal from the gall do so in a different manner from those germinating *in situ*.

Nevertheless, irrespective of the cause of the change in pattern it is important to note the effect that culture conditions may have on spore germination. This is of considerable importance taxonomically and must be borne in mind when interpreting results and making comparisons within the Ustilaginales. It also gives no support to Cunningham's (1924) suggestion of establishing a third family for forms which germinate directly.

C. ULTRASTRUCTURAL CHANGES ASSOCIATED WITH GERMINATION

1. INTRODUCTION

The ultrastructural changes associated with the germination of fungal spores have been briefly reviewed by Hawker (1965, 1966), Bracker (1967) and Bartnicki - Garcia (1968) and much of the literature is summarised by Fletcher (1971).

Many of the studies have been concerned with spores which germinate readily; such spores are often relatively thin-walled. These studies include sporangiospores (Hawker & Abbott, 1963; Ekundayo, 1966; Hawker, 1966; Bartnicki - Garcia et al., 1968; Buckley et al., 1968); conidia (Hawker & Hendy, 1963; Akai et al., 1966; Hawker, 1966; Marchant, 1966 a, b; Tanaka, 1966; Remsen et al., 1967; Campbell, 1970; Border & Trinci, 1970; Hawker et al., 1970; Mitchell & McKeen, 1970; Fletcher, 1971; Gull & Trinci, 1971; McCoy et al., 1971; Richmond & Pring, 1971); ascospores (Hashimoto et al., 1958; Conti & Naylor, 1960; Das & Black, 1971 cited by Black & Gorman, 1971); and basidiospores (Voelz & Niederpruem, 1964; Aitken & Niederpruem, 1970; Stocks & Hess, 1970; Heintz & Niederpruem, 1971).

Thick walled spores, which are often capable of long periods of survival, have been studied less frequently eg. uredospores (Williams & Ledingham, 1964; Manocha & Shaw, 1967; Sussman et al., 1969), aeciospores (Walkinshaw et al., 1967), teliospores (Hess & Weber, 1971; Robb, 1972) and ascospores of Neurospora (Lowry & Sussman, 1968).

Most of these studies examine spores during and shortly after germination (defined as the appearance of a germ tube);

very few examine changes prior to germination (see Robb, 1972).

Spore germination in Entorrhiza has been observed with the electron microscope mainly to elucidate the details of a rather unusual type of germination process (see B. 3) which is difficult to examine at the light microscope level because of the thick spore wall. At the same time it is hoped to add to the knowledge of spore germination in the Ustilaginales and in resting spores generally.

2. MATERIALS AND METHODS

The material of Entorrhiza casparyana used in this study was collected on Juncus articulatus from Smiths Creek and from Erewhon station, Upper Rangitata, Canterbury, 5 December, 1971. Degenerating galls containing germinating spores were selected for study; spores from these galls were also examined by light microscopy (see B.3).

Material of Entorrhiza caricicola on Carex gaudichaudiana, north-west shore of Lake Heron, Canterbury, 5 December, 1971 was also examined briefly.

The material was fixed according to Karnovsky's (1965) schedule:

> 2% glutaraldehyde plus 3% paraformaldehyde in 0.025 M phosphate buffer, pH 7.0, for 4 hours at 4°C, with evacuation for the first 30 minutes at ca. 60 cm Hg pressure; washed in 3 changes of phosphate buffer and left in the last change overnight.

The material was then post-fixed in 1% OsO_4 in 0.025 M phosphate buffer for 3 hours at 20°C, dehydrated in alcohol for $\frac{1}{4}$-1 hour at each of 25% stages and embedded in Spurr's (1969)

resin, polymerised at 70°C for 20 hours.

The material was then sectioned, mounted, stained and examined as described earlier (see Chapt. 3.2).

3. RESULTS

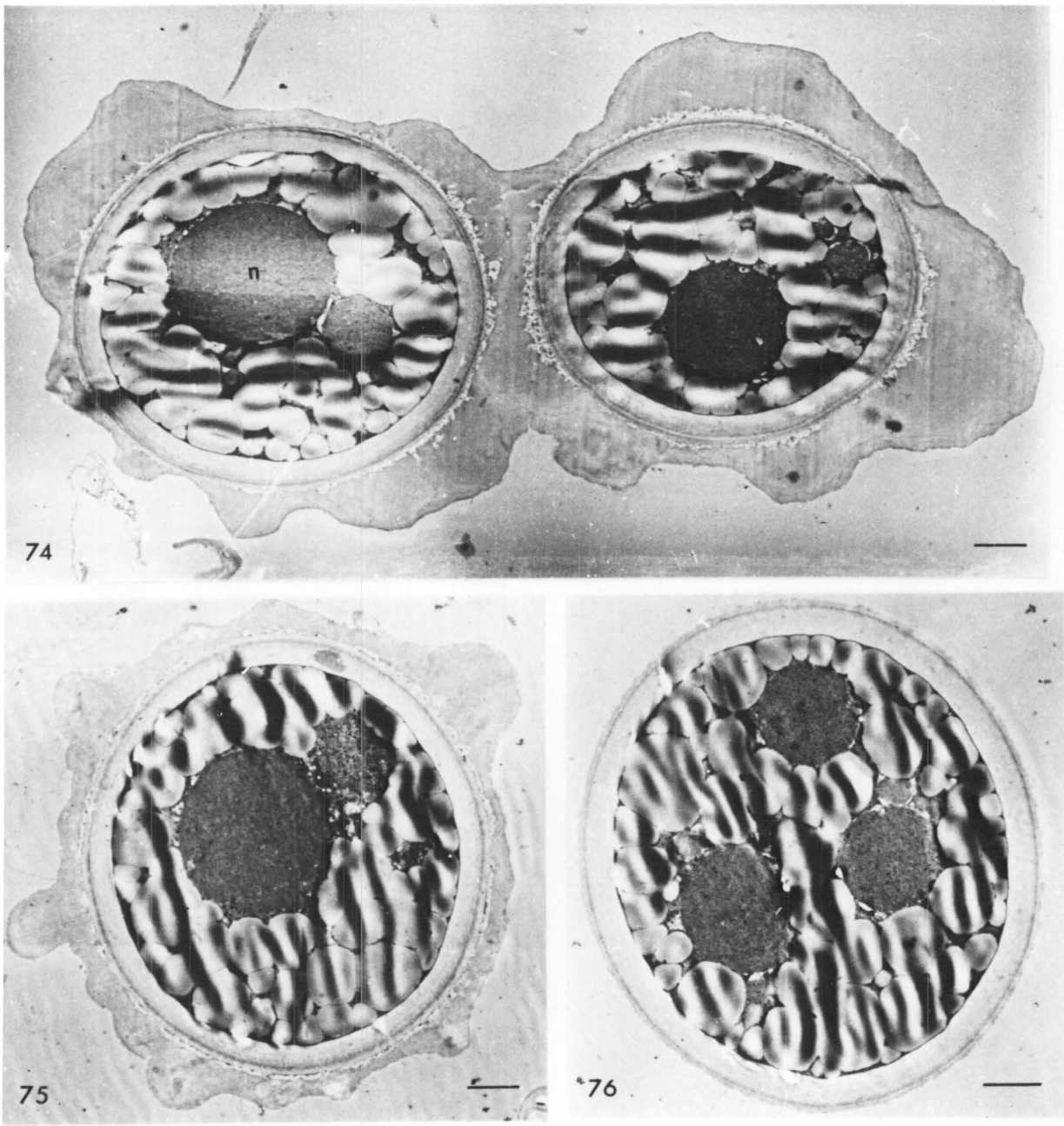
Spores which are preparing for germination are easily distinguished from dormant spores because their contents fix more readily, probably due to the increased permeability of the spore wall associated with germination. Furthermore, there is an increase in cytoplasmic organelles at the expense of lipid which is correlated with the increased activity of the spore. In dormant spores the cytoplasmic contents are extremely difficult to fix and are either lost completely during sectioning or show only a mass of lipid (see Chapt. 3. 3.2.3).

In spores of Entorrhiza there appear to be two main phases of activity preceding the emergence of the germ tube or promycelium; these are karyokinesis followed by cytokinesis.

3.1. Karyokinesis

Spores undergoing nuclear division show from two to seven nuclei in a single section of the spore (Figs 74-79). The nuclei appear to decrease slightly in size as their number increases; where only two nuclei per spore are evident (Figs. 74 & 75) they may be slightly larger than 3.0 µm in diameter; in spores with at least seven nuclei (Fig. 79) they measure 1.2-2.0 µm in diameter. Not all nuclei will be sectioned medianly, however.

The nuclear envelope is not often evident as a double membrane (Fig. 80) although in one spore a nucleus with nuclear



Stages associated with germination in Entorrhiza spores: Figs. 74 - 85 of E. casparyana (cf. Figs. 49-56) and Figs. 86 & 87 of E. caricicola.

Abbreviations: er - endoplasmic reticulum; l - lipid body; m - mitochondrion; n - nucleus; v - vacuole.

Scale: line on prints represents 1 μm.

Fixation: aldehyde - OsO_4 (Karnovsky, 1965).

Figs. 74 - 79 Increase in nuclear number preceding germination: Erewhon collection.

Fig. 74 Nuclei in a double spore.

Fig. 75 Nuclei in a thick walled warty spore.

Fig. 76 Nuclei in a thin walled smooth spore.

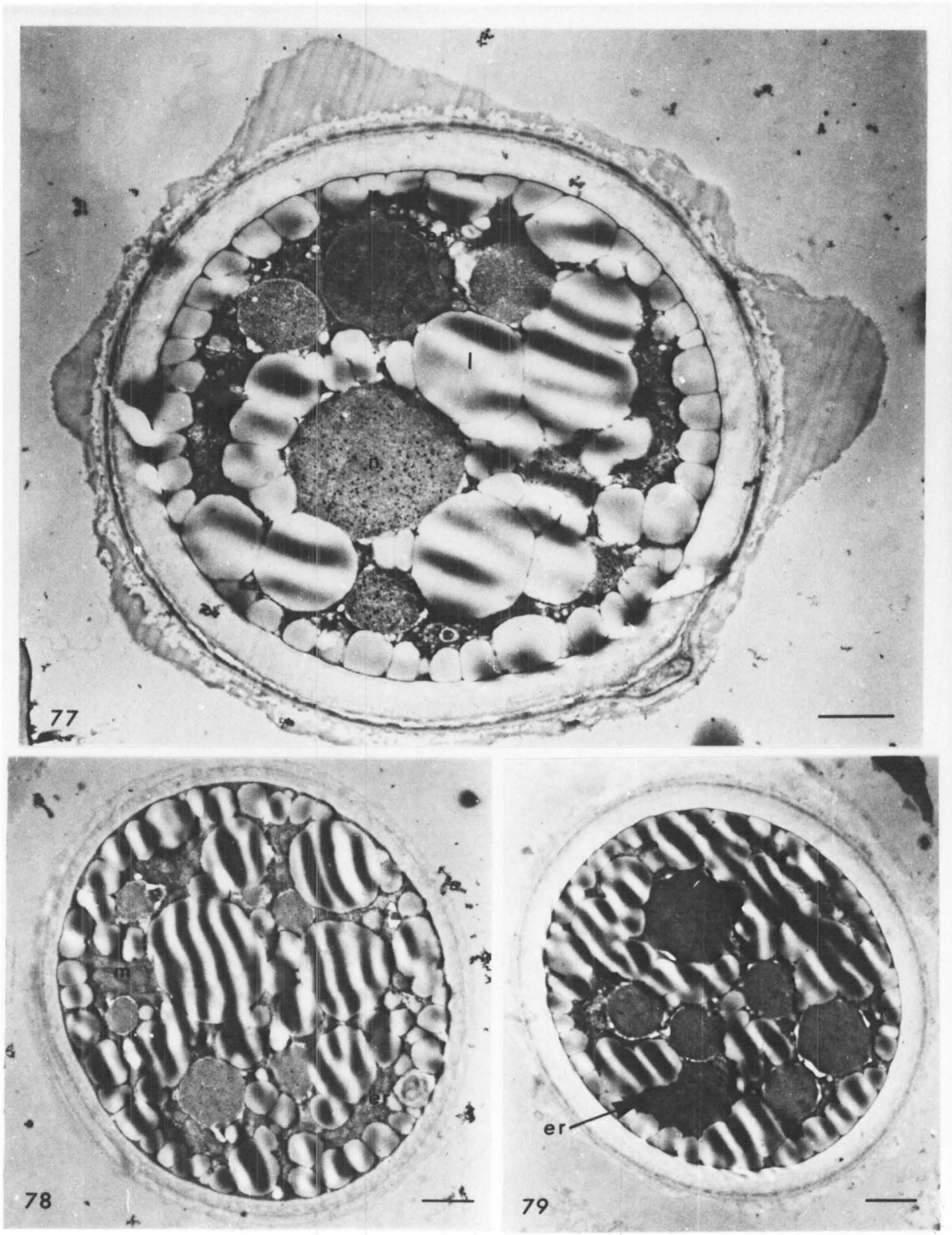


Fig. 77 Several nuclei in a warty spore; note stacks of ER associated with nucleus having a definite nuclear envelope.

Figs. 78 & 79 Several nuclei in smooth walled spores.

Fig. 78 Shows indistinct mitochondria and ER.

Fig. 79 Shows indistinct ER stack adjacent to one nucleus.

envelope is apparent (Fig. 77). At lower magnifications the membranes can sometimes be seen to have separated. This is probably due to fixation problems associated with such thick-walled spores. Nuclear pores and nucleoli are not apparent.

The lipid bodies form the most abundant component of the spore protoplast. Their density is indicated by the chatter produced during sectioning. When only a few nuclei are evident (Figs. 74-76) lipid packs the spore and there is very little sign of other contents. In spores with larger numbers of nuclei (Figs. 77 & 78), lipid and nuclei are not so tightly packed and there is evidence of other organelles and inclusions within the cytoplasm. At this stage there appears to be an increasing tendency for lipid bodies to aggregate around the periphery of the cytoplasm.

ER sometimes appears as conspicuous membrane stacks adjacent to the nuclear envelope (Figs. 77, 79). These may superficially resemble Golgi bodies (dictyosomes). Elsewhere ER is not usually evident although short profiles occur in the cytoplasm of some spores (Fig. 78). At higher magnifications ER can often be seen in close proximity to lipid bodies (Fig. 80).

Mitochondria cannot be demonstrated unequivocally probably due to the tightly packed lipid and the poor preservation of the cytoplasm. However, they appear to be present as lipid decreases and nuclei increase (Fig. 78). Mitochondria are possibly present here as small, oval or elongated organelles eg. ca. 0.25 x 0.5 μm and 0.2 x 1.5 μm.

An examination of the wall structure of the spores shows that nuclear division may occur in spores where the third wall layer has barely begun to develop (Fig. 76) or has developed only slightly (Figs.77-79). During the nuclear division phase the

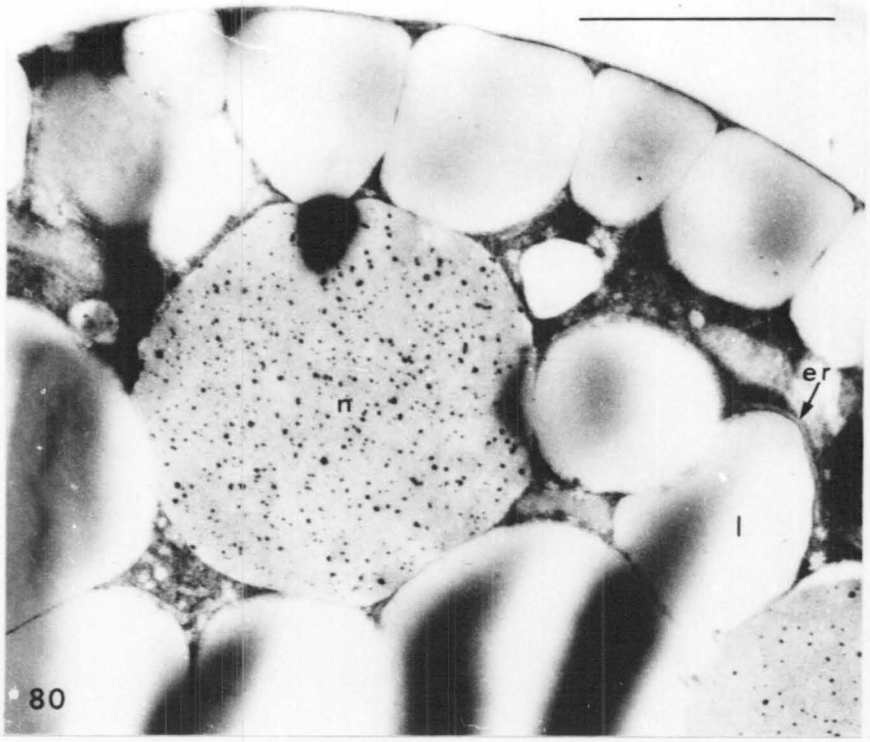


Fig. 80 Higher magnification of pregermination nucleus in a spore; there is no visible nuclear envelope. Note ER ensheathing some lipid bodies.

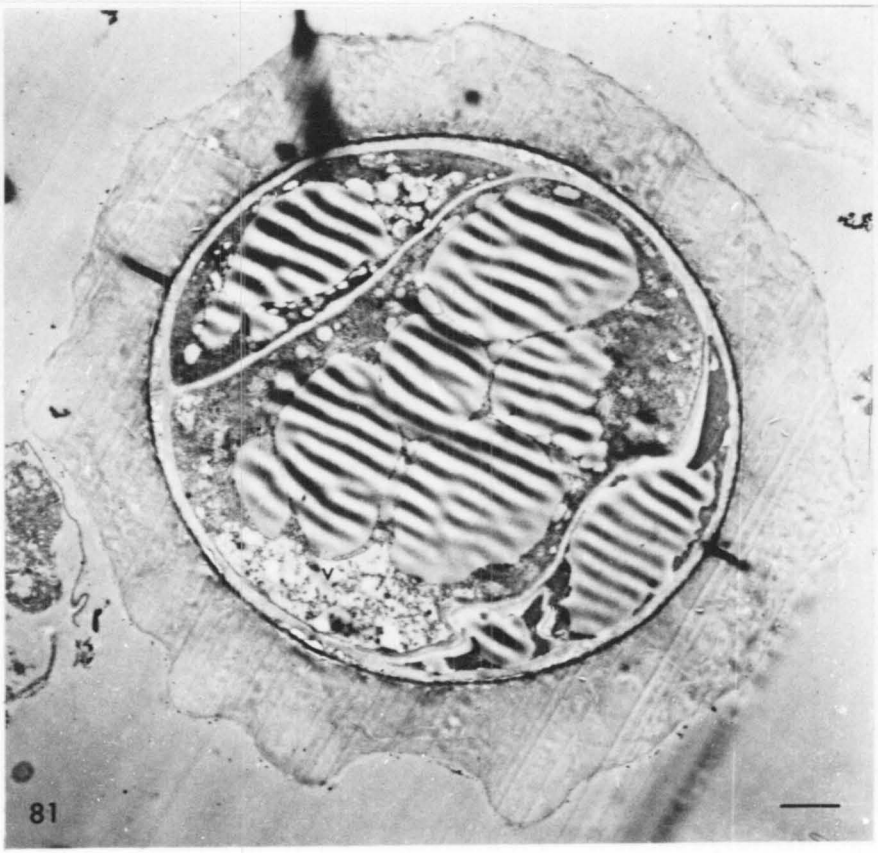


Fig. 81 Pregermination cytoplasmic division: Smiths Creek collection. Vacuoles and coalescing lipid bodies are apparent.

wall structure is basically similar in appearance to that of dormant spores (see Chapt.3. 3.2).

Nuclear division is noted in double spores (Fig. 74) as well as normal spores. The stage of development may be approximately the same in both parts of a double spore (Fig. 74) or different (Fig. 86).

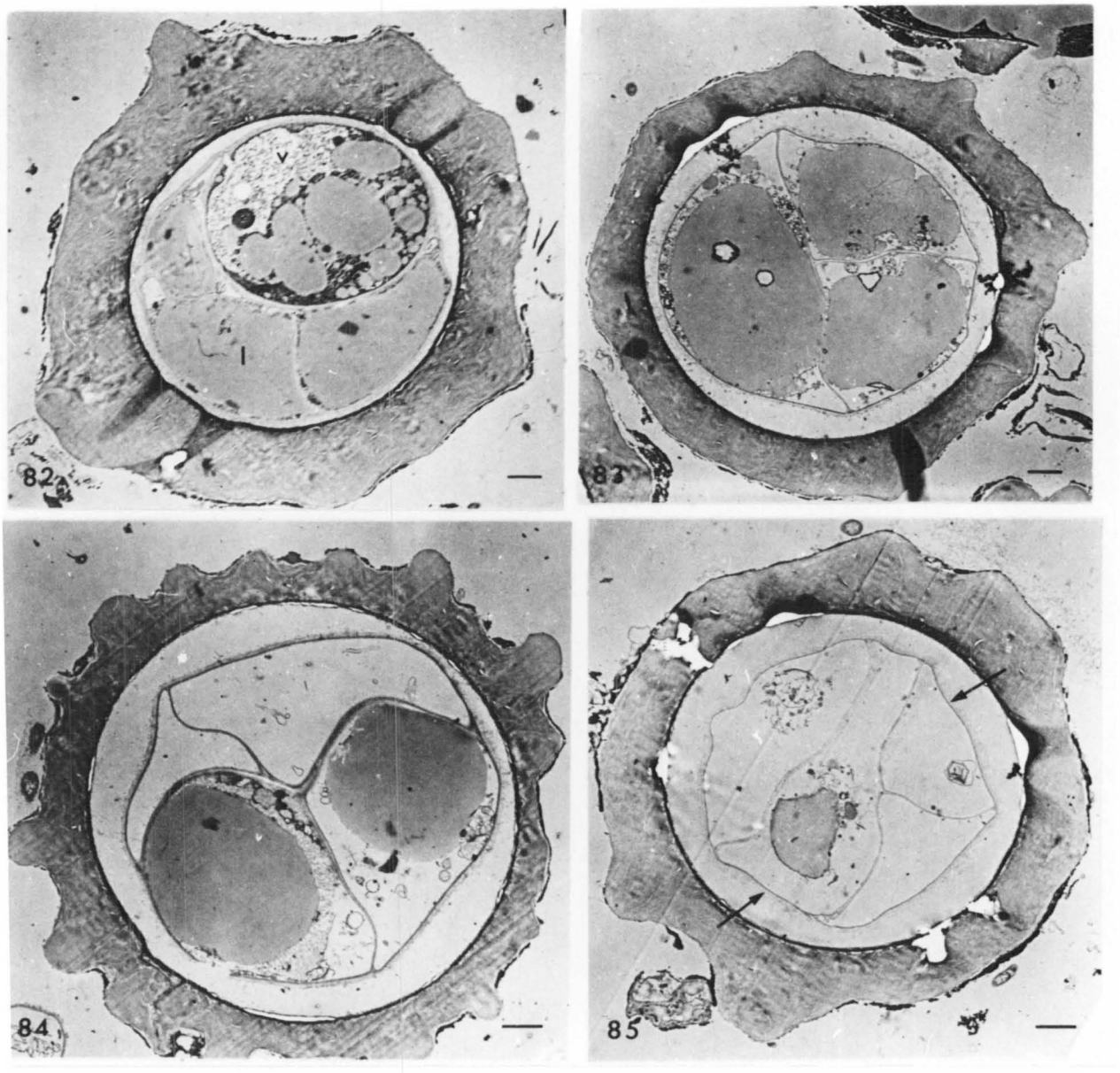
3.2. Cytokinesis

In some spores the contents have cleaved into three or four sectors (Figs. 81-85). The innermost wall layer of the spore is continuous with the wall dividing these sectors (Fig. 81) and is considerably narrower in diameter and less differentiated than it is in dormant spores or spores undergoing nuclear division. The way in which the cytoplasm divides and the mode of formation of the dividing walls has not been observed.

The most conspicuous component of the cytoplasmic sectors is the lipid. In some spores (Fig. 81) this still shows chatter because of its density and is therefore apparently structurally similar to the lipid occurring in dormant spores and during earlier stages of pregermination development. However, the lipid bodies after cytokinesis do appear to have coalesced to a certain extent and are more irregular.

Vacuoles are now present but other organelles and inclusions are difficult to identify. Nuclei are rarely seen in spores undergoing cleavage.

In some spores, one sector has rounded off (Fig. 82). The lipid now appears to have altered in structure as there are no longer chatter lines and it appears more electron dense. A vacuole is conspicuous and occupies approximately one-third



Figs. 82 - 85 Cytoplasmic division associated with germinating spores: Smiths Creek collection.

Fig. 82 One sector has rounded off and shows a conspicuous vacuole and coalescing lipid bodies. Coalesced lipid is the main component of the other sectors.

Figs. 83 - 85 Progressive degeneration of contents following germination; contents shrink from the thick spore wall.

Fig. 83 Coalesced lipid and membrane fragments.

Fig. 84 Coalesced lipid in 2 sectors; others mainly empty.

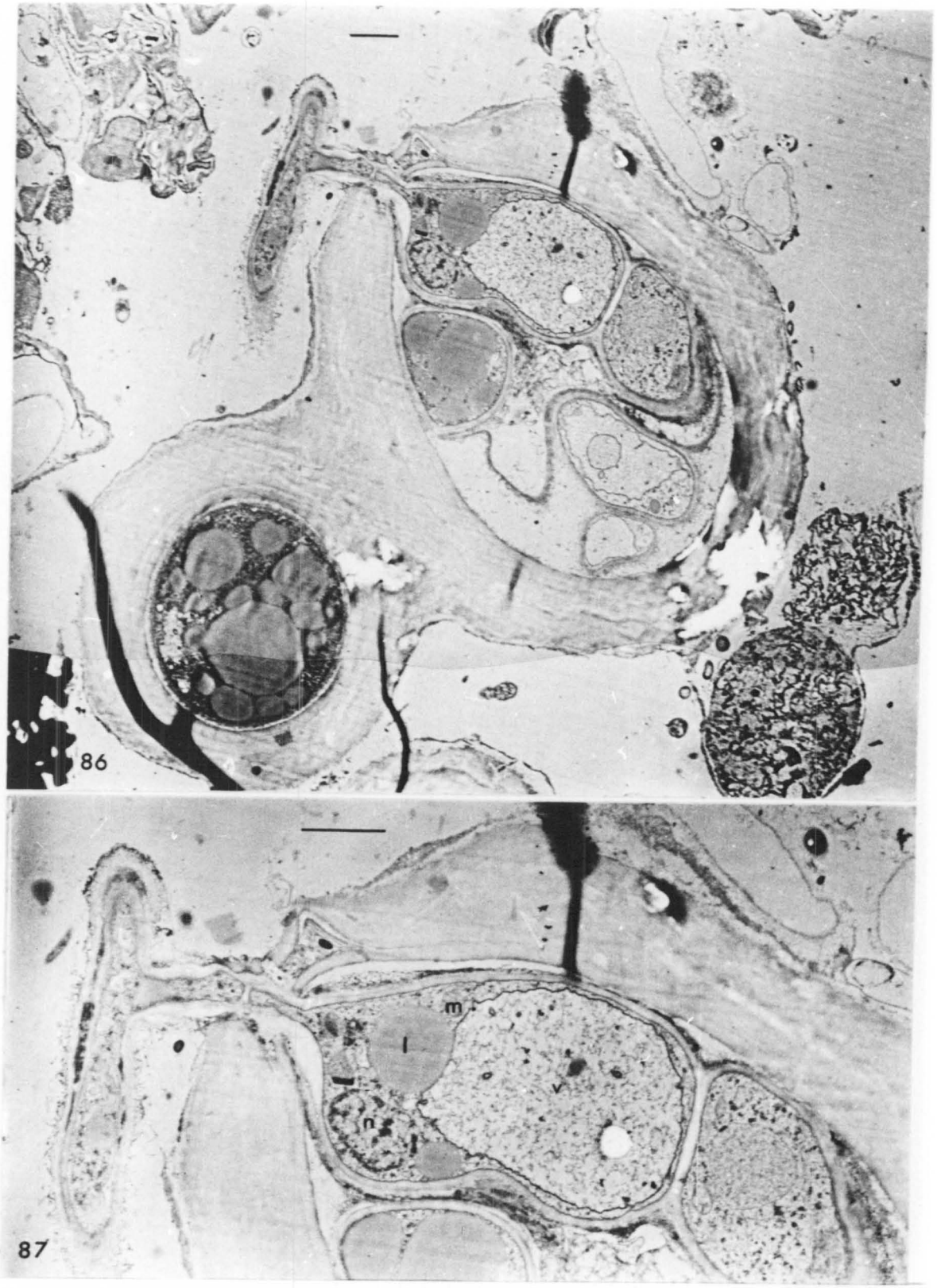
Fig. 85 Sectors with either coalesced lipid, membrane fragments or devoid of contents. Fragments of the inner spore wall layer (arrows) may still be present.

of the volume of the sector. In this spore and others (Figs. 82-85) there appears to be a progressive change in the contents of the sectors. Apart from the rounded off cell in Fig. 82, the sectors show either coalesced electron dense lipid almost filling the cell, membrane fragments or a complete absence of contents. This apparent progressive degeneration of the cytoplasm is shown in Figs. 83-85; in Fig. 83 all cells contain a central coalesced lipid body surrounded by membrane fragments; in Fig. 84, two cells of the four are in this state while the remaining two have completely, or almost completely, lost their contents; in Fig. 85, one cell has the remains of a central lipid body, two have membrane fragments and a fourth is completely empty. In spores with depleted contents the inner spore wall appears to have largely disappeared, although fragments may still be present (Fig. 84). Furthermore, there is shrinkage of the spore contents away from the 2nd and 3rd wall layers leaving them surrounded by a thin wall layer (Fig. 85) and a wide zone of indefinite nature (Figs. 83-85).

3.3. Addendum

During an examination of spores of E. casparyana none were seen in which the promycelium was actually emerging. This stage has, however, been observed for E. caricicola and a description is presented here so that the sequence of events associated with germination may be more complete. Spore germination has not been followed in E. caricicola and the description below is based on a single observation.

In the double spore of E. caricicola shown in Fig. 86 the cytoplasmic contents of one part of the spore have cleaved. The innermost wall of the spore has formed the cleavage walls



Figs. 86 & 87 Spore germination in E. caricicola.

Fig. 86 Germ tube emerging from one part of a double spore; this part has cleaved and sectors show a range of activity associated with germination. The inner spore wall layer forms the cleavage wall.

Fig. 87 Higher magnification of Fig. 86 showing germinating cell with large vacuole, nucleus, small lipid bodies and mitochondria.

(Figs. 86 & 87). From one cell a germ tube has emerged through the spore wall, its wall, although indistinct, being continuous with the inner spore wall.

This cell, in which germination has just taken place, has the following ultrastructural features. The plasmalemma is slightly crenulated. Within the cytoplasm at the end furthermost from the young promycelium is a large vacuole occupying approximately two-thirds of the sector. Lipid is present in its more electron dense, homogeneous form, and possibly glycogen granules. A nucleus approximately 0.75 x 1.25 μm in size and more heterogeneous in organisation than those occurring during karyokinesis is present as well as small mitochondria less than 0.4 μm in length. Short profiles of ER are evident adjacent to the lipid.

There does not appear to be a well-organised germ pore as the spore wall is ruptured where the germ tube emerges, separating the second and third wall layers. The developing germ tube is septate at the point where it begins to emerge from the spore wall.

The germ tube itself is not well differentiated. It contains lipid and various organelles which cannot be identified unequivocally. The germ tube may be slightly unusual in that it branches soon after emergence; this could be correlated with direct germination.

4. DISCUSSION

Although this work is only of a preliminary nature it elucidates and extends observations made with the light microscope.

From electron micrographs a number of stages associated with germination have been noted. The sequence in which these occur must be deduced by comparing the changes which are taking place with what is already known about spore germination in the fungi. It is on this basis that the following interpretation of ultrastructural changes during the germination of E. casparyana spores is given.

Spores of E. casparyana are binucleate (dikaryophase) during early stages of development within the host (see Chapt. 3. 3.2). At some stage nuclear fusion occurs; this stage has not been identified but occurs during the development of the spore in most smut fungi (Ainsworth, 1971). In the present study, during pregermination development, up to seven nuclei per spore were noted. These are presumed to be haploid and to have arisen from the fusion nucleus by meiosis followed by at least one mitosis; not all nuclei in a spore will be observed in one plane of section. From light microscope observations of E. casparyana it is known (see B. 3) that up to four promycelia, each capable of producing up to four sporidia, may be formed so that a second mitosis resulting in a total of 16 haploid nuclei must occur in at least some spores. Irregularities of nuclear division may account for non-multiples of four nuclei.

In the Ustilaginales where the cytological events accompanying germination have been examined, light microscope studies have shown that meiosis, especially in the Ustilaginaceae (Fischer & Holton, 1957), occurs more commonly in the germ tube (or promycelium) after the migration of the diploid nucleus out of the spore eg. Anthracoidea species (Kukkonen, 1972),

Ustilago avenae (Webster, 1970). However, in Ustilago maydis meiosis occurs partly in the spore and partly in the germ tube (Hanna, 1929). In an ultrastructural study of the pregermination development of Ustilago hordei (Robb, 1972) the nucleus remains diploid in the spore; in contrast, Fischer and Holton (1957) have reported that the first nuclear division occurs in the spore. There are also conflicting reports for Tilletia caries, Ainsworth and Sampson (1950) stating that meiosis occurs in the spore and Smith (1955) that it occurs in the promycelium. Holton (1935) found that the distribution of nuclei between spore and promycelium differed in two races of T. caries.

It would appear that very few critical studies of the cytology of germination in the smut fungi have been undertaken. Observations with the light microscope are difficult because the spores have thick, often heavily pigmented walls and studies with the EM have been confined to those of Hess and Weber (1971 unpublished) for T. caries and Robb (1972) for U. hordei. Apart from the teliospores of rust and smut fungi, meiosis does not often accompany spore germination in non-motile fungi; meiosis is involved in the germination of zygospores of some Zygomycetes eg. Rhizopus stolonifer (Webster, 1970) but there is an increasing body of evidence (Sansome, 1961, 1963; Bryant & Howard, 1969; Sansome & Brasier, 1973) that, contrary to previous beliefs (see eg. Allen, 1965), meiosis does not occur in the germination of oospores of the Oomycetes. In the Ascomycetes and Homobasidiomycetes, meiosis occurs during the formation of ascospores and basidiospores respectively.

The present study of pregermination development of E. casparyana spores appears to be the first ultrastructural

account of meiosis accompanying germination.

During nuclear division within the spore there is an increase in cytoplasmic activity. This reflects the increased metabolic activity generally associated with germination (Bracker, 1967) but is at a comparatively low level compared with later stages of preparation for germination. The most interesting phenomenon during this period is the association of stacks of ER membranes with the dividing nuclei. There is very little ER elsewhere in the cytoplasm. Robb (1972) found that in hydrating spores of Ustilago hordei the first formed ER was in close proximity to the nuclear envelope and was also able to demonstrate connections between the nuclear envelope and ER. Richmond and Pring (1971) and Gull and Trinci (1971) have also noted such connections in germinating conidia. Esau and Gill (1971) have shown for higher plants that the nuclear envelope serves as a source of rapidly proliferating ER membranes and the stacks of ER membranes in germinating Entorrhiza spores may arise in this way. These authors have reviewed the literature on the stacking phenomenon. The stacks of ER membranes adjacent to nuclei in E. casparyana spores superficially resemble dictyosomes. Campbell (1968) suggested that similar ER stacks in Alternaria conidia resembled simple Golgi. Black and Gorman (1971) also question whether the lamellar stacks in sporulating Hansenula wingei might not be dictyosomes. However, it is not suggested for E. casparyana that these membrane stacks are the functional equivalents of Golgi. In most fungi the development of stacks of smooth membranes appears to be associated with highly active states including nuclear division (see Black & Gorman, 1971; Gull &

Trinci, 1971; Lerbs, 1971; Robb, 1972) and this is also the situation in E. casparyana spores. In meiotic basidia of Coprinus radiatus ER complexes of a different kind are thought to be associated with restitution of the nuclear envelope (Lerbs, 1971).

Profiles of ER are also found in close association with lipid bodies. This is consistent with the role of lipid during germination; lipid is the major energy reserve utilised during the germination of many fungal spores. In fact, in rust uredospores, lipids are the only constituent which drops appreciably during germination (Allen, 1965). In Entorrhiza, the ER is probably in a position to provide the metabolically active spore with access to these reserve materials. Williams and Ledingham (1964) found a similar situation in germinating uredospores of Puccinia graminis f. sp. tritici. In these spores, vesicular forms of ER were found in direct contact with storage lipid. Nevertheless, in E. casparyana spores, lipid still occupies most of the volume of the protoplast at this stage and appears to be structurally similar to that in dormant spores.

Following nuclear division, activity becomes more pronounced in the cytoplasm. This stage is associated with cleavage of the cytoplasm into three or four sectors. Cleavage of the cytoplasm is consistent with the fact that E. casparyana regularly produces three or four promycelia. Although this is not typical of other genera of the Ustilaginales, it may, however, occur in the spores of smut fungi which occasionally produce more than one promycelium. According to the terminology of Donk and Talbot for the basidium (see Ainsworth, 1971) both probasidium (nuclear fusion) and metabasidium (nuclear division) stages occur in the teliospore of Entorrhiza casparyana. In

this smut fungus, therefore, it is not strictly correct to refer to the germ tube as a metabasidium and it is probably less confusing to use the term promycelium (see also Talbot, 1971).

In Entorrhiza the inner spore wall forms the cleavage walls which are later continuous with the walls of germ tubes. In some germinating spores eg. sporangiospores of Rhizopus sexualis, Rhizopus nigricans and Mucor rouxii a new wall is formed for the germ tube; in other spores eg. conidia of some species of Botrytis and Aspergillus, an inner spore wall layer is extended to form the wall of the growing germ tube (see Hawker, 1966; Bartnicki - Garcia, 1968). Hawker (1966) and Hawker et al. (1970) believe that the origin of the germ tube wall may indicate taxonomic relationships. However, in view of the fact that $KMnO_4$ fixation may not reveal all the details of wall structure and much of the earlier work was done with this fixative (Gull & Trinci, 1971), the distinctions between these two groups of spores may not be clear-cut and some fungi may have to be reassigned to a different group (see also Fletcher, 1971). No information is available for the smut fungi but in the rust fungi both situations seem to occur. The germ tube wall of uredospores of Puccinia graminis f. sp. tritici is derived from pre-existing spore wall material (Williams & Ledingham, 1964) but its precise origin is difficult to ascertain from their description and electron micrographs. Also for uredospores of Melampsora (Manocha & Shaw, 1967) the vegetative wall during germination is an extension of the spore wall. However, for aeciospores of Cronartium fusiforme, Walkinshaw et al. (1967) found that a new wall layer was formed for the developing germ tube. It would appear that there is

insufficient evidence so far to indicate whether the origin of the germ tube wall is of phylogenetic or taxonomic importance.

The change in the cytoplasmic contents of the spore accompanying the actual emergence of the germ tube is striking. Lipid is present but no longer conspicuous, much of it presumably having been used during the germination process; it is present in a structurally different form from that in dormant spores. Entorrhiza spores germinate in water and therefore depend entirely on their reserve materials. The presence of a large vacuole and its position furthermost from the germ tube are probably important factors in maintaining the turgidity of the cell and allowing for cytoplasmic expansion during the emergence of the germ tube. Vacuole formation is normally associated with the change from the resting to the activated condition in spores. The crenulated plasmalemma may be associated with the extension of wall material as it is in growing hyphal tips (Marchant et al., 1967).

It is difficult to determine what the exact sequence of events during germination is from the electron micrographs of cleavage in E. casparyana spores. By analogy with E. caricicola, one sector of one E. casparyana spore appears to be close to the production of a germ tube (see Fig. 82). Other sectors, however, show only a homogeneous mass of lipid, membrane fragments or a total absence of contents. These stages can be directly compared with similar stages observed with the light microscope and probably represent a progressive emptying out of the spore contents during and after the emergence of the germ tube. Whether any reserve lipid is not utilised by the growing germ tube and remains behind in the spore is unknown. Stocks and Hess (1970) found for basidiospores of a Psilocybe sp. that the entire protoplast commonly migrates out of the

old spore wall and leaves the wall as a ghost and this is probably characteristic of most fungal spores during germination.

From the electron micrographs Entorrhiza spores do not appear to have definite germ pores. This confirms light microscope observations.

This ultrastructural investigation has been carried out mainly to elucidate cytological events accompanying germination in spores of E. casparyana. Because spore germination in this species is similar to that of E. aschersoniana at the light microscope level it would seem that the observations on karyokinesis and cytokinesis made with the electron microscope for E. casparyana may be taken as characteristic of the genus. Such information is taxonomically important in view of the lack of knowledge of the details of spore germination in the smut fungi and the present uncertainty as to the taxonomic position of the Ustilaginales as a group (von Arx, 1970; Moore, 1972).

Ultrastructural changes occurring in the cytoplasm of spores preparing for germination have tended to confirm previous observations on the germination of resting spores i.e. that the basic changes taking place involve an increase in metabolic activity concurrent with the utilisation of lipid. The increase in metabolic activity is correlated with an increase in the amount of ER and mitochondria and the appearance of vacuoles but because thick-walled spores are not easily fixed for electron microscopy these features are not always well preserved.

SUMMARY

1. Teliospores germinate in situ in degenerating galls;

however, not all degenerating galls contain germinating spores.

2. Freshly collected spores could not be induced to germinate under any of the laboratory conditions tested.

3. Teliospores from mature galls stored in water or soil extract solution at 7°C for several months were induced to germinate; after 15 months germination was present in 34 of the 41 galls stored at this temperature in soil extract solution.

4. Not all collections subjected to chilling were induced to germinate; spores from infected plants grown in the glasshouse for 8½ months remained dormant. The inoculum for the plants was, however, from the same source as the spores which were induced to germinate (see 3. above). Other factors besides chilling are therefore implicated.

5. Teliospores germinate <u>in situ</u> to produce up to 4 aseptate promycelia each bearing up to 4 falcate sporidia, often distinctively looped or curved; the sporidia are terminal or sub-terminal.

6. Teliospores germinate directly on agar media forming septate branched hyphae; no sporidia are formed.

7. Karyokinesis occurs in spores prior to germination. Division is presumed to be meiotic and to be followed by at least one mitosis; up to 7 nuclei are seen in one plane of section.

8. Cytokinesis follows karyokinesis prior to germination. Up to 4 sectors are formed in the spore.

9. Several ultrastructural changes occur in spores during the pregermination period; ER, mitochondria and vacuoles appear with a concurrent decrease and alteration in appearance of lipid; this is typical of changes associated with germination in other resting spores.

10. It is concluded that Entorrhiza is a member of the Tilletiaceae i.e. promycelia are aseptate and sporidia are terminal. Moreover the genus appears to be characterised by the regular production of more than one promycelium and cytokinesis. Not only does this distinguish Entorrhiza from other genera of the Ustilaginales, including Melanotaenium, but it provides information of importance when considering the taxonomic position of the Ustilaginales as a group.

CHAPTER 6

SCANNING ELECTRON MICROSCOPY OF *ENTORRHIZA* TELIOSPORES

1. INTRODUCTION

The surface ornamentation of fungal spores is one of the main diagnostic characters for determining species. The light microscope with its comparatively low resolving power and narrow depth of focus limits the degree to which differences in spore wall ornamentation can be detected. The transmission electron microscope (TEM) with its higher resolving power has made it possible to explore surface ornamentation at much greater magnifications. However, the usefulness of this instrument has been limited by its effective depth of focus. The best results from the TEM, using modifications of the surface replica method first evolved by Bradley (1954), are only poor imitations of the much superior results that can now be obtained with the scanning electron microscope (SEM), a more recently developed instrument with an exceptionally large depth of focus (Heywood, 1971).

In the Ustilaginales, electron microscopic observations of the surface of spores were published as early as 1956 by Hille and Brandes. These investigators used the replica technique for a study of certain *Ustilago* species. The replica technique has since been used by Swinburne and Matthews (1963) for *Tilletia caries*, by Savulescu *et al.* (1964, 1968) and Dumitras *et al.* (1970) for delimiting species of *Tilletia*, by Banerjee *et al.* (1969) for *T. caries* and some other smut fungi, and by Khanna *et al.* (1966) and Khanna and Payak (1971 a, b) for *Melanopsichium eleusinis*, *Neovossia indica*, *Sphacelotheca reiliana*, *T. caries* and *Ustilago nuda*. The methods used by

these workers were mainly modifications of the carbon replica technique evolved by Bradley and the gum replica technique evolved by Hess and Schantz (1956).

Spore surfaces may also be examined directly with the TEM. Hille and Brandes (1956) observed that this gave reasonable results for Ustilago species. Savulescu et al. (1968) and Dumitras et al. (1970) have also studied Ustilago and Tilletia spores directly with the TEM. They found for Ustilago species that more precise information could be obtained by direct examination than by the examination of carbon replicas because the delicate carbon film was often broken by the spines on the spore surface.

In addition, surface views of fungal spores may be obtained by freeze-etching. Weete et al. (1969) and Allen et al. (1971) have used this technique to study teliospores of Sphacelotheca, Ustilago, Urocystis and Tilletia caries. Although this technique yields good results it is unpredictable as fracturing of the spore may occur at a variety of levels and may only occasionally reveal the spore surface.

The technique which produces the best images of spore ornamentation is scanning electron microscopy. Very little preparation of the material is necessary for examination in the SEM and the results obtained are depicted in 3 - dimensional form. As Hawker (1971) has mentioned, this technique has not yet been widely used for fungal spores. In the Ustilaginales, Kukkonen (1969) used the SEM to study spore ornamentation in Anthracoidea species, Banerjee et al. (1969) examined Tilletia caries and some other smut fungi, Moore and Grand (1970) and Grand and Moore (1970) included Ustilago and Cintractia junci spores in a survey of basidiomycete genera and most recently

Zogg and Schwinn (1971) have surveyed the spores of a large number of genera representative of the Ustilaginales.

The advances that have been made in the techniques available for studying spore ornamentation can probably best be assessed by examining micrographs of Tilletia caries, a species which has been studied using techniques discussed above: a Perspex-Rose's alloy replica method (Swinburne & Matthews, 1963), the gum replica technique (Khanna et al., 1966), the carbon replica technique (Savulescu et al., 1968), freeze etching (Allen et al., 1971), and scanning electron microscopy (Zogg & Schwinn, 1971). With the gum replica technique some distortion is evident and the fine detail present in SEM photographs is not revealed. Swinburne and Matthews' method, the carbon replica technique and freeze etching provide relatively good images but these techniques lack the simplicity of specimen preparation of scanning electron microscopy and the replicating methods are less likely to be free from artefacts.

The work of Zogg and Schwinn (1971) has shown that the SEM reveals some new structures as well as fine detail of already known structures. This increased detail may be of taxonomic value. Earlier, Savulescu et al. (1968) and Dumitras et al. (1970) had attempted to elucidate taxonomic problems involving certain species of Tilletia and Ustilago, using the additional information on teliospore surface markings provided by the TEM. They believe that some species regarded by Fischer (1953) and Duran and Fischer (1961) as synonymous should be retained as separate entities on the basis of small morphological differences revealed by the electron microscope. In their study, however, they do not adequately examine the natural variability within a species; for Tilletia species

variability is assessed only on the basis of one collection cultivated on two different wheat varieties for up to three successive years.

Kukkonen (1969) found for Anthracoidea section Echinosporae that results from the SEM reinforced taxonomic distinctions based on light microscope observations of spore structure, rather than adding new information. In the section studied, structural differences between species were small and apparently showed many gradations. Evidence indicates that the species of the genus Anthracoidea are inbreeding and self-fertile (Kukkonen & Raudaskoski, 1964) which possibly explains these constant but small morphological differences.

The present study was carried out to elucidate taxonomic problems within the genus Entorrhiza. Where possible type material has been compared with collections made on a variety of hosts and/or from several different localities; from this and from complementary light microscope studies the variability of surface ornamentation could be assessed. Species which were apparently synonymous on the basis of observations with the light microscope were critically examined.

Entorrhiza spores have previously been studied briefly with the SEM by Zogg and Schwinn (1971) who included a micrograph of E. aschersoniana in their survey of genera representative of the Ustilaginales.

2. METHODS

The spores were scraped from the galls on to double-sided adhesive tape mounted on standard specimen stubs. Spores

from fresh material were allowed to air dry. The stubs were transferred to a high vacuum (1 x 10^{-5}torr) evaporating unit and lightly coated with about 20 nm of carbon and then 20 nm of gold - palladium alloy (60:40) while being rotated at 150 rpm. The specimens were examined and photographed with a Cambridge Stereoscan Series 2 scanning electron microscope.

Measurements were made, as accurately as the magnifications allowed, from micrographs of single spores (ca. x 4,000 -6,000). Micrographs were taken at lower magnification (ca. x 1,300) to show the variability within individual collections and at higher magnifications (ca. x 10,000 - 11,000) to show surface detail of individual spores.

The collections examined have been listed as identified on herbarium labels; the international symbol of the herbarium in which the material is deposited is given in parentheses. New Zealand collections are listed under the species to which they have been attributed by the author on the basis of light microscope observations; where identification was doubtful the species has not been designated.

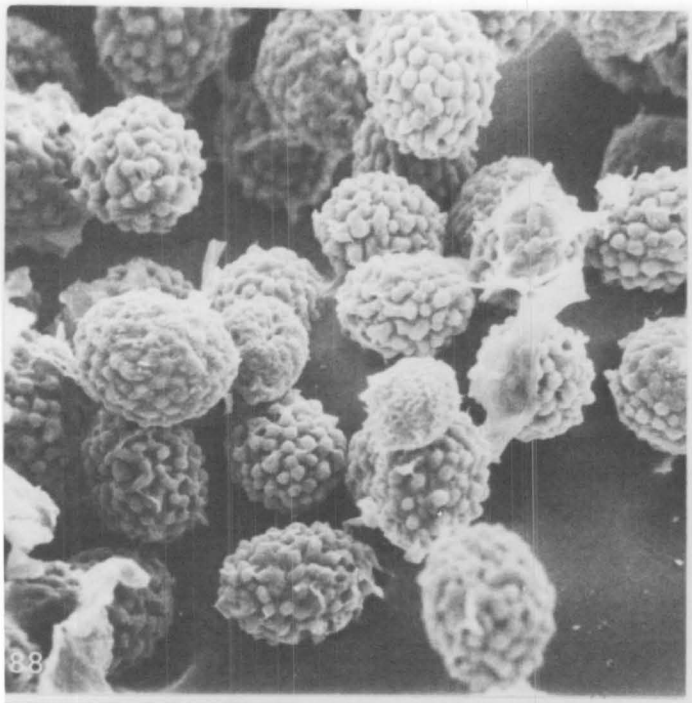
3. RESULTS

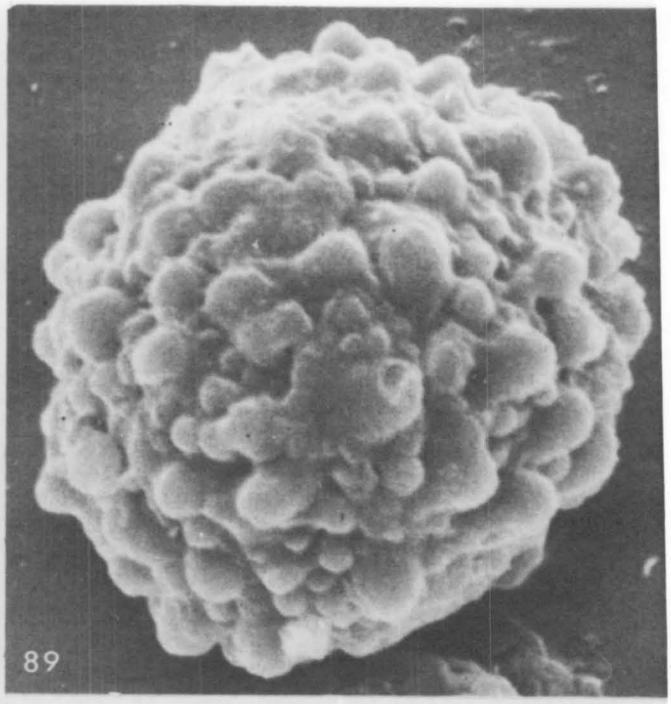
The micrographs taken at higher magnifications provided no additional information and are not included in the results.

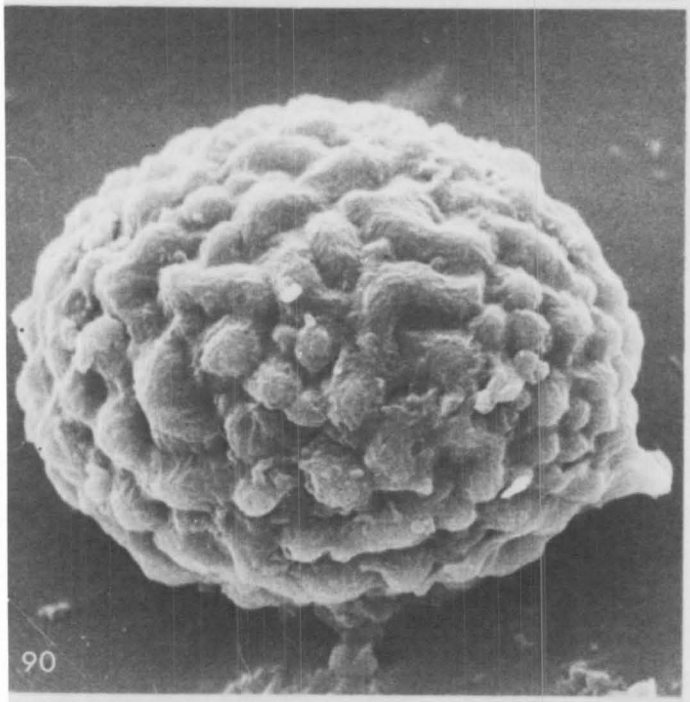
Group 1 - Verrucose, sub-globose to broadly ellipsoidal spores

E. aschersoniana on Juncus bufonius

Collections examined: Grünwald, Berlin, Germany, July 1878, P. Ascherson, HOLOTYPE (HBG); Krieger, Fungi saxonici 467, near Walthersdorf and near Königstein, Aug. 1888 and 1889, W. Krieger (HBG 72); Denniston, Westland, New Zealand, Jan. 1971, J.M. Fineran (CANU).







Figs. 88-90 Entorrhiza aschersoniana from Juncus bufonius

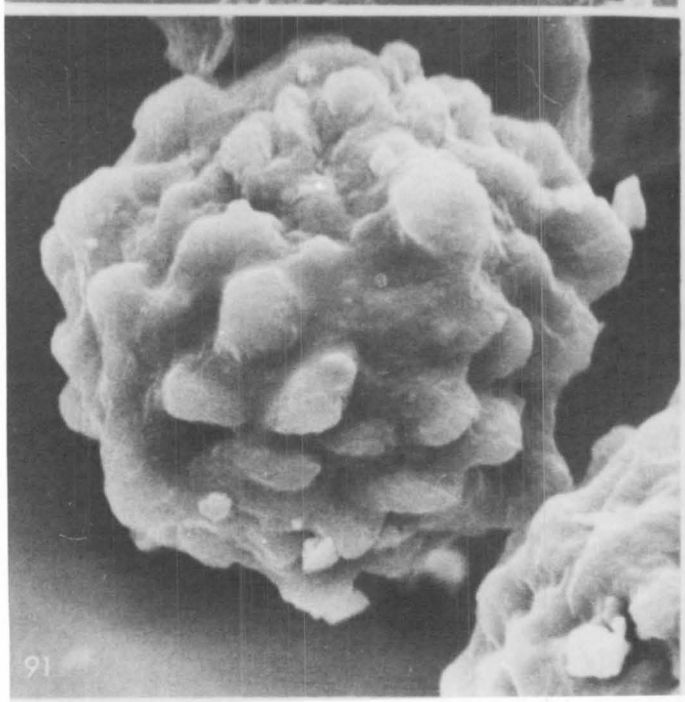
Fig. 88 Krieger, 1888 and 1889, Germany. x1100

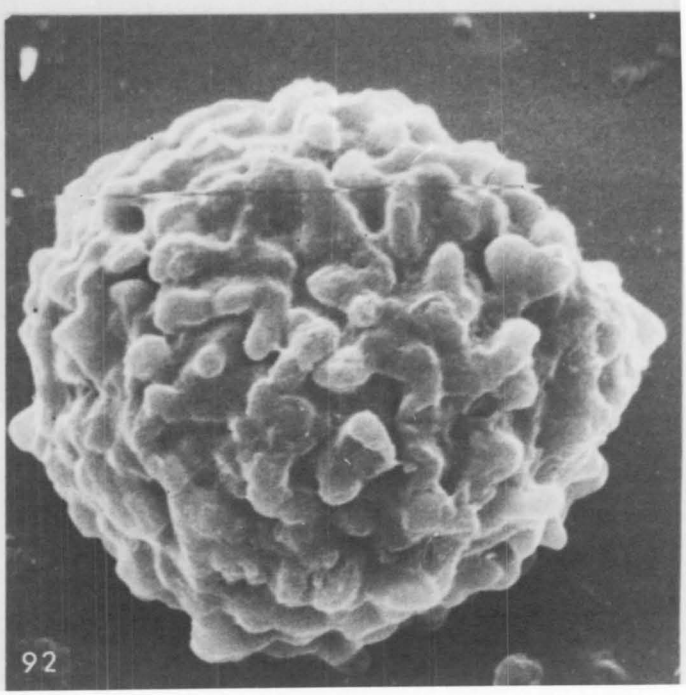
Fig. 89 Ascherson, 1878, Germany, HOLOTYPE. x4600

Fig. 90 Krieger, 1888 and 1889, Germany. x4800

Fig. 91 Entorrhiza from Juncus bufonius; Lagerheim, 1887, Sweden. x5400

Fig. 92 Entorrhiza from Juncus effusus; Fineran, 1971, New Zealand. x5700





Warts unevenly but closely packed on the spore surface (Fig. 88), sometimes varying greatly in size on one spore (Fig. 89), sometimes approximately the same size and occasionally confluent, either at the base or entirely, forming short ridges (Fig. 90); surface of warts often minutely wrinkled. Warts rounded at the apex, broadening at the base, from <1.0 µm - 2.5 µm in diameter, projecting up to 1.0 - 2.0 µm.

Group 2 - Verrucose, globose to sub-globose spores

a) Entorrhiza on Juncus bufonius (Fig. 91)

Collections examined: as E. aschersoniana, Warberg, Sweden, Aug. 1887, N. & G. Lagerheim (HBG 38); seepage area, Summit Rd., Banks Peninsula, Canterbury, New Zealand, Feb. 1971, J.M. Fineran (CANU).

The spores examined show the same range of variation as E. digitata on J. articulatus (see below).

b) Entorrhiza on Juncus effusus (Fig. 92)

Collections examined: Denniston Rd., Westland, New Zealand, Jan. 1971, J.M. Fineran (CANU).

The spores have warts at the lower end of the size range for E. digitata on J. articulatus (see below) i.e. <1.0 - 1.5 µm in diameter, projecting up to 1.5 to 2.0 µm from the spore surface. The size of the warts is similar to that recorded for some globose spores from J. bufonius.

c) E. casparyana on Juncus tenageia

Collections examined: Pyritz (near Hinterpommern, Germany), 1863, Meyer, LECTOTYPE (HBG); Sa. Teresa Gallura, by Tempio (Sardinia), June 1881, Reverchon, SYNTYPE (HBG); Hoyerswerda, (Germany), Aug. 1891, P. Ascherson (HBG 44).

Warts unevenly but closely packed on the spore surface, sometimes varying greatly in size on one spore (Fig. 93), sometimes approximately the same size and occasionally confluent forming short ridges (Fig. 94); surface of warts minutely wrinkled. Warts rounded at the apex, broadening at the base,

Figs. 93 & 94 *Entorrhiza casparyana* from *Juncus tenageia*

Fig. 93 Meyer, 1863, Germany, LECTOTYPE. x3700

Fig. 94 Reverchon, 1881, Sardinia, SYNTYPE. x5900

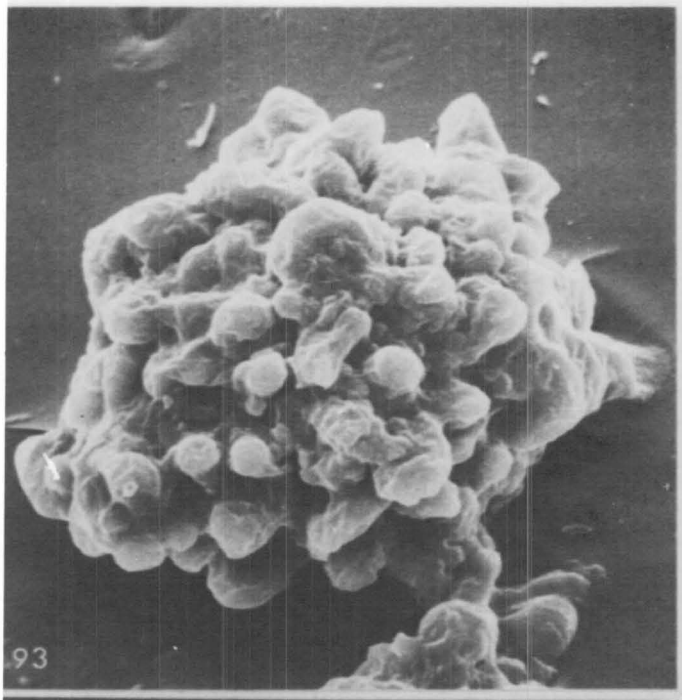
Figs. 95-98 *Entorrhiza digitata* from *Juncus articulatus*

Fig. 95 Correns, 1903, Germany. x4600

Fig. 96 Correns, 1903, Germany. x4800

Fig. 97 Fineran, 1971, New Zealand. x1100

Fig. 98 Correns, 1903, Germany. x1100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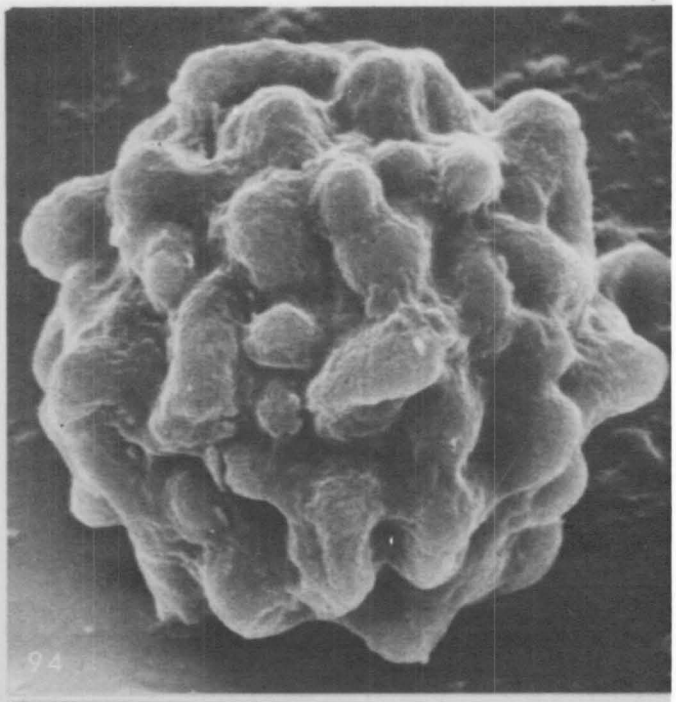
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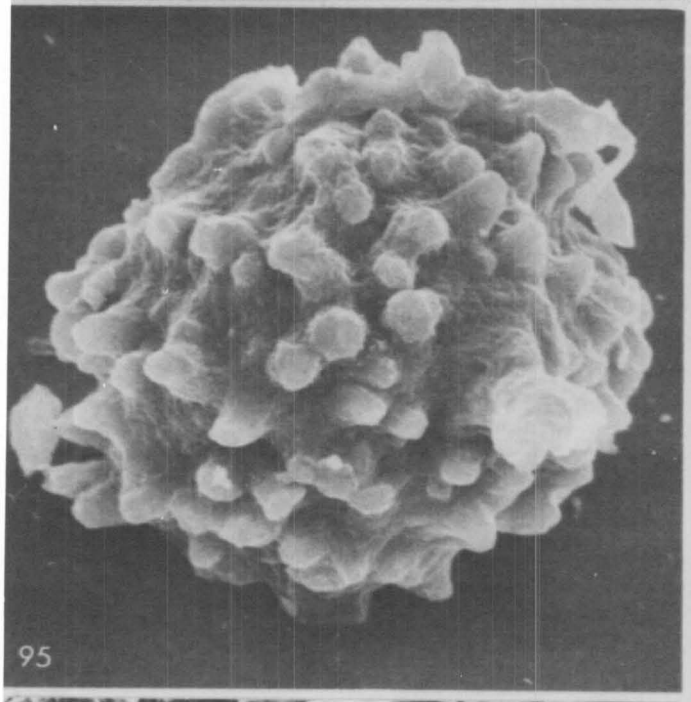
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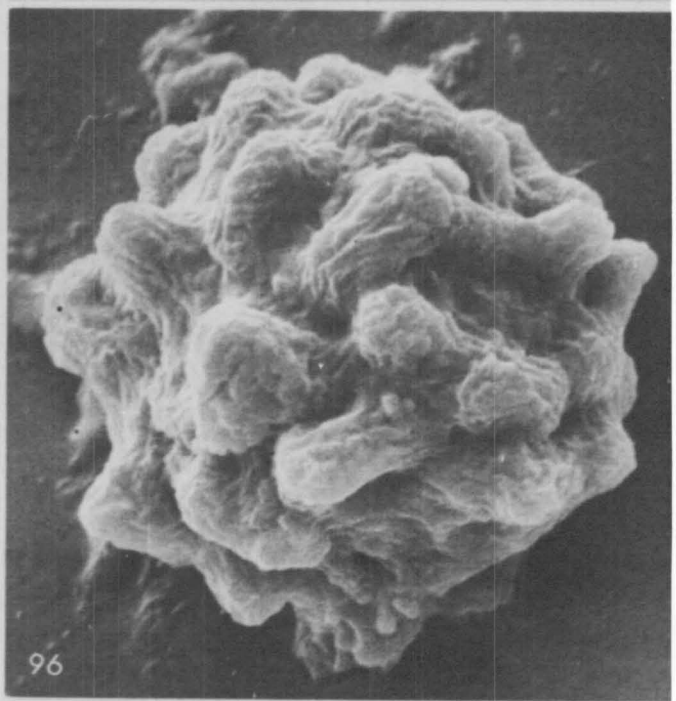
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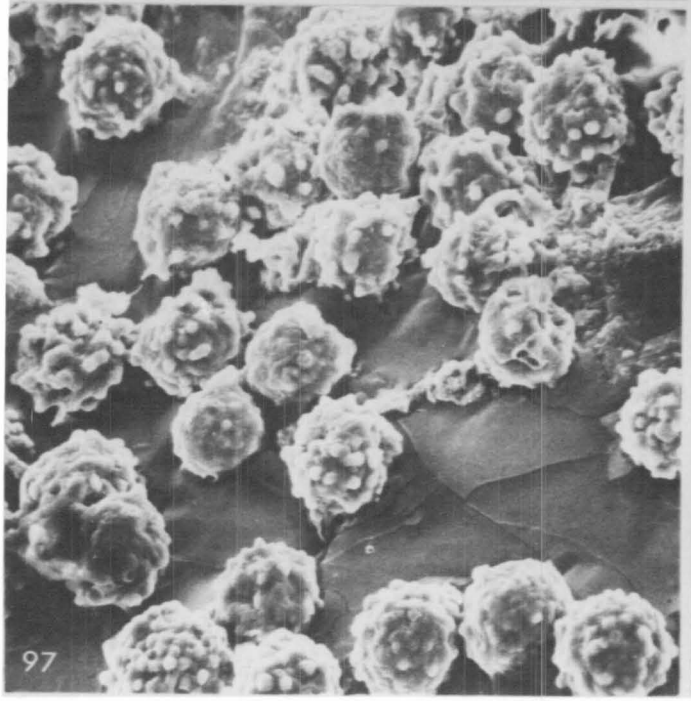
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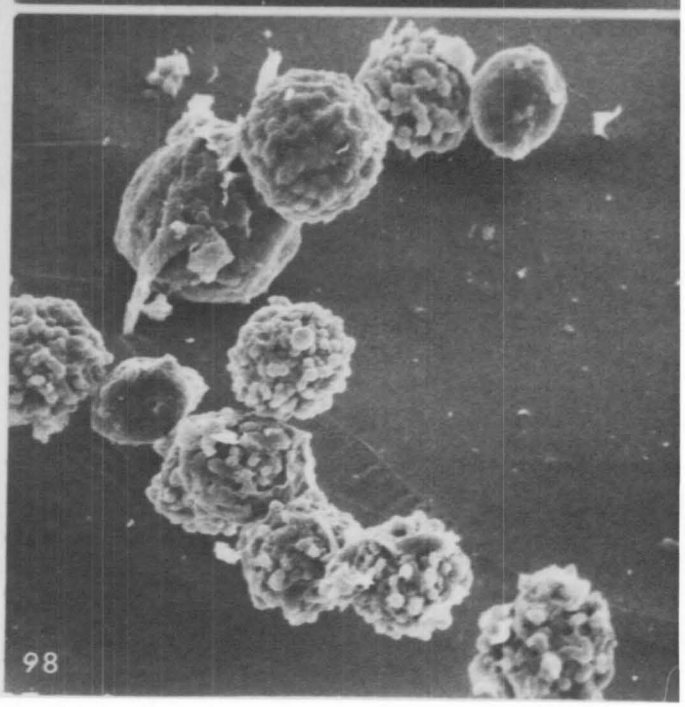

98

from <1.5 µm - 5 µm in diameter, projecting up to 1.5 - 4.0 µm.

d) E. digitata on Juncus articulatus

Collections examined: Tambach, Thüringer Wald, (Germany), Oct. 1903, C. Correns (HBG); Waikouaiiti River, Bucklands Crossing, Otago, New Zealand, Dec. 1970, J.M. Fineran (CANU); Okains-Stony Bay Rd., Banks Peninsula, Canterbury, New Zealand, Feb. 1971, J.M. Fineran (CANU).

Warts unevenly but closely packed on the spore surface (Figs. 95, 97), usually separate, but occasionally confluent (Fig. 96), sometimes undeveloped (Fig. 98); surface of warts minutely wrinkled. Warts rounded at the apex, broadening at the base, from <1.0 µm - 4.0 µm in diameter, projecting up to 1.5 - 3.0 µm.

Group 3 - Striate, ellipsoidal spores

E. scirpicola on Scirpus basilaris, S. cernuus and S. setaceus

Collections examined: on Scirpus basilaris, Turakina beach, near Wanganui, New Zealand, Nov. 1967, A.P. Druce (CANU); on Scirpus cernuus, Sink Hole Flat, Snares Is., New Zealand, Mar. 1971, D.S. & C.J. Horning (CANU); on Scirpus setaceus, Blowing Pt. Bridge, Canterbury, New Zealand, Dec. 1971, J.M. Fineran (CANU); Smiths Creek, Canterbury, New Zealand, Dec. 1971, J.M. Fineran (CANU).

Ridges on spores usually extending most of the length of the spore, discontinuous when developing (Fig. 99), usually evenly spaced (Fig. 100), occasionally branching (Fig. 101), almost parallel to the long axis of the spore but tending to skew slightly to the left (Fig. 102). Ridges ca. 0.75 - 1.5 µm wide, 1.0 - 2.5 µm apart, projecting about 1.0 - 1.5 µm.

Group 4 - Smooth to reticulate ornamentation, broadly ellipsoidal to ellipsoidal spores

E. cypericola on Cyperus flavescens

Collections examined: Halensee, Grünwald, Berlin, (Germany), Aug. 1876, C. Müller, HOLOTYPE (HBG); Sydow, Myc. March. 1138, Halensee, Berlin, Aug. 1886, P. Sydow (HBG).

Figs. 99-102 Entorrhiza scirpicola from Scirpus spp.

Fig. 99 Horning, 1971, Snares Is., N.Z.; Scirpus cernuus. x1200

Fig.100 Fineran, 1971, New Zealand; Scirpus setaceus. x3300

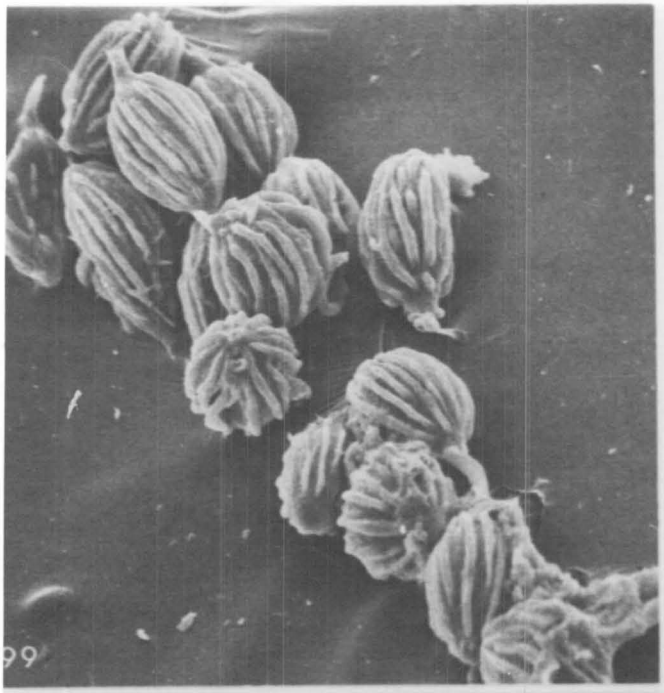
Fig.101 Horning, 1971, Snares Is., N.Z.; Scirpus cernuus. x3900

Fig.102 Druce, 1967, New Zealand; Scirpus basilaris. x5800

Figs.103 & 104 Entorrhiza cypericola from Cyperus flavescens

Fig.103 Sydow, 1886, Germany, TYPE locality. x5600

Fig.104 Sydow, 1886, Germany, TYPE locality. x4600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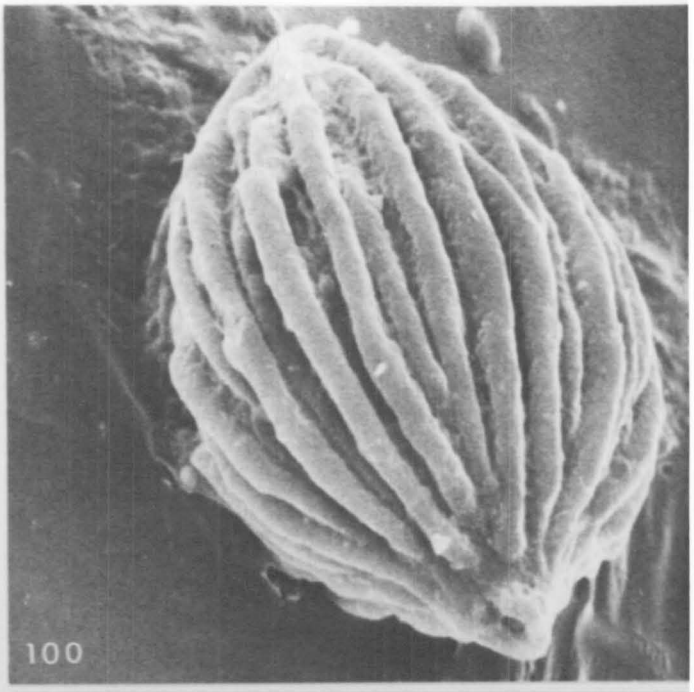
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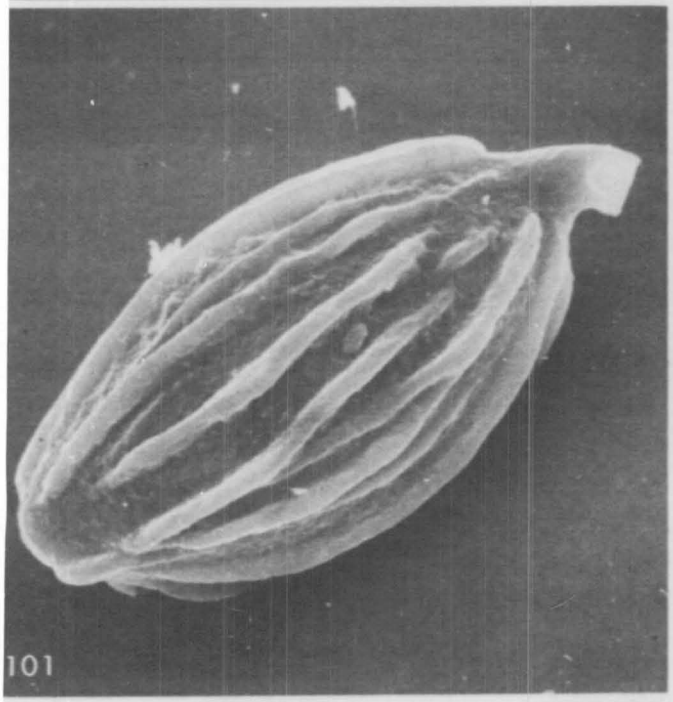
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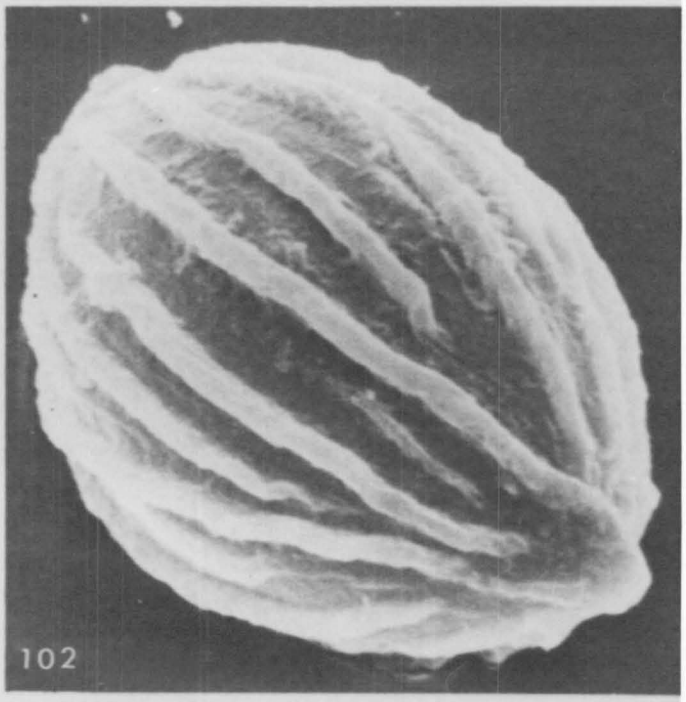
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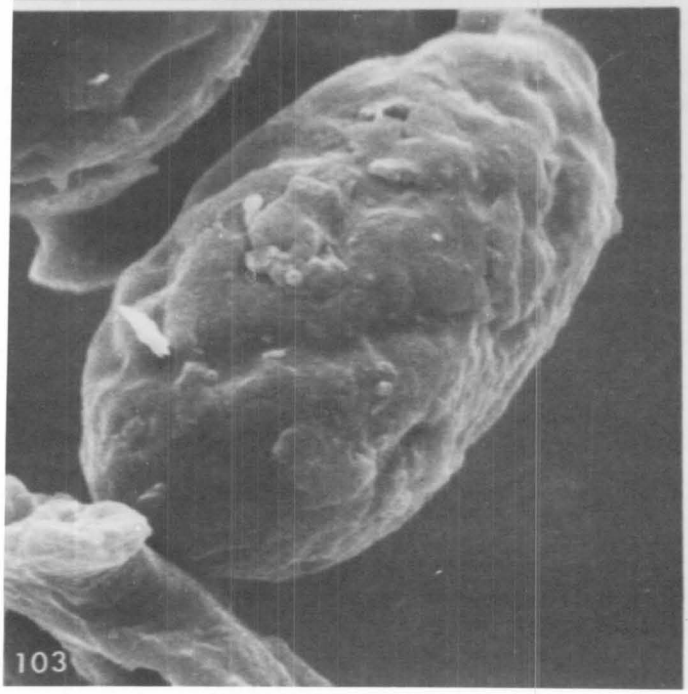
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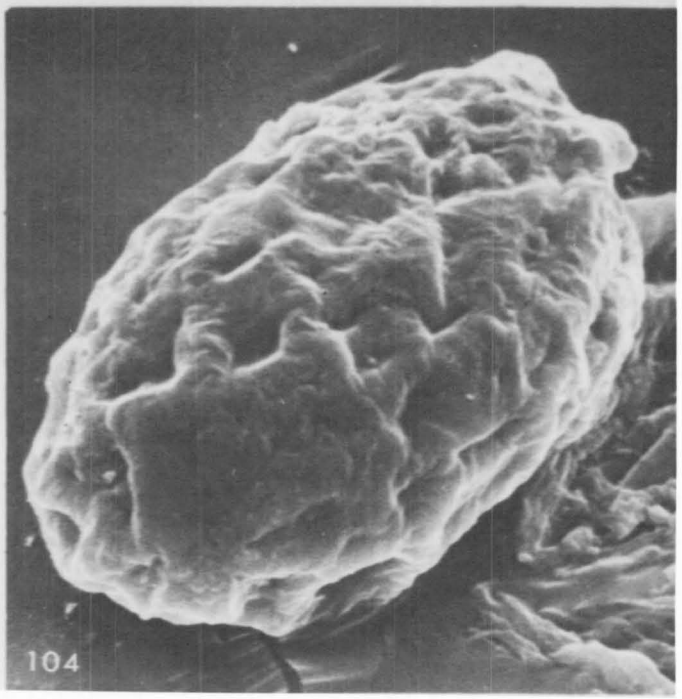

104

Spore wall almost smooth with irregular shallow troughs (Fig. 103) or irregularly ridged, with ridges reticulating, rounded, ca. 1.5 - 2.0 µm wide (Fig. 104).

Group 5 - Smooth to rugulose - undulate, ellipsoidal to oval spores

a) E. caricicola on Carex limosa

Collections examined: E. Rostrups Svampesamling, Sjall., Lyngby Mose, Sept. 1893, K. Ravn, HOLOTYPE (C) and ISOTYPES (H, HBG 54); Selandia, Lyngby, Oct. 1895, C. Ostenfeld - Hansen, PARATYPE (HBG 53).

Walls almost smooth (Figs. 105-107) or with more definite fine wrinkles (Fig. 108), sometimes irregular (Fig. 109).

b) Entorrhiza on Juncus filiformis

Collections examined: as E. juncicola, Sweden, Skane, July 1932, H. Christoffersson (PPC); as E. digitata, Fennia, Kuopio, Palosaari, July 1937, J.I. Liro and H. Roivainen (H).

Walls almost smooth, slightly irregular (Figs. 110 & 111)

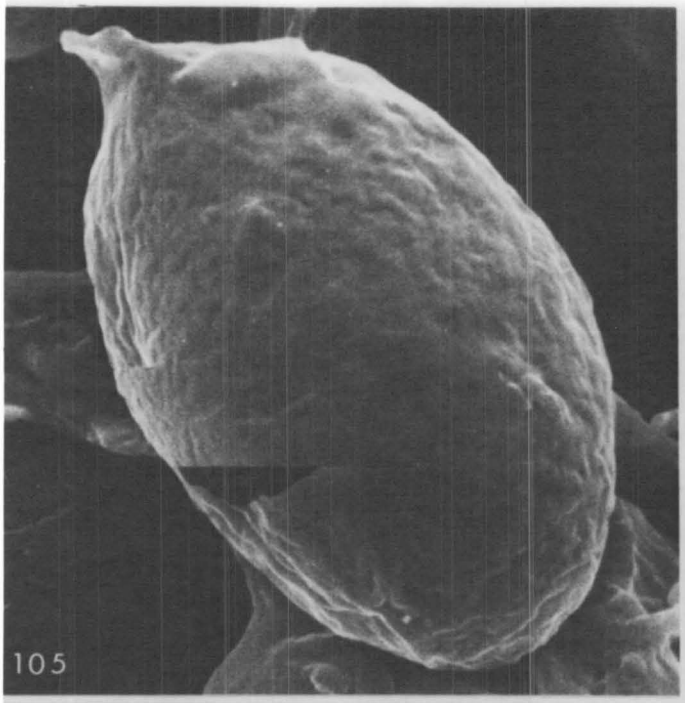
c) Entorrhiza on Carex resectans, Carex sinclairii, Eleocharis gracilis and Juncus pusillus

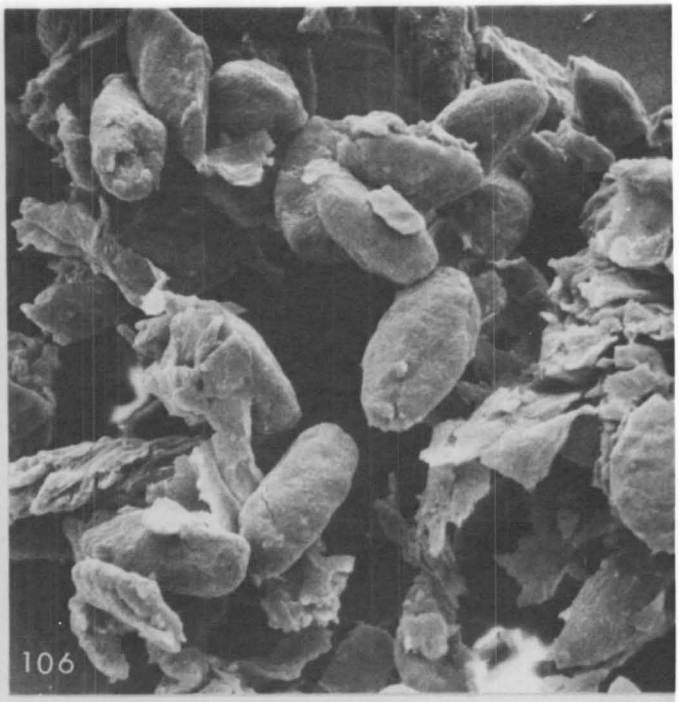
Collections examined: on Carex resectans, Kettlehole, Fagan's Downs, Sth. Ashburton River, Canterbury, New Zealand, Jan. 1971, L.B. Moore (CANU); on Carex sinclairii, Lookout Hill Bog, north of Lake Te Anau, Otago, New Zealand, Feb. 1971, C.J. Burrows (CANU); on Eleocharis gracilis, Lookout Hill Bog, north of Lake Te Anau, Otago, New Zealand, Feb. 1971, A.T. Dobson (CANU); on Juncus pusillus, Kettlehole, Fagan's Downs, Sth. Ashburton River, Canterbury, New Zealand, Jan. 1971, L.B. Moore (CANU).

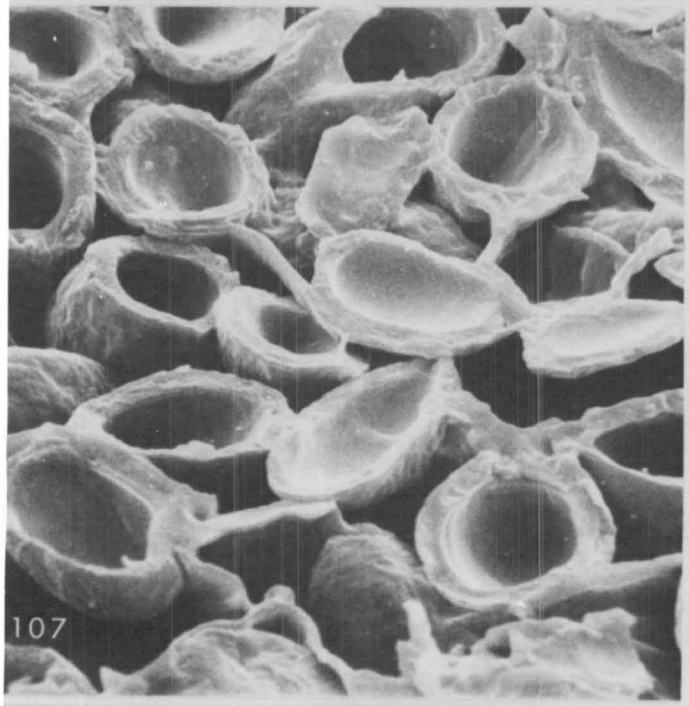
Walls slightly irregular to almost smooth (Fig. 112), finely wrinkled (Fig. 113), with indentations (Fig. 114) or irregular ridges (Fig. 115). Ridges ca. 2.0 µm wide.

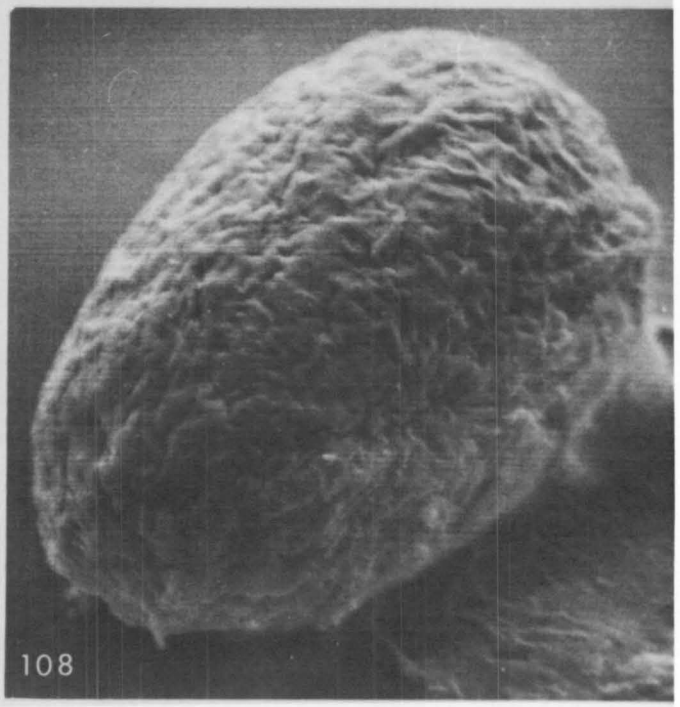
DISCUSSION

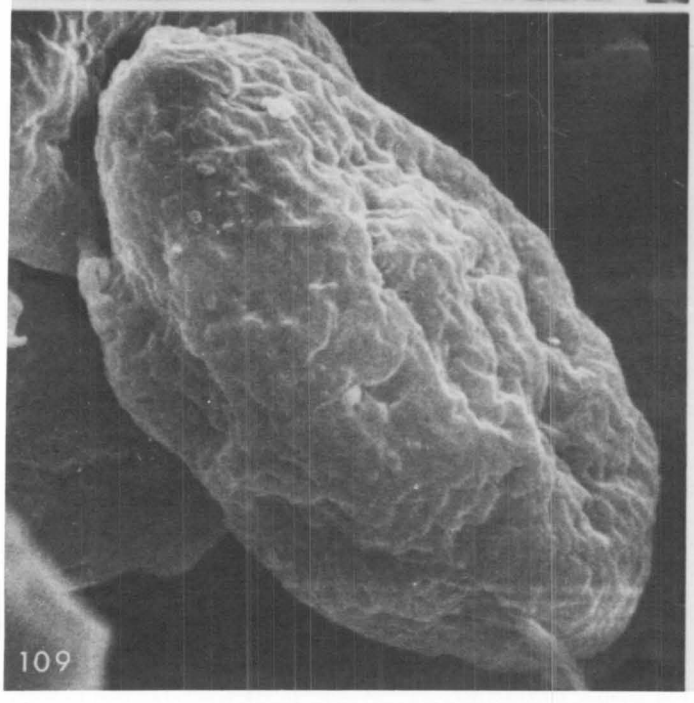
For species having spores with a definite pattern of sculpturing i.e. E. aschersoniana, E. casparyana, E. digitata











Figs. 105-109 Entorrhiza caricicola from Carex limosa

Fig. 105 Ravn, 1893, Denmark, HOLOTYPE. x4500

Fig. 106 Ravn, 1893, Denmark, ISOTYPE (H). x1100

Fig. 107 Ravn, 1893, Denmark, HOLOTYPE. x1900

Fig. 108 Ostenfeld-Hansen, 1895, Denmark, PARATYPE (HBG). x4800

Fig. 109 Ravn, 1893, Denmark, ISOTYPE (HBG). x4900

Figs. 110 & 111 *Entorrhiza* from *Juncus filiformis*

Fig. 110 Liro & Roivainen, 1937, Finland. x1000

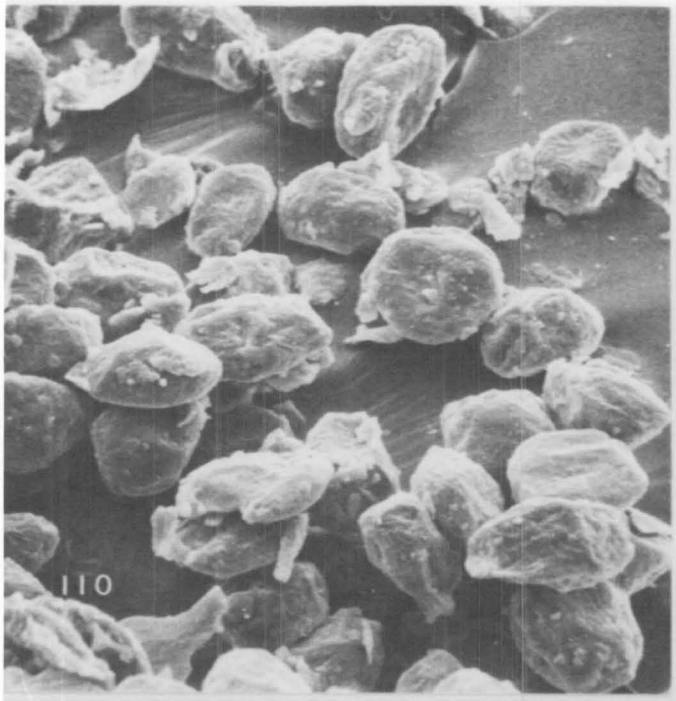
Fig. 111 Christoffersson, 1932, Sweden. x5000

Fig. 112 *Entorrhiza* from *Juncus pusillus*; Moore, 1971, New Zealand. x3900

Fig. 113 *Entorrhiza* from *Eleocharis gracilis*; Dobson, 1971, New Zealand. x3700

Fig. 114 *Entorrhiza* from *Carex sinclairii*; Burrows, 1971, New Zealand. x5100

Fig. 115 *Entorrhiza* from *Carex sinclairii*; Burrows, 1971, New Zealand. x5300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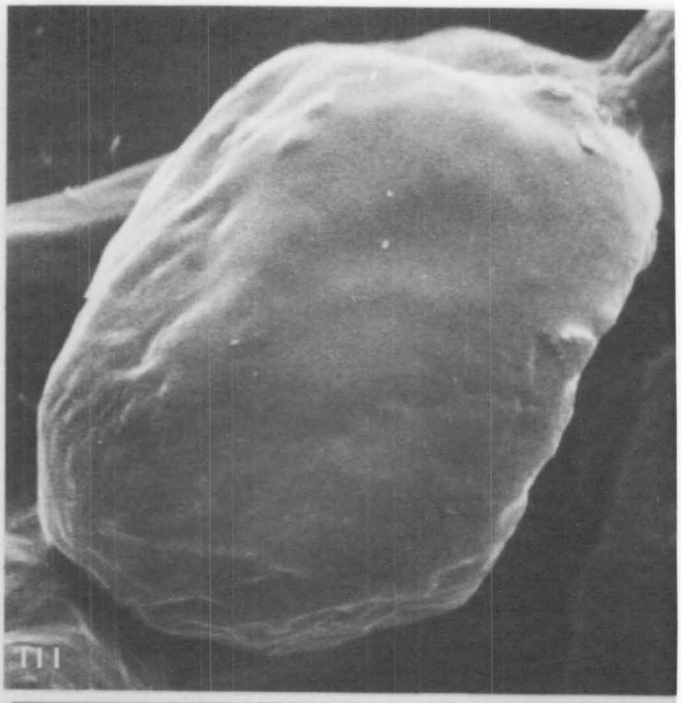
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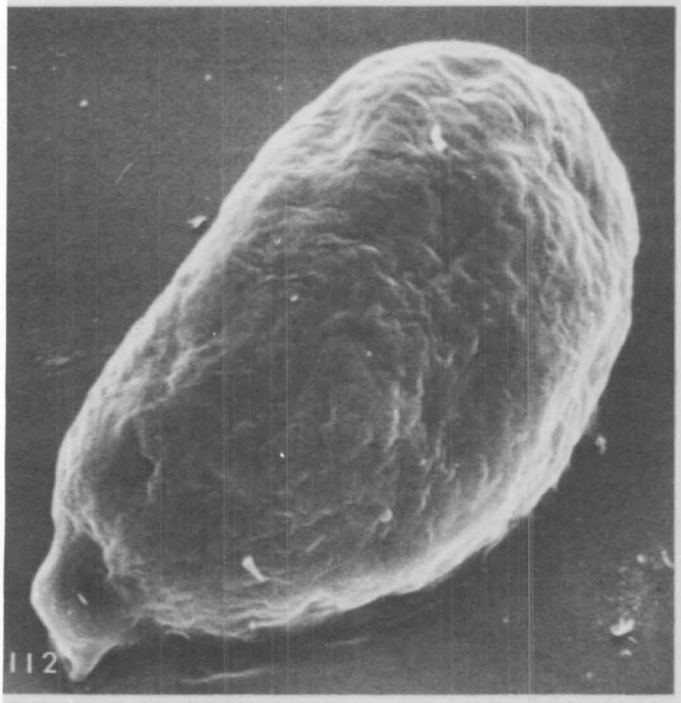
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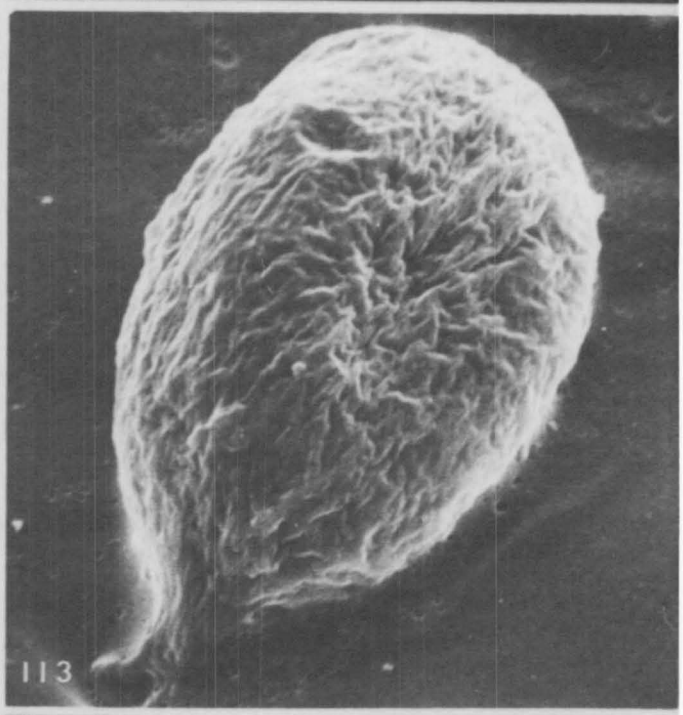
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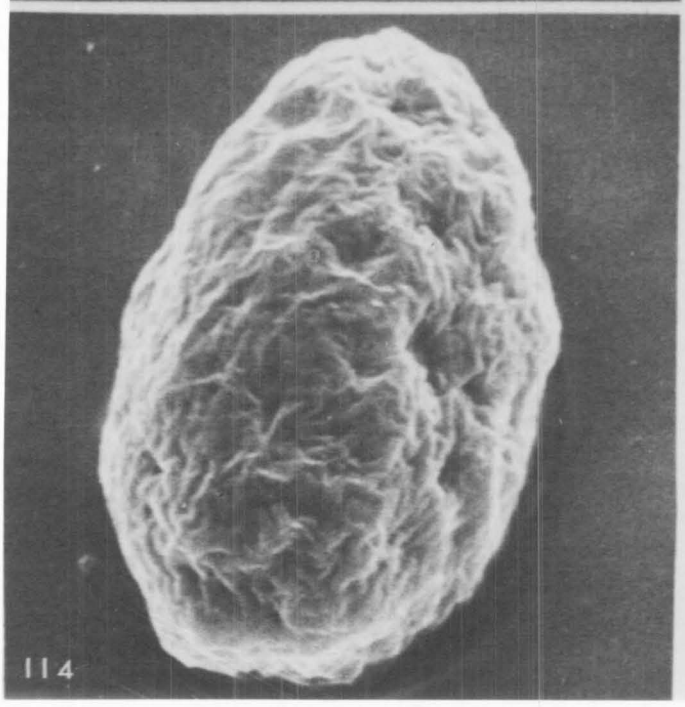
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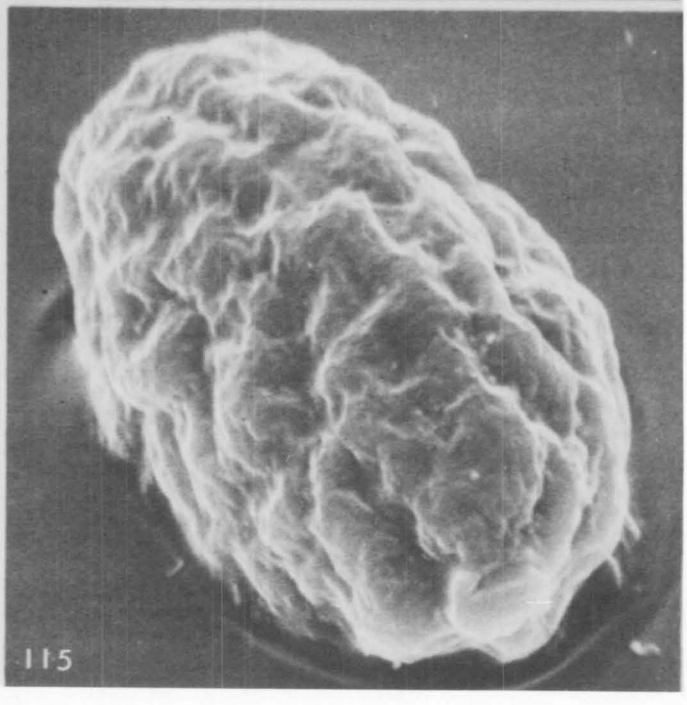

115

and E. scirpicola the SEM confirms observations made with the light microscope. The pattern of sculpturing is similar in the first three species. E. aschersoniana can, however, be distinguished from the other two species on the basis of spore shape. For E. casparyana and E. digitata, information from the SEM supports evidence from light microscope studies (see Chapt. 7) that these species should be united under E. casparyana. The SEM also confirms that globose, verrucose spores present in the galls of some collections of Juncus bufonius are indistinguishable from E. casparyana although they have been identified as E. aschersoniana. The species of Entorrhiza on Juncus effusus has spores with warts slightly smaller than those normally recorded for E. casparyana. However, as wart size appears to be extremely variable in E. casparyana, the fungus on Juncus effusus should be included in this species.

E. scirpicola is a species with distinctively sculptured spores. The SEM confirmed that there were no fundamental differences in spore wall ornamentation between spores from different host plants.

The most difficult groups of species taxonomically are the groups having spores with rather indefinite sculpturing or with almost smooth walls. Included here are E. cypericola, E. caricicola, Entorrhiza on Juncus filiformis and a number of collections on New Zealand hosts with spores resembling those of E. caricicola.

With E. caricicola on Carex limosa an examination of holotype, isotype and paratype material showed no sign of regular ornamentation. This may have been due to the immaturity of the spores - as shown by the wrinkling of the spore surface in some specimens. On the other hand, a section through a number of

holotype spores showing the thickness of the spore wall to be up to ca. 2.5 µm, and the absence of collapsed spores in this material, indicates that the smooth-walled spores present are probably mature or nearly so. No other collections on Carex limosa are available to substantiate these results.

There are also very few collections of Entorrhiza on Juncus filiformis available for comparative purposes. The SEM confirms, for the two collections examined, that this species, too, has basically smooth-walled spores, although the spore wall may sometimes show irregularities. The SEM therefore supports light microscope observations (see Chapt. 7) that this species should not be separated from E. caricicola.

The Entorrhiza species on various New Zealand hosts has some spores which show almost no sculpturing and others with a definite but irregular pattern of ridges. Whether the spores are extremely variable in their sculpturing or whether different stages of development are represented is difficult to assess. Because of the similarities at the light microscope level (see Chapt. 7) and because smooth-walled and wrinkled spores are indistinguishable from those of E. caricicola on Carex limosa, the Entorrhiza on the New Zealand hosts should be regarded as E. caricicola.

In E. cypericola the development of an irregular but discernible pattern of reticulating ridges distinguishes this species from E. caricicola where ridges are present but not interlocking. Nevertheless, with the SEM the similarity in the pattern of sculpturing is great and, because of the variability of spore wall ornamentation in both species, it is difficult to decide from the material examined whether these species should

be considered synonymous. Observations from the light microscope reveal, however, that the spore wall in E. cypericola has a distinct inner reticulated layer which is sometimes covered by a smooth layer (see Chapt. 7). This particular type of two layered wall distinguishes this species from other Entorrhiza species. Observations from the electron microscope for E. casparyana show that there may be a superficial layer of host cell wall material sheathing some spores (see Chapt. 3) and this type of host cell reaction may also account for the smooth wall of E. cypericola spores in Cyperus flavescens. These results indicate that spore surface sculpturing should not be given undue emphasis as a taxonomic character unless SEM and light microscope observations of the surface topography of spores are associated with studies of thin sections of the spore wall by light and/or electron microscopy.

This study has shown that for Entorrhiza the SEM can consolidate observations made with the light microscope. No new structures have been found; the SEM has merely provided a clearer basis for comparative studies. In those taxonomically difficult species i.e. those with indefinite sculpturing, studies with the SEM have so far provided very little new information which will help to decide the species limits.

5. SUMMARY

1. The SEM confirms that the pattern of sculpturing is similar for spores of E. aschersoniana, E. casparyana and E. digitata; however, E. aschersoniana can be distinguished from the other two species on the basis of spore shape.

2. The SEM supports evidence from light microscope studies that E. digitata is indistinguishable from E. casparyana.

3. The SEM confirms that there are no fundamental differences in spore wall sculpturing for E. scirpicola on a variety of host plants.

4. For species with less distinctive sculpturing of the spore wall i.e. E. caricicola, E. cypericola and Entorrhiza on Juncus filiformis, the SEM has been unable to provide any new information on which to base species limits. It has, however, consolidated observations on the surface structure of the spores made with the light microscope.

5. Scanning electron microscopy should be used in conjunction with light microscopy and transmission electron microscopy to provide information of value for taxonomic studies. Observations from the SEM alone may be misleading as spores with a similar surface topography eg. E. caricicola and E. cypericola may have walls with a completely different internal organisation.

CHAPTER 7

A TAXONOMIC REVISION OF THE GENUS *ENTORRHIZA*

1. INTRODUCTION

"Many mycologists feel that the taxonomy of fungi is in greater need of synthesis than of more analysis, and a competent study of old genera and species, with much elimination" is required (Bisby & Ainsworth, 1943). In the parasitic fungi, especially, taxonomic revisions are important because, in much of the early work, species were delimited on the basis of presumed host specialisation with very little regard to morphology. This occurred in the genus *Entorrhiza*.

The only taxonomic investigations of the genus *Entorrhiza* are those of Ciferri (1963) and Thirumalachar and Whitehead (1968). Ciferri made several alterations at the specific level. He reduced *E. raunkiaeriana* to synonymy with *E. scirpicola* and indicated with his trinary system of nomenclature that *E. aschersoniana* should be included as an infraspecific taxon under *E. casparyana*. Thirumalachar and Whitehead reviewed the generic position of the group and reduced *Entorrhiza* to synonymy with *Melanotaenium* de Bary. However, in neither case did these authors attempt a complete revision of the genus and it is with this purpose that the present study has been carried out.

1.1. The Species Concept in Fungi

Before embarking on a taxonomic revision, the criteria for taxonomic decisions must be established. There are two main approaches to the delimitation of species; one is to use

a morphological species concept, the second is to use a biological species concept.

Morphology as a taxonomic criterion in the fungi has recently been reviewed by Chesters (1968). He notes the value of being able to use "the total morphological criteria accumulated from field and laboratory study". Those criteria which are less subject to environmental pressures are of most importance taxonomically but as wide a range of characters as possible should be employed as long as their variability is known. The taxonomy of the fungi is based largely on morphological characteristics and Chesters believes this is justified on the grounds that "the morphology of an individual is the ultimate expression... of all its complex relationships with its normal habitat".

It is well known that reproductive phases of organisms are more constant in their characteristics and hence of more value taxonomically than vegetative phases. In the fungi, this means that characters associated with spore structure and development are the most important. Moreover, observations on fungi from the field are extremely valuable especially for obligate or near-obligate parasites. The exclusive habitats occupied by such fungi mean that there is very little environmentally induced variation in nature. In obligate parasites variability at the level of the individual, species, genus or family is mainly host induced.

For parasitic fungi, host specialisation is often the basis of the biological species concept. It is regarded as the ultimate expression of the physiological, genetical and ecological attributes of the species. The question of host specialisation as a taxonomic criterion has often been discussed

(eg. Ainsworth, 1962; Johnson, 1968). Johnson points out that parasitic fungi vary far more in their pathogenicity than in morphological characteristics and for this reason he believes that species should be based on morphological criteria with pathogenic races being distinguished as formae speciales (f. spp.). Furthermore, from the practical point of view, the morphological species concept is much easier to apply.

Although morphological characters in toto are regarded as being more constant than physiological characters, in many taxonomic treatments the variability of individual morphological characters has not been adequately studied. Genetical investigations have shown that some characters, such as the pigmentation of the spore wall, widely used in classification are potentially variable; in some fungi, pigmentation is controlled by a single gene eg. Sordaria macrospora (Esser & Kuenen, 1967).

In recent years, a number of studies have been carried out on the genetical basis of pathogenicity. Flor (1956) has demonstrated experimentally in the flax rusts that extension of the pathogenic range occurs by single mutational steps. This is probably how resistance in cultivated varieties of cereals is overcome by races of cereal rusts in nature. Furthermore, an extension in host range depends on the mutability of genes not only of the parasite but also of the host. These two facts taken together underline the necessity for placing more emphasis on a combination of morphological characters rather than on pathogenicity for delimiting species.

In higher plants the biological species concept is usually based on reproductive isolation. This concept has not been widely applied to fungi although, in fact, reproductive isolation is often a consequence of host specialisation in

parasitic fungi. Nelson (1963) has pointed out that cross-sterility as a criterion for reproductive isolation is difficult to apply to the fungi because of the complexity of the sexual mechanisms. Furthermore, interfertility tests to determine species limits are laborious and have the disadvantage that they are invariably done in culture (Talbot, 1971).

The attitude towards the taxonomy of fungi has changed since the early part of the century. As Snyder and Hansen (1954) stated, originally the systematist looked not so much for similarities between the individuals of a species as for differences of any kind. When only a few collections were examined the differences often seemed large enough to constitute specific differences.

With further work on fungal taxonomy it has been demonstrated that morphological variability is much greater than was previously supposed; variability in the smut fungi has recently been reviewed by Holton *et al.* (1968). Taxonomists now stress the importance of examining as many collections as possible to assess the variability of certain characters and to determine species limits. Among parasitic fungi these principles may also be applied to the designation of *formae speciales*. These specialised forms (f. spp.) may overlap so that a thorough knowledge of the extent of variability in host specialisation is necessary to assess the limits of each *forma specialis*. *Formae speciales* may be further subdivided, as in the cereal rust fungi, into physiological races which may be regarded as the ultimate taxonomic unit. Minor morphological differences correlated with these specialised forms or physiological races are usually only evident to the specialist and then possibly with the assistance of statistics. They are therefore of

little importance to the general taxonomist.

1.2. The Species Concept in Smut Fungi

The general principles outlined above have been applied to the Ustilaginales by Fischer and Shaw (1953), who believe that these fungi are in need of a critical revision at all levels. They suggest that the major emphasis in designating species of smut fungi should be placed on morphological criteria. However, they point out that because there are very few morphological characters on which to distinguish species it is also necessary to consider host specialisation. If no consideration is taken of host specialisation, the merging of some probably unrelated species would be inevitable. Their compromise decision was that the species concept in the smut fungi should be based "primarily on morphology but taking into account also host specialisation at the host - family level". Thus, two smut fungi of similar morphology and symptomology found on members of different host families would be treated as different species. Nevertheless, they realise that even this species concept is not infallible.

Within the Ustilaginales, the genera are separated mainly on the basis of soral position and structure, on whether the spores are single or arranged in spore balls of various types and frequently also on spore germination. There are very few characters on which to separate the species. Although the structure of the sorus (eg. size and covering) is of some importance, the characters used for delimiting species are usually the size, shape, colour and ornamentation of the spores. This means that the smut fungi may be very conveniently studied from herbarium material as spore characters remain almost unaltered for long periods of time.

1.3. Taxonomic Criteria in the Genus Entorrhiza

In Entorrhiza the following characters have been considered in delimiting the species:

a) Soral characters

The position of the sorus in the roots is a generic character and cannot be used to differentiate species. The galls in which the sori form are mainly terminal; when they are lateral they are probably morphologically still a lateral branch rather than an amorphous growth of the cortex. The nature of the sorus with the spores remaining permanently embedded in the host tissue is also distinctive for the entire genus. However, soral structure, particularly the form of the gall in which the sorus is produced and the type of branching, may be of minor importance in differentiating species. Soral structure may be used as a taxonomic criterion but its use is limited because it appears that the ultimate shape of the gall in which the sorus is produced is probably due to a combination of a certain species of Entorrhiza with a particular host species. Species of Entorrhiza with a wide host range may therefore be found to possess a diversity of soral shape, each form being distinctive for a certain host species or group of species.

The size of the sorus is not an important taxonomic character. The host root meristem continues to function as spores develop and the ultimate size of the sorus probably depends largely on the environment (degree of soil moisture, nutrients etc.), on whether the host is a perennial or annual and on the longevity of the roots.

b) Spore characters

(i) Spore size. Spore size may be an extremely variable character. With Entorrhiza infections sori do not erupt and release spores at maturity, so many spores examined may not be fully developed. Moreover, spores examined from the base of the sorus near the point of attachment of the gall to the root will be much more advanced in their development than spores nearer the apex of the sorus. On one host plant each sorus is probably the result of a separate infection and will be at a different stage of development, explaining the differences in mean spore size among duplicate collections. Much of the variability in spore size within one sorus is due to the wide discrepancies in spore wall thickness. For these reasons spore size must be taken from as many collections as possible to give a meaningful range for the species.

(ii) Spore shape. This is regarded as a more important character than spore size, being relatively constant throughout the development of the spore and also being more consistent for spores from different collections.

(iii) Spore ornamentation. This is an important specific character but is subject to different degrees of variability in various species. Part of the variability in spore ornamentation is due to the deposition of an outer wall layer which is usually smooth and appears to be due to host-parasite interaction (see Chapt. 3). This 'sheath' is not found on all spores and varies considerably in degree of development. It appears more frequently in some species than others. The sheathing material is not analogous to the gelatinous

sheath characteristic of the spores of many smut fungi. Striate, verrucose, rugulose and smooth walled spores occur in the genus.

(iv) Spore wall pigmentation. The degree of pigmentation depends on the maturity of the spore. The mature spore mass for all species in the genus is a light, mustard brown so that colour cannot be used to distinguish species.

(v) Spore formation. Throughout the genus spores develop terminally; they usually arise singly but occasionally spore pairs are formed. It is possible that each cell in these 'double spores' arises independently and that two spores are united by the sheathing material mentioned earlier or by spore wall material. Double spores occur to a limited extent in most species and are not of diagnostic value.

(vi) Spore germination. The cleavage of the spore contents and the formation of up to four promycelia per spore and up to four sporidia per promycelium appears to be a generic character. The shape of the sporidium also appears to be distinctive for the genus. Sporidial size may, however, be of use in separating species. Spore germination has been documented only twice (for Entorrhiza aschersoniana and E. casparyana), so there is insufficient data to assess the significance of differences in sporidial dimensions or in other aspects of spore germination.

2. MATERIALS AND METHODS

All species which have been described or combined under the genus Entorrhiza are considered in the taxonomic revision below.

The taxonomic decisions have been based on a morphological species concept. The relationships have been established by examining the extent of variation among different collections. Within one species there is continuous overlapping variation, but between species there is discontinuity either in spore ornamentation or spore shape; spore size has not been a criterion for delimiting species but further studies may show whether it should be used to delimit infraspecific taxa.

Spore germination could not be used for determining species limits as data is available for only two species.

Cross inoculation studies have not been carried out. The current view of mycologists is that these determine infraspecific taxa, in particular formae speciales, so that species delimited in this study should not be altered as a result of the discovery of differences in the pathogenicity of individual collections.

2.1. Collections Examined

As many collections as possible were sampled. Most of these were from European herbaria listed at the end of this section. However, fresh material of several species was available for examination. A total of ca. 150 specimens of Entorrhiza (including all the type material available) was examined. The collections are listed under the species to which they have been assigned. The international symbol of the herbarium in which

the material is deposited is given in parentheses.

Citations of the data from these collections were transcribed as they appeared on the packets with the following modifications: translation into English (where appropriate), abbreviation of the date to month and year, additional information on locality (added in brackets). Where the original labels were not in English and handwritten, transcriptions were sometimes difficult and may not always be accurate. The title and number of an exsiccatus has been given.

2.2. Preparation of Material

A slide was prepared for each accession. With a needle or sharp scalpel, material was scraped or cut, usually from the base of a sorus. Sometimes spores were easily freed by scraping but in most sori spores were firmly held in the tissue. The spores were mounted in lactic acid and the preparation sealed with nail lacquer. Measurements of spores from type material in lactic acid correlated well with those given in the original descriptions, indicating that there was little, if any, shrinkage over long periods of time.

Photographs of these spore mounts were taken with a Leitz Orthoplan microscope fitted with an automatic camera, using x40 high dry objective and x10 oculars. Each field was chosen to include, wherever possible, at least 10 well separated spores. However, where spores were widely dispersed, sometimes more than one photograph was necessary to obtain a sample of 10 spores. The data on spore size and shape for one sorus from each collection was then compiled by measuring 10 spores at random from one (or occasionally more than one) photographic enlargement of known magnification. A photograph of a micrometer slide at the same enlargement was used to calibrate the measurements.

To assess the variability in spore size between different collections, the mean spore size for each collection was then calculated. The mean spore size was taken to be representative of the size range for that collection as variability was low within a sample. The variability in spore shape (expressed as a length: breadth ratio) was also assessed. In this case, however, the mean ratio for the sample of 10 spores was not calculated; instead, the ratio was taken directly from the mean spore size for each collection. This was an approximation for the true mean ratio; however, the ratio could only usefully be expressed to one decimal place (due to the accuracy of the measurements) and at this level the approximation was identical to the true mean for a number of collections where these figures were compared. Extremes of spore size and shape and details of spore ornamentation and colour were noted from the total spore mount.

The semi-permanent slides and the photographic negatives from them are housed in the herbarium at the Botany Department, University of Canterbury (CANU).

2.3. Species Descriptions

These are compiled entirely from material seen and studied microscopically by the author. The terminology for spore shape is taken from Ainsworth (1971). The range in spore size represents the range in mean spore size for the collections examined; the extremes (in brackets) are measurements of individual spores.

2.4. Illustrations

Illustrations present in the literature are cited. For this revision each species is represented by a photomicrograph of the spores (magnification x 1450). The usual host is illustrated, showing the form of the galls in which the sori are produced. Type material has been used wherever possible.

2.5. Host Plants and Geographical Distribution

The host plants and the geographical distribution for each species of Entorrhiza are listed after the species description. Where these records have been confirmed from herbarium material they are given without literature reference. Where a reference is cited the report has not been personally substantiated. Records marked with an asterisk are regarded by the author as doubtful, being either ambiguous reports in the literature or possible misidentifications.

Host plants have been checked critically only when their identification has been in doubt.

2.6. Synonymy

True synonyms are listed chronologically, misidentifications follow. Taxonomic synonyms are not indented; nomenclatural synonyms are indented to show their relationship to the taxonomic synonym.

Herbaria which lent material for examination or made their facilities available for use (†) with their standard abbreviations as they appear in the Index Herbariorum Part 1, are listed below:

Botanisches Museum, Berlin (B); Botanical Museum and Herbarium, Copenhagen (C); Department of Plant Pathology, Copenhagen (CP);

Royal Botanic Garden, Edinburgh (†E); Botanical Museum, Helsinki (H); Staatsinstitut für allgemeine Botanik und Botanischer Garten, Hamburg (HBG); The Herbarium and Library, Kew (K); Botanische Staatssammlung, München (M); Botanical Research Institute, National Herbarium, Pretoria (PRE); Botanical Department, Naturhistoriska Riksmuseum, Stockholm (S); Institute of Systematic Botany, University of Uppsala (UPS); Institut für Spezielle Botanik der Eidg. Technische Hochschule, Zürich († ZT).

Herbaria which furnished lists of their accessions but from which material was not examined are cited below. In most cases specimens are duplicates of those examined from the herbaria mentioned above. Those herbaria holding specimens which are not duplicated in the above collections are marked with an asterisk.

The National Fungus Collections, Crops Research Division, Plant Industry Station, Beltsville, Maryland (*BPI); Institutul de Biologie "Tr. Savulescu" al Academici RPR, Bucuresti (*BUCA); Farlow Library and Herbarium of Cryptogamic Botany, Harvard University, Cambridge, Massachusetts (FH); Conservatoire et Jardin botaniques, Genève (G); Botanical Museum, Lund (LD), (few collections - probably duplicates of material at Stockholm, Uppsala or Copenhagen); University Herbarium, University of Michigan, Ann Arbor, Michigan (MICH); The New York Botanical Garden, New York (NY); Botanisk Museum, Oslo (O); Museum National d'Histoire Naturelle, Laboratoire de Cryptogamie, Paris (PC), (probably all duplicates); Herbarium of the University of California, Berkeley, California (UC); Department of Plant Pathology, Washington State University, Pullman,

Washington (WS); Botanischer Garten und Institut für Systematische Botanik der Universität, Zürich (Z).

3. TAXONOMY

3.1. Description of the Genus

Entorrhiza Weber

Bot. Ztg. 42 : 378 (1884)

Schinzia Naegeli - Linnaea 16:281 (1842) non Dennstätt (1818)

Melanotaenium de Bary - Bot. Ztg. 32:105 (1874)

Sori in terminal, occasionally lateral, galls on roots; galls clearly demarcated from the uninfected root. Root meristem functional at the apex of the gall, sometimes branched abnormally, gall with indeterminate growth. Infection restricted to the gall.

Gall normally less than 2 cm in length, width dependent on extent of branching. Gall light coloured at the apex becoming dark brown at the base as it matures. Spores embedded in the host cortex with the outer 3-5 cell layers of the host forming a protective covering to the sorus.

Spores formed intracellularly, terminally on the hyphae, usually single, occasionally surrounded by sheathing material (apparently of host origin), spore-wall yellow brown to chestnut brown. Spores immature at the apex of the sorus, maturing towards the base; released passively by the decay of the gall.

Spore germination, where known, by the production of up to 4 aseptate promycelia bearing 1-4 terminal sporidia. Sporidia aseptate, slender, falcate, often with a single loop in the middle.

Known only from the Cyperaceae and Juncaceae.

Type species: Entorrhiza aschersoniana (Magn.) Lagerh.

Discussion:

There has been some confusion as to the type species of this genus. At the time Weber introduced the genus Entorrhiza with the single species E. cypericola, the fungus on both Cyperus flavescens and Juncus bufonius was believed to be the same species, Schinzia cypericola Magn. Weber was unable to obtain material on Cyperus flavescens and based his study entirely on infected material of Juncus bufonius. He examined two collections, one sent from de Bary in Strasbourg and the second from the locality (Halensee, Grünwald, Berlin) cited by Magnus (1878). As a result of his work Weber erected the genus Entorrhiza, naming the species on Juncus bufonius (and Cyperus flavescens) as Entorrhiza cypericola (Magn.) Weber.

In 1888, Magnus reported that the fungi in these two hosts were in fact different species. He did not accept Weber's generic change and gave the name Schinzia aschersoniana to the fungus on J. bufonius. This is therefore the type species of the genus; the new combination Entorrhiza aschersoniana was made later in the same year independently by both Lagerheim (1888) and de Toni (1888). Although E. aschersoniana has been recognised as the type species by most authors, several workers including Ainsworth and Sampson (1950), Fischer (1951), Talbot (1956), Fischer and Holton (1957) and Thirumalachar and Whitehead (1968) have incorrectly cited the species on Cyperus flavescens as the type.

In 1968 Thirumalachar and Whitehead proposed that the genus Entorrhiza should be merged with Melanotaenium, a genus characterised by having dark sori embedded in the host tissue in leaf parts, stems and, in some species, in the upper roots

(Fischer & Holton, 1957). They believed that the development of sori in the root tissues and the presence of light-brown coloured spores were not sufficient reasons for retaining Entorrhiza as a separate genus. Thirumalachar and Whitehead claimed that the soral characters in the two genera were identical and that the range in teliospore colour from light brown in Entorrhiza to dark brown in Melanotaenium was similar to that occurring in some other genera eg. Entyloma.

However, Thirumalachar and Whitehead based their decisions on inadequate studies. Melanotaenium is a little known genus and many aspects of its biology have not been documented. There was also very little known about Entorrhiza and the examination of material by these two authors appears to have been superficial. For these reasons and because the genus Entorrhiza appears to delimit a natural group of fungi, Thirumalachar and Whitehead's decision to merge the two genera has not been accepted.

3.2. Artificial Key to Species of Entorrhiza

1. Spores globose or sub-globose, spore wall smooth to verrucose.....E. casparyana

   Spores broadly ellipsoidal, ellipsoidal or oval, occasionally sub-globose..... 2

2. Spore wall distinctly ridged.....E. scirpicola

   Spore wall not distinctly ridged..... 3

3. Spore wall verrucose, no smooth layer.....E. aschersoniana

   Spore wall not verrucose, often smooth..... 4

4. Spore wall smooth to rugulose, length: breadth ratio of spore usually >1.4.....E. caricicola

   Spore wall with an inner reticulated layer often surrounded by a smooth layer, length:breadth ratio of spore usually <1.4.....E. cypericola

3.3. Species Descriptions

Entorrhiza aschersoniana (Magn.) Lagerh.

Hedwigia 27 : 262, Sept. - Oct., 1888.

Figs. 116-121

Schinzia cypericola Magnus p.p. - Verh. bot. Ver. Prov. Brandenb., 20:53 (1878)

Entorrhiza cypericola (Magn.) Weber - Bot. Ztg. 42:378 (1884)

Schinzia aschersoniana Magnus - Ber. dt. bot. Ges. 6:103 (1888)

Entorrhiza aschersoniana (Magn.) de Toni - Sacc. Syll. Fung. 7(2):497, 28 October (1888)

Entorrhiza junci Brefeld - Unters. Gesammt. Myk. 15:80 (1912) - nom. nud.

Melanotaenium aschersonianum (Magn.) Thirum. & Whitehead Am. J. Bot., 55(2):184 (1968)

Lectotype : on Juncus bufonius L., Germany, Berlin, Halensee, Grunewald, July 1878, P. Ascherson (HBG 19).

Sori in galls, oval to elongate, up to 11 mm long by 4.5 mm wide, occasionally branched. Spores ellipsoidal, occasionally sub-globose, (12.5 -) 14.5 - 21.0 (- 25.5) x (11.0 -) 12.5 - 17.0 (- 21.5) µm; spore wall verrucose, usually regular.

Illustrations: Weber (1884) Figs. 1-23; Magnus (1888) Figs. 3,4; de Toni (1888) Figs. 43,44; Schwartz (1910) Fig. 26 (see discussion); Schellenberg (1911) Fig. 54b; Lindau (1912); Gutner (1941) Fig. 112; Ainsworth and Sampson (1950) Fig. 12; Thirumalachar and Whitehead (1968) Fig. 2.

Host plants: *Juncus articulatus L. (syn. J. lamprocarpus Ehrh.) (Schwartz, 1910; Zillig, 1932), Juncus bufonius L., *Juncus bulbosus L. (syn. J. uliginosus Roth) (Cameron, 1886; Lindeberg, 1959; Buhr, 1965), *Juncus squarrosus L. (Cameron, 1886).

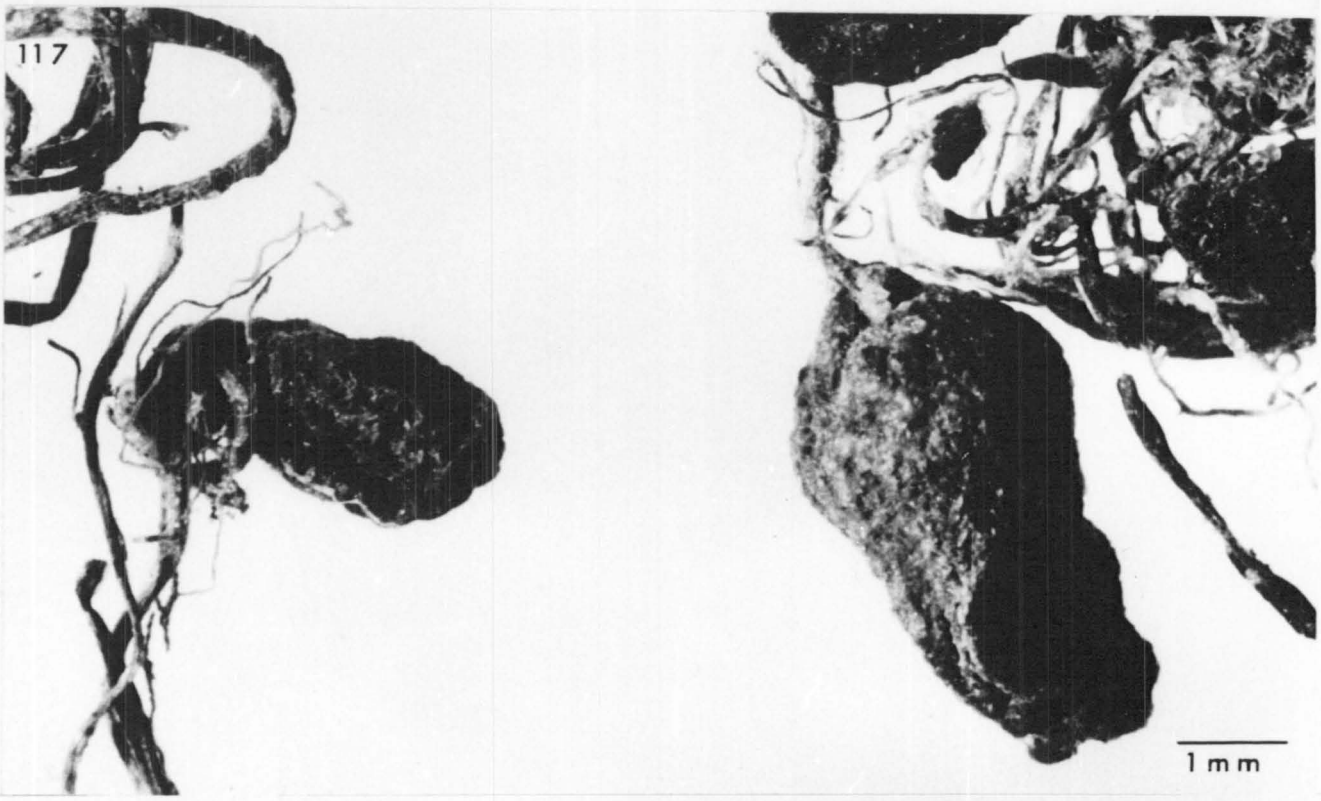


Fig. 116 Entorrhiza aschersoniana (Magn.) Lagerh. on Juncus bufonius L., LECTOTYPE.

Fig. 117 Galls of the lectotype at higher magnification.

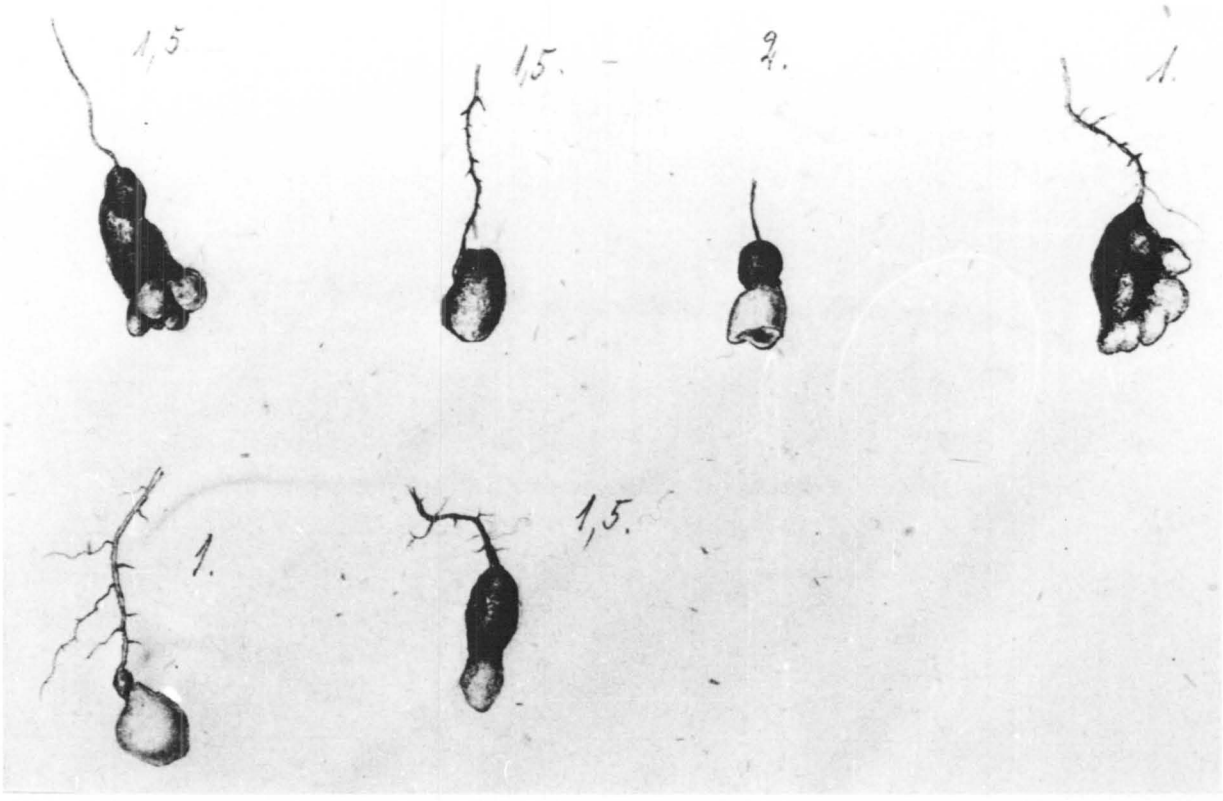


Fig. 118 Galls produced by Entorrhiza aschersoniana on Juncus bufonius, Altenhausen, Germany, 1894; a sketch by Magnus (HBG 40). x2

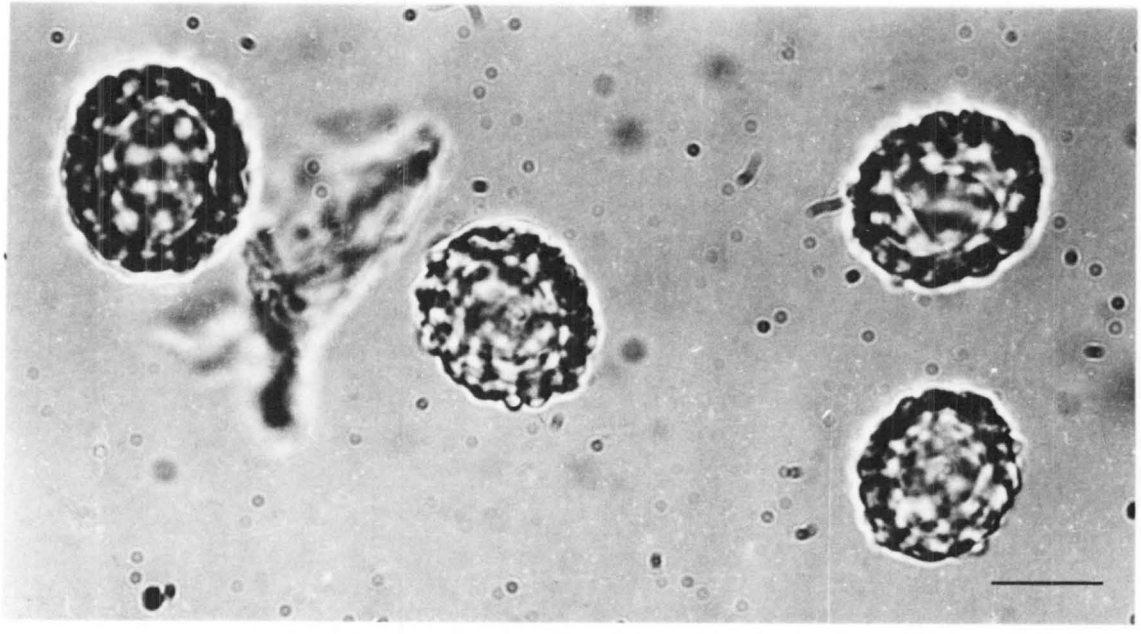


Fig. 119 Spores of Entorrhiza aschersoniana; LECTOTYPE.

Scale: line on figures of spores i.e. 119, 123, 134, 135, 140 & 141 represents 10 µm.

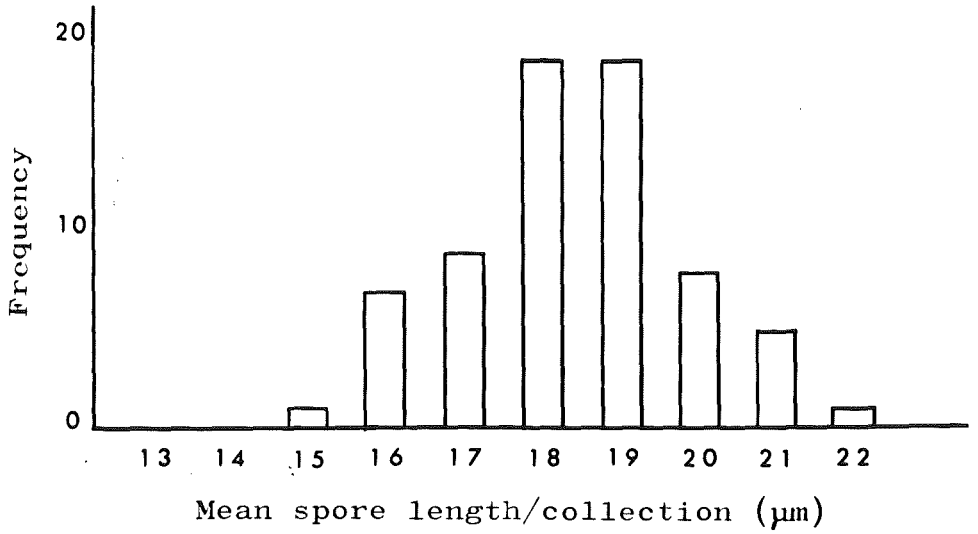


Fig. 120 Histogram to show range in mean spore length for collections of Entorrhiza aschersoniana on Juncus bufonius.

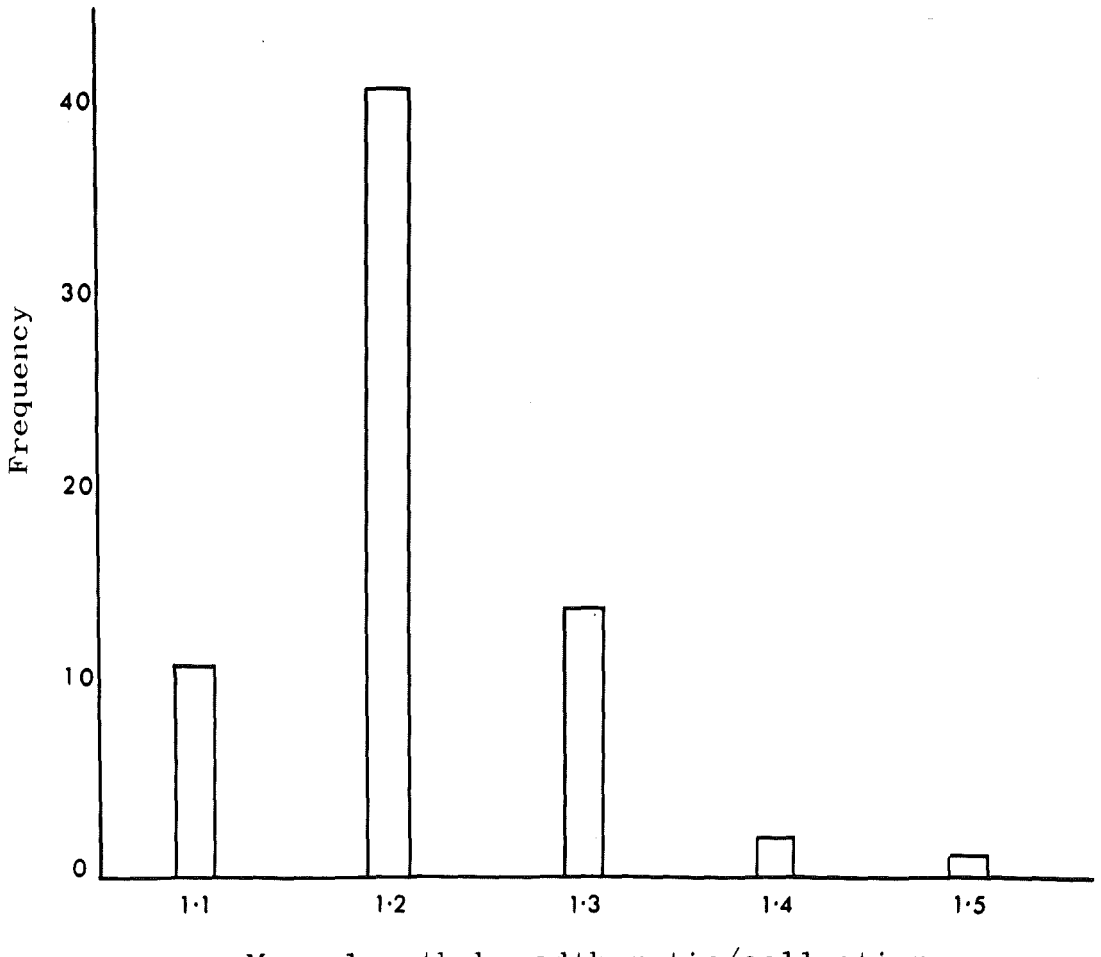


Fig. 121 Histogram to show range in mean length: breadth ratio for collections of Entorrhiza aschersoniana on Juncus bufonius.

Geographical distribution: on Juncus bufonius; Czechoslovakia, Denmark, *England (Schwartz, 1910), Finland, *France (Weber, 1884; Viennot-Bourgin, 1956), Germany, Italy, Netherlands, New Zealand, Norway (Magnus, 1902; Jørstad, 1963), Poland, Roumania, Scotland, Sweden, Switzerland, U.S.S.R. (Ulyanischev, 1968).

Collections examined: on Juncus bufonius

ex Herb. J.I. Liro, Sb., Kuopio, (Finland), Aug. 1856, M. Runeberg (H); ex Herb. Magnus, Grünwald, Berlin, (Germany), July 1878, P. Ascherson, LECTOTYPE (HBG); ex Herb. Magnus, Gaschwitz near Falkenberg, Silesia,(Germany), Aug. 1884, J. Schroeter, SYNTYPE (HBG); E. Rostrups Svampesamling, Charlottenlund, Sjaelland, (Denmark), July 1885, E. Rostrup (C); Herb. Rostrup, Randbøldal, Jylland, (Denmark), Sept. 1885, Rostrup (C); E. Rostrups Svampesamling, Treldeskov, Jylland, (Denmark), July 1886, E. Rostrup (C); Herb. Plowr., Links, Old Aberdeen, (Scotland), Sept. 1886, J.W.H. Trail (K); ex Herb. Sydow, Halensee, Berlin, (Germany), Aug. 1887, P. Sydow (S); ex Herb. Syd. Ustilag. 237, Varberg,(Halland), Sweden, Aug. 1887, G.v. Lagerheim (S); Varberg, Halland, (Sweden), Aug. 1887, G.v. Lagerheim or C.A.M. Lindman ? (S); Vestergr. Micr. rar. sel. 451, Varberg, Halland, Sweden, Aug. 1887, G.v. Lagerheim (UPS); ex Herb. Magnus, near Walthersdorf, (Germany), Aug. 1888, W. Krieger (HBG); ex Herb. Magnus, Statzer See, Oberengadin, Schweiz, Aug. 1888, G. Lagerheim (HBG); ex Herb. Magnus, Sarnthein Weg Nordhein,(Italy), Aug. 1888, Regenhart (HBG); Krieg. Fung. sax. 467, near Walthersdorf and near Königstein, (Germany), Aug. 1888 and 1889, W. Krieger (ZT); ex Herb. Syd. Ustilag. 47, Jungfernheide, Berlin, (Germany), June 1889, P. Sydow (S); ex Herb. Syd. Myc. march. 2615, Jungfernheide, Berlin, (Germany), July 1889, P. Sydow (S); Krieg. Fung. sax. 468, Muldenthale near Nossen,(Germany), Aug. 1889, W. Krieger (ZT); ex Herb. Magnus, Aug. 1889, P. Hennings (HBG); ex Herb. Magnus, near Nossen, (Germany), Oct. 1889, W. Krieger (HBG); ex Herb. Magnus, Bad Gastein hinter Ryrker Cafe, (Germany), Aug. 1890, P. Magnus (HBG); ex Herb. Magnus, near Walthersdorf, (Germany), June 1891, P. Magnus (HBG); ex Herb. Magnus, near Pohlitz near Greiz, (Germany), Aug. 1891, F. Ludwig (HBG); near Königsheim, Lachsen, (Germany), Aug. 1891, W. Krieger (S); ex Herb. Rehm, Rab. Fung. eur. 3902, Greiz i. V., (Germany), Sept. 1891, F. Ludwig (S); Herb. Rostrup, Klitmøller, (Jylland, Denmark), July 1894, Rostrup (C); ex Herb. Magnus, Ober Lansitz, Kr. Hoyerswerda, (Germany), July 1894, Barber (HBG); Herb. Tavel, plateau behind Gabistorfer-Horn, Aargau, (Switzerland), June 1895, F. v. Tavel (ZT); ex Herb. Magnus, Tupadeler Moor, Kr. Putzig, West Prussia, (Germany), June 1895, P. Graebner (HBG); ex Herb. Magnus, Karwenbruch, Kr. Putzig, West Prussia, (Germany), June 1895, P. Graebner (HBG); ex Herb. Magnus, Bielawa Bruch, Slawoschin, Kr. Putzig, West Prussia, (Germany), July 1895, P. Graebner (HBG); ex Herb. Sydow, Meiskau, V.L. Aecker, (Germany), Aug. 1895, P. Sydow (S); ex Herb. Magnus, near Triglitz,

(Germany), Oct. 1895, O. Jaap (HBG); ex Herb. Magnus, Neuruppin, (Germany), June 1896, C. Warnstorf (HBG); Herb. A. Ludwig, near Altendorf, (Germany), May 1898, Krieger (B); ex Herb. Magnus, near Schandem, July 1898, P. Magnus (HBG); ex Herb. J.I. Liro, N., Espoo, Kaitans, (Finland), 1902, A.O. Kairamo (H); ex Herb. J.I. Liro, N., Lappvik, Högsand, (Finland), 1907, O. Sundvik (H); Herb. Reg. Monacense, Herb. Bavaricum 1918 No. 1, Bernau, Oberbayern, (Germany), Sept. 1909, H. Paul (M); Suec. Köping, Öland, (Sweden), July 1910, G.E. du Rietz (UPS); Triglitz i.d. Prignitz, (Germany), Sept. 1910, O. Jaap (HBG); Rørvig, (Sjaelland, Denmark), June 1914, C. Ferdinandsen (C); ex Herb. J. I. Liro, N., Porvoo, Emsalö, Lervik (Finland), July 1915, Ch. & Fr. Boldt (H); Myc. fenn. 2:57, Lapinlahti, Helsinki, Nylandia, Finland, Aug. 1915, J.I. Liro (UPS); Väröbacka, Halland, (Sweden), June 1917, Erdtman (S); ex Herb. J.I. Liro, Ab., Skuru, (Finland), July 1917, J.I. Liro (H); ex Herb. J.I. Liro, N. Kirkkonummi, Bergstad, (Finland), July 1919, J.I. Liro (H); Fennia, Alandia, Foglo, Bano, July 1919, T. Putkonen (H); Herb. A. Ludwig, Kruty, (Czechoslovakia), July 1924, R. Picbauer (B); Herb. A. Ludwig, Fl. mor. Konty n. D, (Moravia, Czechoslovakia), July 1924, R. Picbauer (B); Petr. Fl. boh. and mor. 40: 1962, Gr. Kessel, (Czechoslovakia), July 1924, R. Picbauer (S); Fung. scand., Sweden, July 1927, K. Falck (UPS); ex Herb. J.I. Liro, Scandia, Svalöf, (Sweden), Sept. 1928, H. Christoffersson (H); ex Herb. J.I. Liro, Ob., Tervola, Loue, Saari, (Finland), Aug. 1933, H. Roivainen (H); ex Herb. J.I. Liro, Kb., Pielisjärvi, Kitsinvaara, Haukiaho, (Finland), July 1936, M. Laurila (H); Fung. fenn. 5:209, Valkeakoski, Finland, Aug. 1937, L.E. Kari (ZT); ex Herb. J.I. Liro, Ok., Paltamo, Kiehimä, Killimaki, (Finland), Aug. 1937, H.Roivainen & J. I. Liro (H); Ustilag. pol. 67, Krukienice distr. Mosciska, (Poland), Aug. 1938, J. Kochman (ZT); Herb. Fahrendorff, Kr Westhavelland, Paulinenaue, (Germany), July 1940, Fahrendorff 1153 (B); Fung, suec. 1288, Kall, Lillanjeskutan, above Lake Baksjön, Jämtland, (Sweden), Aug. 1942, J. A. Nannfeldt, 5938 (S); Fung. neer., Comm. ex Herb. Lugduno-Batavo, Leiden, Zuid-Holland, July 1955, C. Bas 961 (UPS); K. Vanky Ustilag. 19, Transsilvania, Hargita, pr. Tusnad, Roumania, Aug. 1956, K. Vanky (ZT); Plantae ex Herb. Univ. Helsingiensis, Nybacka, Alikerava, Kerava, Nylandia, Finland, June 1960, L. and H. Roivainen (UPS); Calgary, Isle of Mull, (Scotland), July 1968, R.W.G. Dennis (K); Einsiedeln, Switzerland, Sept. 1968, H. Seitter (ZT); EH Hollola, Laasonpohja, (Finland), 6769: 421, July 1970, V. Haikonen (H); Denniston Rd., Westland, New Zealand, Jan. 1971, J.M.F. (CANU); Stony Bay Rd., Akaroa, Canterbury, N.Z., Jan. 1972, J.M. Fineran, (CANU); Herb. A. Ludwig, Pütz, Pütnitz near Ribnitz, (Germany), Nov. ?, Lroy ? (B).

Discussion:

This species was reported first by Magnus (1878) on Juncus bufonius in a postscript to a paper describing a new fungus Schinzia cypericola on Cyperus flavescens. Magnus recorded that

the fungus on Juncus bufonius was also Schinzia cypericola. Weber (1884) investigated the fungus in the roots of Juncus bufonius in much greater detail. He studied material from two localities, including Halensee from where the material examined by Magnus had been collected. On the basis of his observations Weber erected a new genus Entorrhiza for these root-infecting fungi, with the single species Entorrhiza cypericola. In 1888 Magnus reported that the fungus infecting Juncus bufonius was distinct from the fungus in Cyperus. He described it as Schinzia aschersoniana, regarding the new genus Entorrhiza Weber as unjustified.

There has been some confusion as to whether Lagerheim (1888) or de Toni (1888) first published the combination Entorrhiza aschersoniana (see Ainsworth & Sampson, 1950). Lagerheim's report, dated August 1888, was printed in the Sept.-Oct. issue of "Hedwigia", however the exact date of publication cannot be ascertained (Prof. J. Gerloff, "Nova Hedwigia", pers.comm.) De Toni was published on 28 October, 1888. In view of the earlier date on Lagerheim's report Entorrhiza aschersoniana (Magn.) Lagerh. has been accepted as the correct author citation for this combination.

Entorrhiza aschersoniana is the most frequently recorded species of Entorrhiza. The following authors have contributed information on the geographical distribution of the species: Schroeter (1887), Lagerheim (1888), Magnus (1893, 1894, 1895a, b, 1896, 1902, 1905), Jaap (1897, 1899, 1901, 1905, 1907, 1917), Volkart (1903), Schellenberg (1911), Brefeld (1912), Lindau (1912), Lind (1913), Ferdinandsen and Winge (1914), Cruchet et al. (1915), Bubák (1916), Baudyš and Picbauer (1925), Hruby (1927), Picbauer (1933), Ciferri (1938), Liro (1938), Kochman (1939, 1960),

Gutner (1941), Ainsworth and Sampson (1950), Zundel (1953), Viennot-Bourgin (1956), Savulescu (1957), Lindeberg (1959), Jørstad (1963), Scholz (1968), Ulyanischev (1968), Dennis (1971).

All material of Entorrhiza aschersoniana examined was on Juncus bufonius. Occasionally, however, this species has been reported on other hosts; as far as the author is aware no herbarium material is available to confirm these reports. Zillig (1932) has published a photograph which appears to be an authentic record on Juncus articulatus. However, the reports by Plowright (1889), Massee (1891), Swanton (1912), Massee and Massee (1913) and Zundel (1953) for this host are almost certainly based on incorrect assumptions from Trail (1884) and Cameron (1886) (see Trail, 1890, 1903). The report of Schwartz (1910) of Entorrhiza cypericola on both Juncus bufonius and Juncus articulatus is ambiguous because it is not clear whether he failed to distinguish between two different species of Entorrhiza or whether both host plants were infected by the same species. It is impossible to determine which species of Entorrhiza was present on Juncus bufonius as the spores were not described. The species on Juncus articulatus has been interpreted as E. digitata by Liro (1938) and Fischer (1951), however the spores illustrated are almost certainly those of E. aschersoniana. Lindeberg (1959) mentions that there is a doubtful record (no access to specimens) of E. aschersoniana on Juncus bulbosus. She notes that the same host (i.e. J. uliginosus Roth) is recorded as infected in Great Britain (Cameron, 1886). The record for Juncus squarrosus (Cameron, 1886) is also doubtful (Trail, 1890). Massee (1891) suggested that the species mentioned by Cameron on Juncus squarrosus and J. uliginosus was probably Entorrhiza casparyana.

Ciferri (1963) has referred E. aschersoniana to E. casparyana using his trinary system of nomenclature (see Ciferri, 1932); under this system the species is listed as Entorrhiza casparyana (Magn.) Lagerh. - aschersoniana (Magn.) Cif. However, this is not a valid synonym because the status of the trinomial is not indicated and no type is designated. Nevertheless, Ciferri has shown by using this nomenclature that he recognised that E. aschersoniana was more closely related to E. casparyana than to any other species of Entorrhiza. He failed to note, however, the much closer similarity between E. casparyana and E. digitata.

Most of our knowledge of the biology of the species is due to Weber (1884), Trail (1884) and Brefeld (1912). This is the only species in which spore germination has been confirmed (Weber, 1884; Brefeld, 1912; Thirumalachar & Whitehead, 1968). According to Weber, who gives the most complete description, spores germinate by producing 1-4 promycelia, usually 3. The promycelia are usually aseptate and normally bear sporidia singly in a terminal position. The sporidia are falcate, looped in the middle and 7-9 µm long (not taking account of the loop). Thirumalachar and Whitehead (1968) describe the production of up to 3 sporidia. In Brefeld's studies the spores germinated directly.

In the present study 69 collections of E. aschersoniana from 12 countries have been examined; these include collections from countries as geographically isolated as Finland and New Zealand. These collections were entirely on one host species, Juncus bufonius, thus lending more weight to the analysis of variability in both sorus and spore structure.

Entorrhiza aschersoniana is readily identifiable by its

verrucose, oval spores. Although individual spores may sometimes approach a globose shape, the mean ratio of length:breadth for spores in any one collection is always found to be greater than 1.1. There is no overlap with Entorrhiza casparyana where this ratio is always less than 1.1.

A number of authors in addition to those mentioned in the above discussion have referred to E. aschersoniana, but they give very little new information: Tubeuf (1895), Frank (1896), Correns (1897), Lemmerman (1906), Lindau (1908), Migula (1910, 1912), Ross (1911), Kelley (1950), Fischer (1951), Talbot (1956), Fischer and Holton (1957).

Entorrhiza casparyana (Magn.) Lagerh.

Hedwigia 27 : 262, Sept. - Oct., 1888.

Figs. 122-130

Schinzia casparyana Magnus - Ber. dt. bot. Ges. 6:103 (1888)

Entorrhiza casparyana (Magn.) de Toni - Sacc. Syll. Fung. 7(2):497, 28 October (1888)

Melanotaenium casparyanum (Magn.) Thirum. and Whitehead-Am. J. Bot., 55(2):185 (1968)

Entorrhiza digitata Lagerh. - Hedwigia 27:264 (1888)

Schinzia digitata (Lagerh.) Magnus - Jahresb. Naturf. Ges. Graub. N.F., 34:7 (1890)

Melanotaenium digitatum (Lagerh.) Thirum. and Whitehead-Am. J. Bot., 55(2);185 (1968)

Lectotype: on Juncus tenageia Ehrh., Pyritz near Hinterpommern, Germany, 1863, Meyer (HBG 42)

Sori in galls, oval to elongate, often digitate or dichotomous up to 15 mm long with branches spreading to 15 mm wide. Teliospores globose or sub-globose (12.0-) 13.5-23.0 (-28.0) µm in diameter, occasionally much larger, yellow to chestnut brown;

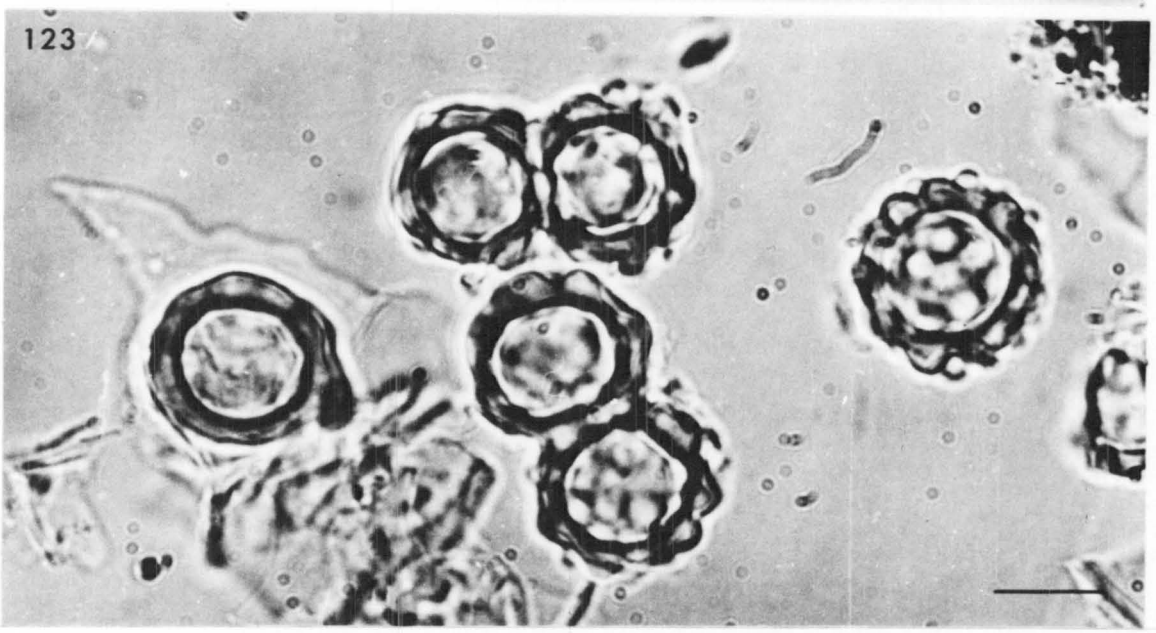
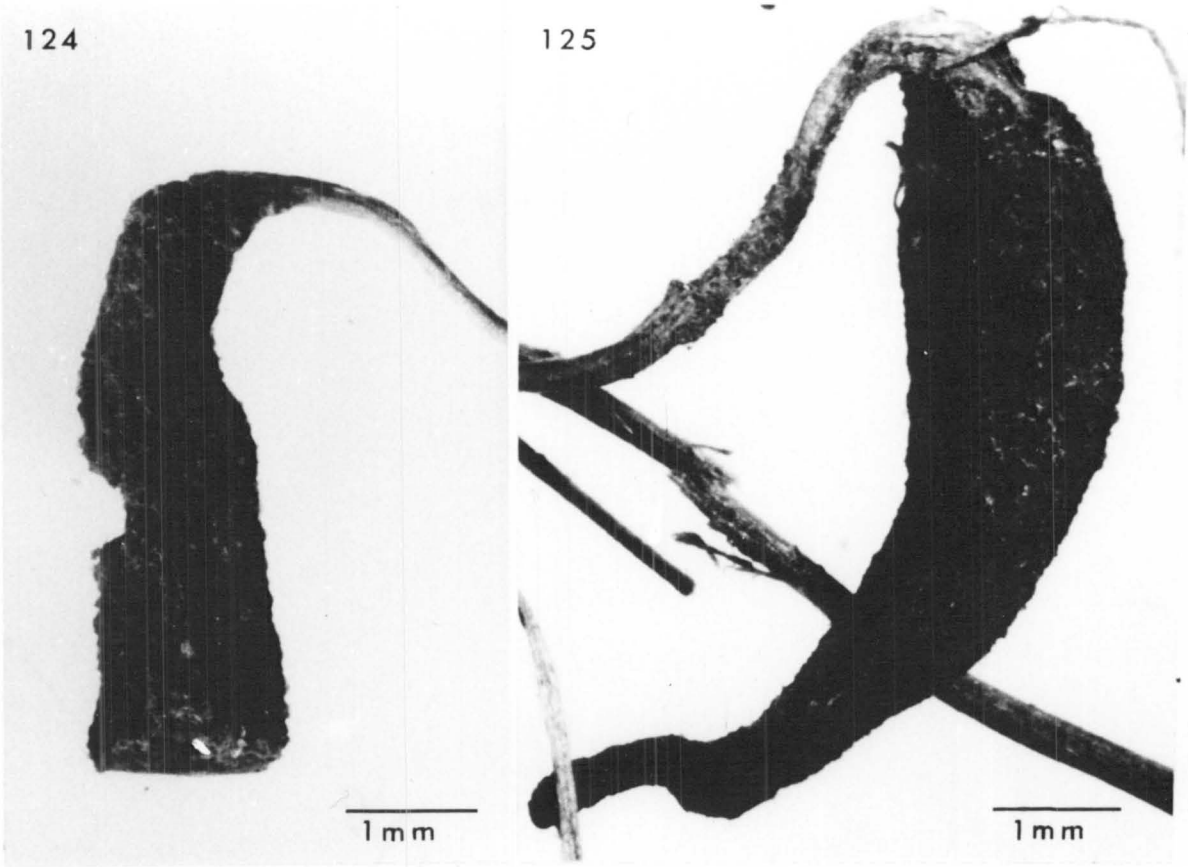


Fig. 122 Entorrhiza casparyana (Magn.) Lagerh. on Juncus tenageia Ehrh., Ascherson, 1891, Hoyerswerda, Germany.

Fig. 123 Spores of Entorrhiza casparyana from Juncus tenageia, Germany, collector and date uncertain (HBG 36).



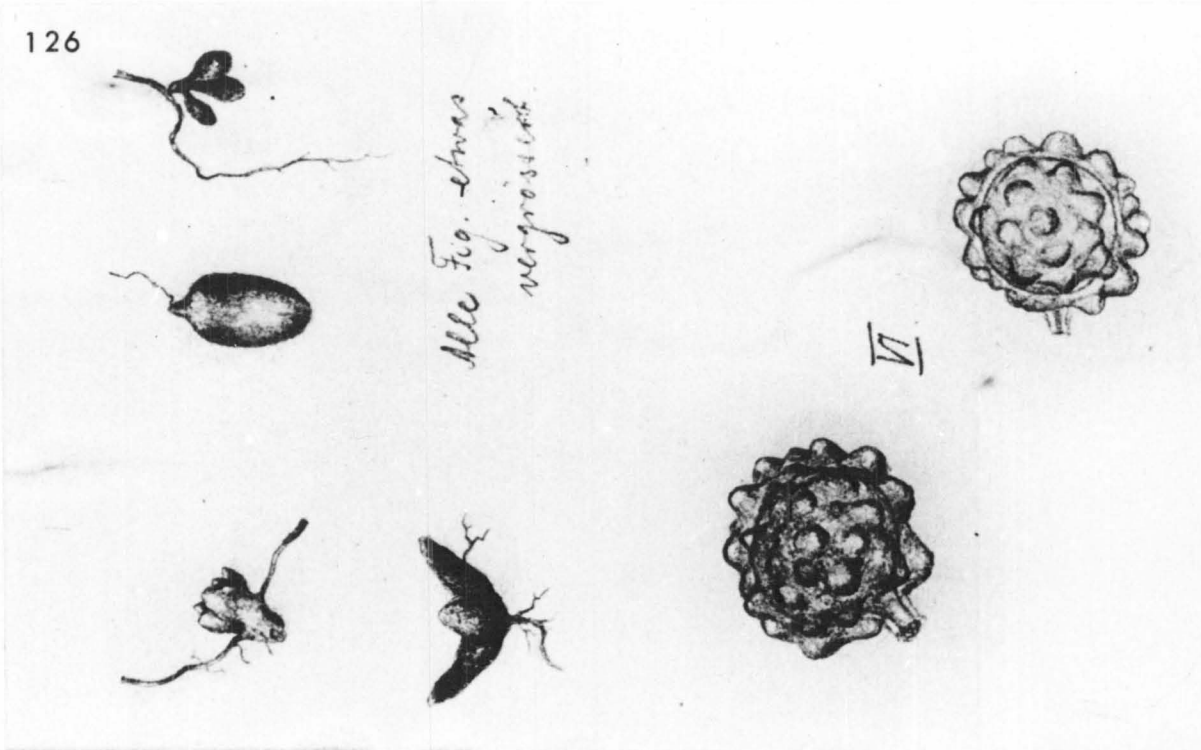


Fig. 124 Gall produced by Entorrhiza casparyana on Juncus tenageia; LECTOTYPE.

Fig. 125 Gall produced by Entorrhiza casparyana on Juncus tenageia; Germany (HBG 36).

Fig. 126 Galls and spores of Entorrhiza casparyana (syn. E. digitata Lagerh.) on Juncus articulatus L, Aberdeen, Scotland, Trail, 1899; a sketch by Magnus (HBG 40); x2.

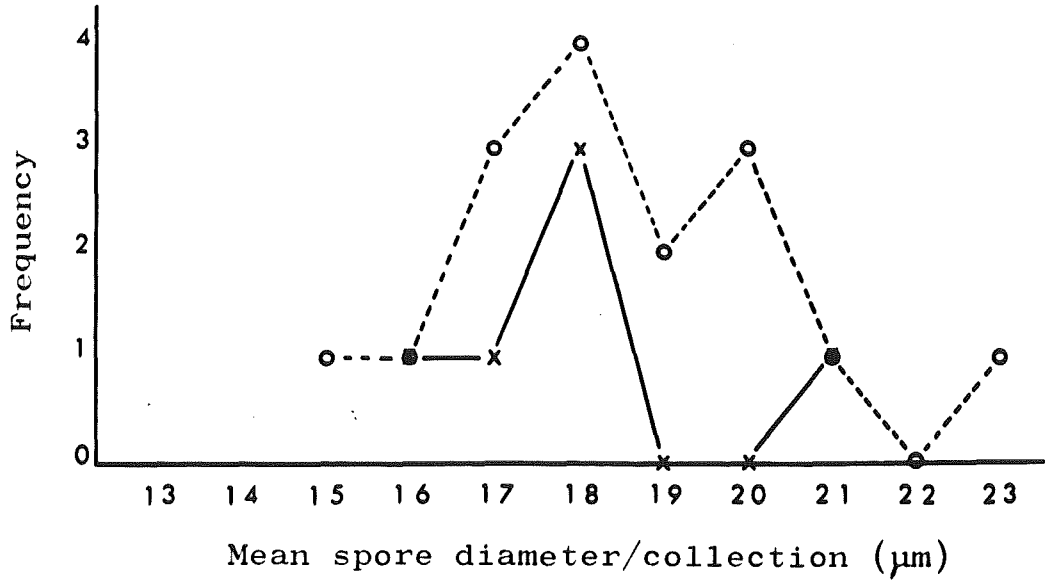


Fig. 127 Graph to show overlap in mean spore diameter for European collections of Entorrhiza digitata from Juncus alpinus and Juncus articulatus (broken line) and of Entorrhiza casparyana from Juncus tenageia (continuous line).

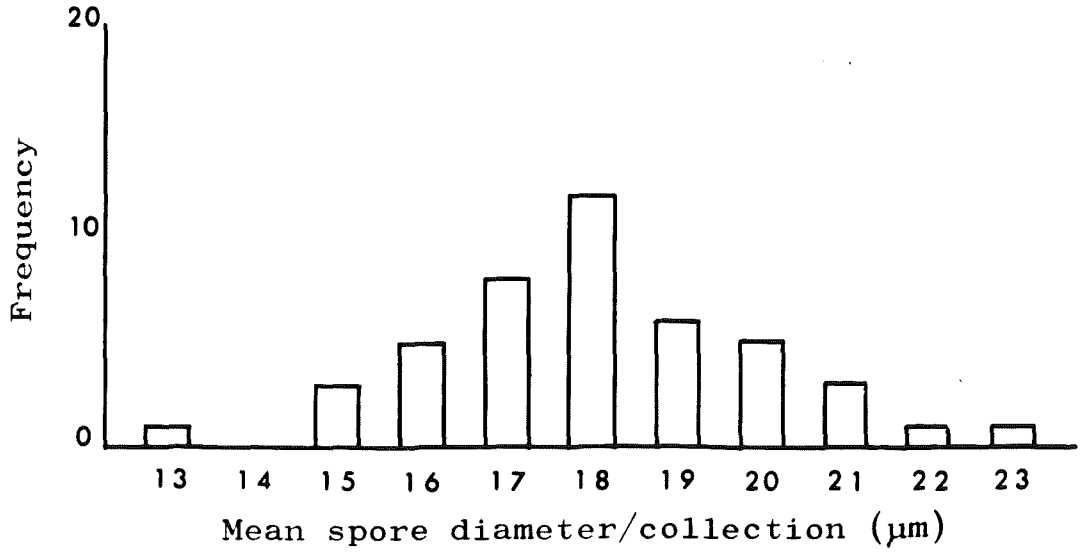


Fig. 128 Histogram to show range in mean spore diameter for collections of Entorrhiza casparyana (syn. E. digitata) on various species of Juncus.

Fig. 129 Entorrhiza casparyana (syn. E. digitata) on Juncus articulatus; Correns, 1903, Germany.

Fig. 130 Galls of the above at higher magnification.

spore wall smooth or verrucose, regular or irregular.

Illustrations: Magnus (1888) Figs. 5 & 6; Lagerheim (1888), Ferdinandsen and Winge (1914) Figs. 1, 8 (after Magnus); Liro (1938) Figs. 1, 5 (galls); Thirumalachar and Whitehead (1968) Fig. 4.

Host plants: *Eriophorum vaginatum L. (Ciferri, 1963), Juncus alpinus Vill., Juncus arcticus Willd. (Schellenberg, 1911), Juncus articulatus L. (syn. J. lamprocarpus Ehrh.), *Juncus atricapillus Drej. (Ciferri, 1963), Juncus bufonius L., *Juncus conglomeratus L., Juncus effusus L.; Juncus gregiflorus L. Johnson, Juncus inflexus L.; Juncus squarrosus L. (Ciferri, 1963); Juncus tenageia Ehrh.; Juncus tenuis Willd. (BUCA - not examined); Juncus uliginosus Roth (Ciferri, 1963), Juncus sp. (unidentified).

Geographical distribution: Czechoslovakia (Bubák, 1916, BPI), Denmark (Ferdinandsen and Winge, 1914), England (Liro, 1938; Fischer, 1951 from Schwartz, 1910), Faeroe Is. (Rostrup, 1901, Möller, 1945), Finland, *France (Viennot-Bourgin, 1956; Lindeberg, 1959), Germany, New Zealand, Norway (Jørstad, 1963), Poland (Kochman, 1960), Sardinia, Scotland, Sweden, Switzerland, U.S.S.R. (Ciferri, 1963; Ulyanischev, 1968).

Collections examined:

1. on Juncus alpinus

ex Herb. J.I. Liro, Sb. Maaninka, Petäinen, Naarvanlahti, (Finland), Aug. 1910, O. Kyyhkynen (H); ex Herb. J.I. Liro, Ta. Janakkala, Monikkala, (Finland), Aug. 1915, Fr. Elfving (H); Myc. fenn. 8:359, Nylandia, Pornainen, Kirveskoski, Finland, Aug. 1916, J.I. Liro and T. Putkonen (UPS); ex Herb. J.I. Liro, Ab. Lohja, (Finland), July 1917, J.I. Liro (H); ex Herb. J.I. Liro, N. Pornainen, Kirveskoski, (Finland), Aug. 1918, J.I. Liro (H); ex Herb. J.I. Liro, Germany, Sachsen, Oberlausitz, Aug. 1922, Richter (H); ex Herb. J.I. Liro, Sweden, Scania,

Ängelholms havsbad, Aug. 1931, H. Christoffersson (H); St. Jamijarvi, (Finland), Aug. 1936, M. Laurila (H). ex Herb. J.I. Liro, Sb. Kuopio, Palosaari, (Finland), July, 1937, J.I. Liro (H); Fung. suec., Lycksele l. c. Lappmark:Tarna s:n Gardiken-magasineto strand, dar landsvagen tangerar sjon, c. 6 km ovanfor Forsmark, Oct. 1969, G. Lohammar (UPS); Val Rosegg near Pontresina, Switzerland, Oct. 1969, J.M. Fineran (ZT).

2. on Juncus articulatus
ex Herb. Syd. Ustilag. 238, Thiessow ins. Rugiae, Germany, July, 1899, P. Sydow (S); ex Herb. Magnus, Tambach, Thüringer Wald, (Germany), Oct. 1903, C. Correns (HBG); Tlumia, N. Borgnas, Aug. 1916, Liro (S); Herb. A. Ludwig, Ostseebad, Ribuitz, Mecklenburg, (Germany), Aug. 1929, Buhr (B); Glen Cruaidh Choillie, Kinlochewe, W. Ross, (Scotland), Aug. 1963, R.W.G. Dennis (K); near Outram, Otago, New Zealand, ca. 540 m.a.s.l., March, 1968, N.C. Lambrechtsen (CANU); Pigeon Bay-Port Levy, Canterbury, New Zealand, Sept. 1970, J.M. Fineran (CANU); between Hurunui and Motunau, North Canterbury, New Zealand, Nov. 1970, J.M. Fineran (CANU); Bucklands Crossing, Waikouaiiti R., Otago, New Zealand, Dec. 1970, J.M. Fineran (CANU); Trotters Gorge, near Palmerston, Otago, New Zealand, Dec. 1970, J.M. Fineran (CANU); Cass Creek near Field Station, Canterbury, New Zealand, Jan. 1971, J.M. Fineran (CANU); foot of Porters Pass, Canterbury, New Zealand, Jan. 1971, J.M. Fineran (CANU); Mitchells, near Moana Kotuku Lodge, Westland, New Zealand, Jan., 1971, J.M. Fineran (CANU); Okains-Stony Bay coastal Rd., Canterbury, New Zealand, Feb. 1971, J.M. Fineran (CANU); Groynes, Waimakariri riverbed, Canterbury, New Zealand, Nov. 1971, J.M. Fineran (CANU); Blowing Point Bridge, Canterbury, New Zealand, Dec. 1971, J.M. Fineran (CANU); Lake Heron, Canterbury, New Zealand, Dec. 1971, J.M. Fineran (CANU); Smiths Creek, Canterbury, New Zealand, Dec. 1971, J.M. Fineran (CANU); Erewhon, Canterbury, New Zealand, Dec. 1971, J.M. Fineran (CANU).

3. on Juncus bufonius
Herb. Karsten 3179, Varberg, Sweden, G.Lagerheim (H); Fl. suec.,J. Eriksson:F. Par. Scand. 265, Varberg, Halland (Sweden), Aug. 1887, N. & G. Lagerheim (HBG); ex Herb. Syd., Wardan Doloner Gritz bei Halle, July 1896, Aeckel (S); ex Herb. J.I. Liro, N. Inkoo, Elisö, June 1911, W. Brenner (H); Summit Rd. between Hilltop and Okains, Canterbury, New Zealand, Feb. 1971, J.M. Fineran (CANU).

4. on Juncus effusus
near Rahu Saddle, Westland, New Zealand, Jan. 1971, J.M. Fineran (CANU); Denniston Rd., Westland, New Zealand, Jan. 1971, J.M. Fineran (CANU).

5. on Juncus gregiflorus
Akaroa, Canterbury, New Zealand, June 1968, J.M. Fineran (CANU); Pigeon Bay-Port Levy, Canterbury, New Zealand,

Sept. 1970, J.M. Fineran (CANU).

6. on Juncus inflexus
Fusio, Tessin, Switzerland, Nov. 1969, J.M. Fineran (ZT).

7. on Juncus tenageia
ex Herb. Magnus, Pyritz, (Hinterpommern, Germany), 1863, Meyer, LECTOTYPE (HBG); ex Herb. Magnus, Sa. Teresa Gallura, par Tempio (Sardinia), June 1881, Reverchon, SYNTYPE (HBG); ex Herb. Magnus, Hoyerswerda, Schlesien, (Germany), Aug. 1891, P. Ascherson (HBG); ex Herb. Magnus, (a) Graben bei Kuhnisch, Hoyerswerda (Germany), Aug. 1891, P. Ascherson & (b) Herb. P. Ascherson, Kaiser-Teihler Uhyst, ob. Lansitz, Hoyerswerda, (Germany), July 1893, E. Barber (HBG)- may be a mixed collection; ex Herb. J.I. Liro, Kaiser-teich b. Uhyst, (Germany), July 1893, Barber (H); ex Herb. J.I. Liro, Commerau b. Klise, b. Bautzen, Sachsen, Germany, Aug. 1922, K. Richter (H).

8. on Juncus conglomeratus or J. effusus ?
ex Herb. Magnus, bei Nossen, (Germany), Sept. 1889, W. Krieger (HBG) (see Magnus, 1902).

9. on Juncus sp. (unidentified)
Porters Pass, Canterbury, New Zealand, Dec. 1959, W.R. Philipson (CANU).

Discussion:

Entorrhiza casparyana was first collected by Meyer on Juncus tenageia in Prussia in 1863. However, it was recorded first by Grütter (1886) as Schinzia cypericola on Juncus tenageia from Kreise Schwetz, Schiroslaw, Prussia (see also Kelley, 1950). Magnus (1888) later described this species as Entorrhiza casparyana basing his description on material from Prussia and Sardinia. In 1887 Lagerheim (see collections examined) had collected this species on Juncus bufonius from Warberg, Sweden. It was not recognised as a new species, however, and was identified as Entorrhiza aschersoniana presumably because of its association with Juncus bufonius. Even Magnus was swayed by the belief, widely held among mycologists at that time, that species of parasitic fungi were confined to single host species; a duplicate

of Lagerheim's material from Warberg in Magnus' herbarium has written on it, apparently in Magnus' hand-writing, "Schinzia casparyana Magn !! auf Praeparate". This identification was subsequently crossed out and the fungus was accepted as E. aschersoniana.

In 1888 Lagerheim described a new species, Entorrhiza digitata, on the roots of Juncus articulatus from Titisee, Germany and Val Rosegg, Switzerland. He noted the similarity of the spores to E. casparyana (and E. aschersoniana) but believed that the spore wall did not have such large or irregular warts as E. casparyana.

Some mycologists have had difficulty separating E. digitata from E. casparyana using Lagerheim's description. The species of Entorrhiza on Juncus articulatus from the Faeroes (Rostrup, 1901) was first identified as E. casparyana. It has since been referred to E. digitata (Magnus, 1902; Liro, 1938; Möller, 1945) apparently merely on the basis of its association with J. articulatus.

In the present study the type material of E. casparyana has been compared with specimens of E. digitata from the type host (J. articulatus) and the type locality (Val Rosegg, Switzerland). The type material of E. digitata could not be located. In addition as many herbarium collections of the two species as possible have been examined as well as a large number of collections of fresh material. Observations on this material show that the ornamentation of the spore wall is extremely variable and that there is continuous variation among spores from one host species to the next. There is therefore no support for Lagerheim's decision to distinguish E. digitata from E. casparyana. An examination of the type material of E. casparyana indicates

that Magnus probably overemphasised the irregular nature of the spore wall.

However, despite the obvious similarities between the species there has been no suggestion up till the present time that the species were synonymous. In fact Ciferri (1963) has shown by his trinary system of nomenclature that he considers E. casparyana to be more closely related to E. aschersoniana than to E. digitata.

In E. casparyana spore dimensions are extremely variable, a fact already noted by Ferdinandsen and Winge (1914). There is, however, no correlation between spore size and the host species infected. E. casparyana is distinguishable from all other species of Entorrhiza in that the spores are basically globose, often smooth walled (although verrucose ornamentation is typical) and sometimes occur in pairs. Extremely thick-walled spores are probably more frequently found in this species than in other species of Entorrhiza.

Gall shape is another variable character in E. casparyana. At present there is insufficient evidence to correlate gall form with the host species but further observations, particularly from cross-inoculation studies, will show whether such a correlation exists. So far it appears that galls on J. tenageia are elongated and unbranched whereas those on J. articulatus (and possibly J. bufonius) are mainly digitate and those on J. effusus and J. gregiflorus are finely divided and predominantly dichotomous.

There are very few records of Entorrhiza casparyana in the literature. Apart from a few collections made soon after Magnus described the species (Magnus,1893, 1895a; Lindau, 1912) the only new records on Juncus tenageia are those of Kochman (1960)

for Poland and Ciferri (1963) for the USSR. Ciferri's record is not substantiated by Ulyanischev (1968). Reports by Zundel (1953) and Ciferri (1938, 1963) of its occurrence on Juncus tenageia in Czechoslovakia are probably based erroneously on Bubák (1916).

Although Lagerheim (1888) was the first to identify the species of Entorrhiza on Juncus articulatus the existence of root swellings on this host had been recorded earlier in Britain by Trail (1884). The species was variously reported as E. cypericola (Magn.) Weber (syn. Schinzia cypericola) (Plowright, 1889; Swanton, 1912) and E. aschersoniana (Massee, 1891; Massee & Massee, 1913). In 1903 Trail noted the species Entorrhiza digitata in Britain for the first time. Schwartz (1910) studied Entorrhiza cypericola Weber in the roots of Juncus articulatus, J. bufonius and J. lamprocarpus in Britain. Most of his observations were carried out on J. articulatus. The fungus he observed on this host has been interpreted as E. digitata (Liro, 1938; Fischer, 1951). However, Schwartz' illustrations are almost certainly of E. aschersoniana. In the absence of specimens it is not possible to decide whether this constitutes a new host record for E. aschersoniana or whether Schwartz' illustrations were inaccurate. E. digitata on J. articulatus has since been recorded in Britain by Dennis (1971).

Only a limited number of collections on Juncus articulatus are available in European herbaria. The majority of collections of Entorrhiza casparyana are on Juncus alpinus (as E. digitata). A number of early workers (eg. Ferdinandsen & Winge 1914; Liro, 1938) believed E. digitata was restricted to the Juncus alpinus group and went so far as to suggest that plants identified as J. articulatus should probably be J. alpinus.

Entorrhiza casparyana has a wider host range than has been recorded for other species of Entorrhiza. However, some records have not been confirmed. The record for Juncus atricapillus (Ciferri, 1963) may be a misinterpretation of Ferdinandsen and Winge (1914) and Liro (1938). These authors stated that plants of Juncus atricapillus grew alongside infected specimens of Juncus articulatus (Juncus alpinus) without becoming infected. Schellenberg (1911) recorded Juncus arcticus as the host for Lagerheim's collection from Val Rosegg. As no herbarium material can be found, it is not possible to verify this report; both J. arcticus and J. articulatus occur in this locality. J. arcticus is, in fact, quite frequent in the Val Rosegg area eg. at 2000 m in alluvium (Flora von Graubünden), although it is fairly rare elsewhere in Switzerland.

Early collections, identified as Entorrhiza cypericola Weber, on Juncus squarrosus and Juncus uliginosus (Cameron, 1886) were tentatively assigned to E. casparyana (Massee, 1891) and are listed under this species by Ciferri (1963). Ciferri also lists Eriophorum vaginatum as a host for E. casparyana but this record must be regarded as extremely doubtful. According to Cameron this plant has "almost identical tumours" to those caused by Entorrhiza "on the stem quite close to the roots". This type of infection is, however, unlike those known to be caused by species of Entorrhiza.

The record for Juncus filiformis (Liro, 1938) is also in error. An examination of herbarium material shows that the spores on this plant cannot be distinguished from those of E. caricicola.

Entorrhiza casparyana is recorded on Juncus bufonius which is normally host to a different species, Entorrhiza aschersoniana. Although the first collections of E. casparyana

on J. bufonius appear to have been made by Lagerheim (see earlier discussion) Kochman (1960) was the first to record this host-parasite relationship (as E. digitata).

Apart from the original brief descriptions of E. casparyana on Juncus tenageia (Magnus, 1888) and Juncus articulatus (Lagerheim, 1888) there is very little information on this species from the literature. Ferdinandsen and Winge (1914) observed spore formation and illustrated the wide range of spore size, form and sculpturing which may occur. Liro (1938) also examined new material and discussed gall shape and host specificity in infections of the Juncus articulatus - Juncus alpinus group.

In addition to the authors referred to in the above account a number of others have mentioned E. casparyana (syn. E. digitata) but add very little new information : Trail (1890), Magnus (1890, 1893, 1895 b), Tubeuf (1895), Frank (1896), Correns (1897), Sydow (1900), Ascherson and Graebner (1904), Lemmerman (1906), Migula (1910, 1912, 1917), Ross (1911), Hruby (1927), Ainsworth and Sampson (1950), Talbot (1956), Fischer and Holton (1957), Buhr (1965). Further references are cited by Liro (1938).

Entorrhiza caricicola Ferd. & Winge

Dansk bot. Ark. 2(1) : 10(1914)

Figs. 131-137

Melanotaenium caricicola (Ferd. & Winge) Thirum. & Whitehead- Am. J. Bot., 55(2) : 185 (1968)

Entorrhiza juncicola Ferd. & Buchw. in Lindeberg, Symb. bot. upsal. 16 : 26 (1959) - nom.nud.

Entorrhiza cypericola auct. non Weber : Rostrup, Bot. Tidsskr. 19:36 (1894); Lind, Danish Fungi : 271 (1913)

Entorrhiza cypericola auct. non de Toni : Rostrup, Bot. Tidsskr. 19:48 (1894); Lindau, Kryptogamenflora der Mark Brandenburg, 5a (1) : 64 (1912)

131

132

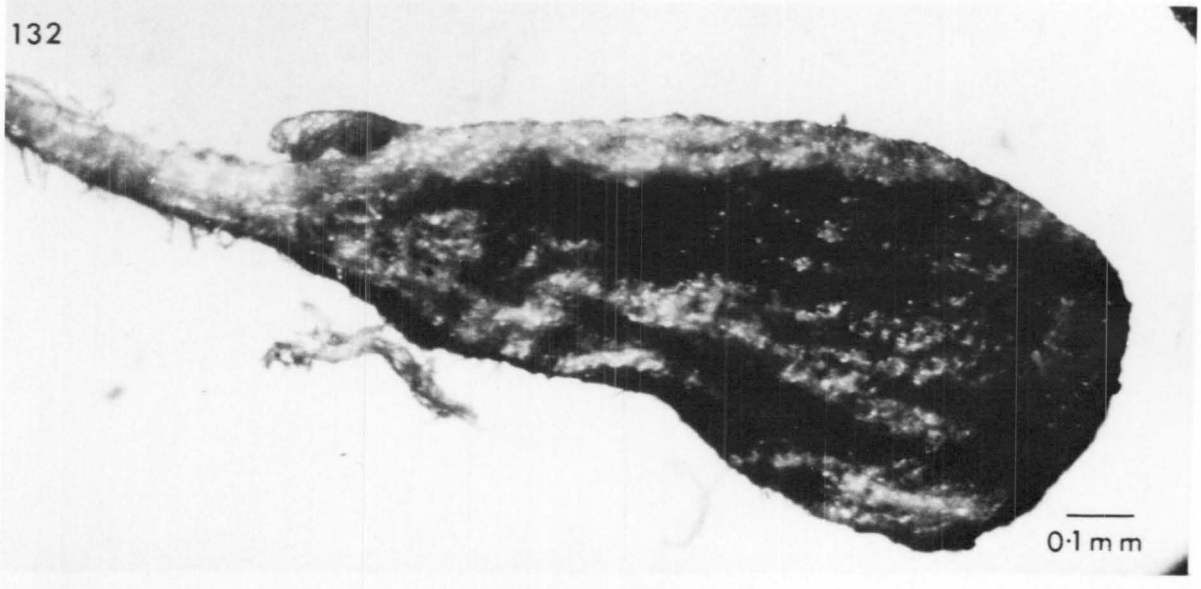


Fig. 131 Entorrhiza caricicola Ferd. & Winge on Carex limosa.; ISOTYPE (HBG 54). ca. x1

Fig. 132 Gall of the above at higher magnification.

Fig. 133 Entorrhiza caricicola on Carex resectans Cheesem.; L.B. Moore, 1971, New Zealand.

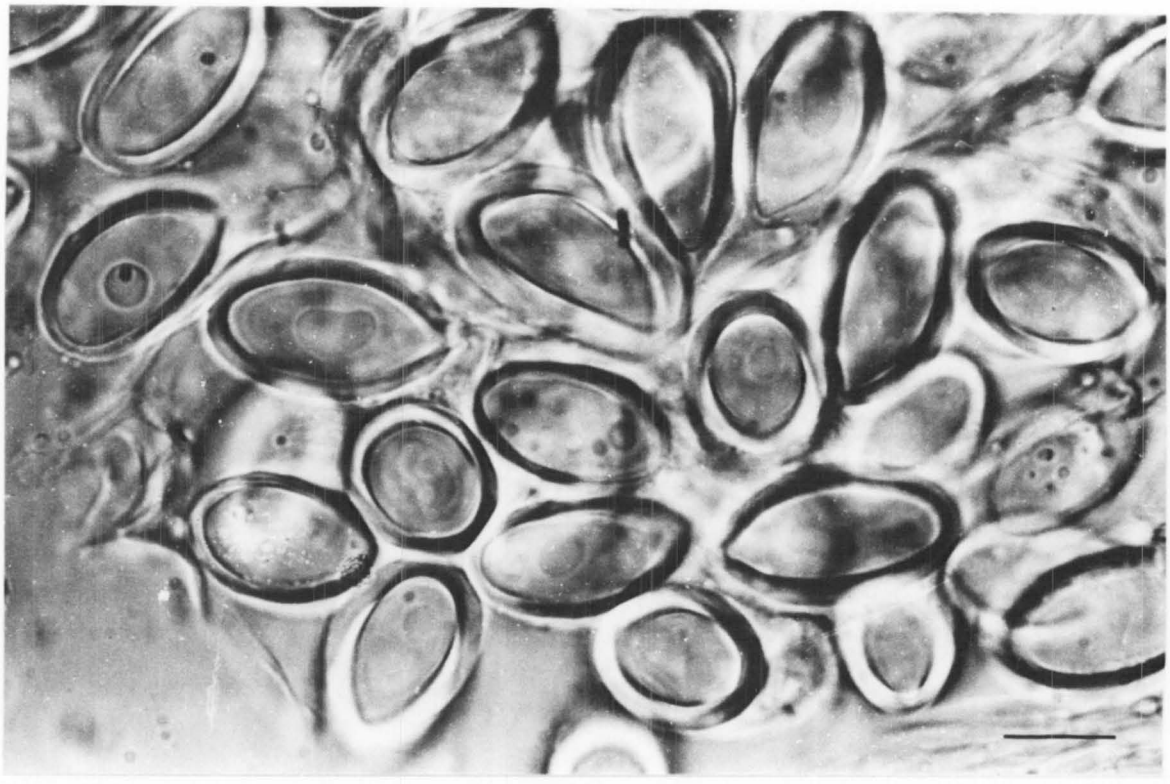


Fig. 134 Spores of Entorrhiza caricicola from Carex limosa; HOLOTYPE.

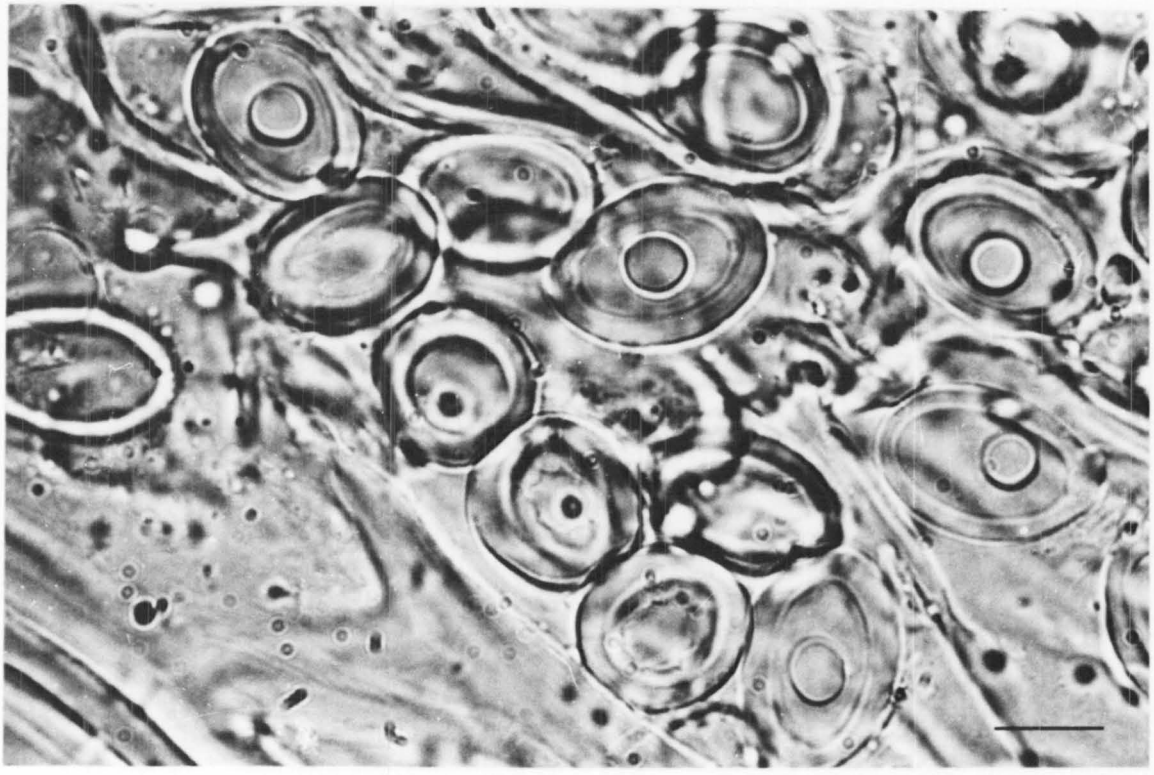


Fig. 135 Spores of Entorrhiza caricicola (syn. E. juncicola Ferd. & Buchw. in Lindeberg, nom.nud) from Juncus filiformis L.; Christoffersson, 1932, Sweden.

Entorrhiza digitata auct. non Lagerh. : Liro, Die Ustilagineen Finnlands 2 : 533 (1938).

Holotype: on Carex limosa L., Denmark, Zealand, 'Lyngby Mose', September, 1893, F.K. Ravn, 'specimen originale' (C).

Sori in galls, oval to elongate, sometimes pyriform, sometimes tapering towards the apex, usually unbranched, up to 2 mm wide x 6 mm long. Teliospores ellipsoidal to oval or elongate, often tapering towards the stalk, (16 -) 19.5 - 24.0 (-26.5) x (9.5 -) 10.5 - 16.5 (- 18.5) µm, sometimes asymmetrical, pale yellow; spore wall rugulose - undulate (especially in immature spores) to smooth.

Illustrations: Ferdinandsen and Winge (1914), Fig. 7.

Host plants: Carex gaudichaudiana Kunth, Carex limosa L., Carex resectans Cheesem., Carex sinclairii Boott in Cheesem., Eleocharis gracilis R. Br., Juncus filiformis L., Juncus pusillus Buch., Juncus sp.

Geographical distribution: Denmark, Finland, New Zealand, Sweden, U.S.S.R. (Ciferri, 1963).

Collections examined:

1. on Carex limosa
E. Rostrups Svampesamling, Lyngby Mose, Sjaelland, (Denmark), Sept. 1893, K. Ravn, HOLOTYPE (C); Cryp. dan., Lyngby, (Sjaelland, Denmark), Oct. 1895, C. Ostenfeld - Hansen, PARATYPE (C).

2. on Carex resectans
Kettlehole, Fagan's Downs, Sth. Ashburton River, Canterbury, New Zealand, Jan. 1971, L.B. Moore (CANU).

3. on Carex sinclairii
Lookout Hill Bog, north of Lake Te Anau, Otago, New Zealand, Feb. 1971, C.J. Burrows (CANU).

4. on Carex gaudichaudiana
Bog, north-west shore of Lake Heron, Canterbury, New Zealand, Dec. 1971, J.M. Fineran (CANU).

5. on Eleocharis gracilis
Lookout Hill Bog, north of Lake Te Anau, Otago, New Zealand, Feb. 1971, A.T. Dobson (CANU).

6. on Juncus filiformis
Fl. Suec., Osby, Skåne, (Sweden), July 1932, H. Christoffersson (UPS); Myc. fenn. 8 : 360, Palosaari, Kuopio, Finland, July 1937, Roivainen and J.I. Liro (H); Tb. Killinkoski, Pappila, (Finland), July 1960, Laila and H. Roivainen (H).

7. on Juncus pusillus
Kettlehole, Fagans Downs, Sth. Ashburton River, Canterbury, New Zealand, Jan. 1971, L.B. Moore (CANU).

8. on Juncus sp.
Stony Bay Rd., Akaroa, Canterbury, New Zealand, Jan. 1972, J.M. Fineran (CANU).

Discussion:

This species was recorded first by Rostrup (1894) as Entorrhiza cypericola (Magn.) Weber on Carex limosa. However, according to Ferdinandsen and Winge (1914), Rostrup noted that the spores were larger (20 - 25 µm x 12 - 15 µm) and had a different structure from those described by Magnus (1878) for E. cypericola and therefore possibly belonged to a distinct species.

In 1914 Ferdinandsen and Winge re-examined the material described by Rostrup and compared it with specimens of E. cypericola from the type locality. On the basis of differences in spore shape and sculpturing they erected a new species E. caricicola for the fungus on Carex limosa. This species had relatively narrower spores than E. cypericola (1.7 : 1.0 compared with 1.4 : 1.0) and the wall was not as thick or as coarsely sculptured.

Since 1914 very little has been added to our knowledge of E. caricicola. Liro (1938) revised Ferdinandsen and Winge's description of the species, presumably on the basis of a re-examination of the Danish material; he noted that the galls were up to 8 mm in length and that the spores were often smaller

than described by Ferdinandsen and Winge.

Ciferri (1963) has recorded E. caricicola on Carex limosa for the U.S.S.R. This record was not confirmed by Ulyanischev (1968). There are, however, roots of Carex livida from the U.S.S.R. in the herbarium at Helsinki (H) and these may be infected by Entorrhiza. The slightly swollen root apices appear similar to those induced by species of Entorrhiza but no spores could be found. Davies et al. (1973) have recently reported swollen lateral roots on the Cyperaceae which were free of endophytes and these roots may be analogous to those of Carex livida.

Entorrhiza caricicola on Carex limosa is recorded only from the type locality near Copenhagen, Denmark, where, according to Lindeberg (1959) "it has been collected for several years". Herbarium material consists entirely of specimens collected by Ravn in 1893 and by Ostenfeld - Hansen in 1895.

In the present study a detailed examination was made of the holotype and paratype from Copenhagen (C) and the observations confirmed on duplicates from Hamburg, Stockholm, the Plant Pathology Department, Copenhagen and Helsinki (isotype material only). The material is rather immature making comparisons with other collections of Entorrhiza more difficult. Nevertheless, the spores are much longer (20.0 - 21.5 µm) than those of E. cypericola (generally 17 - 18 µm) and have a mean length:breadth ratio of 1.8 - 1.9 in comparison with 1.3 for the 12 collections of E. cypericola examined. Furthermore, although both species are basically smooth-walled, E. caricicola can be distinguished from E. cypericola because the wall is not differentiated into an inner reticulated wall layer and an outer smooth layer and never possesses such marked wrinkling or ridging

as is found in small spores of E. cypericola.

These results confirm the decision made by Ferdinandsen and Winge to regard this as a separate species. The decision is supported by an examination of several collections of fresh material from New Zealand, not only on species of Carex but also on another genus of the Cyperaceae, Eleocharis, and on a member of the Juncaceae, Juncus pusillus. The spores found on these New Zealand hosts are identifiable as Entorrhiza caricicola mainly because of their ellipsoidal or oval to elongated shape and lack of any definite pattern of sculpturing at any stage of development.

Also included in the host range for Entorrhiza caricicola is Juncus filiformis. Entorrhiza was first collected on this host by Christoffersson from Sweden in 1932. Ferdinandsen and Buchwald tentatively assigned the fungus on Juncus filiformis to a new species Entorrhiza juncicola. They used this name only on herbarium sheets but it has since been published by Lindeberg (1959). The same fungus has been collected on Juncus filiformis in Finland in 1937 (Liro and Roivainen) and 1960 (L. & H. Roivainen) and incorrectly identified as Entorrhiza digitata Lagerh.

An examination of these 3 collections including duplicates of the 1932 and 1937 material (at CP & UPS respectively), shows that the fungus is not a separate species. The spores on Juncus filiformis closely resemble those of E. caricicola. Although there is continuous variability in spore shape, size and ornamentation for spores on Juncus filiformis and on other plants invaded by E. caricicola, there appears to be a larger proportion of less elongated spores on Juncus filiformis. This is shown by the fact that mean length:breadth ratios

recorded for spores from Juncus filiformis are between 1.3 and 1.5 whereas for the remainder of E. caricicola hosts these ratios are between 1.4 and 1.9. In the fungus on Juncus filiformis there also appears to be less tendency for the spore to taper towards the stalk and the stalk is more often inserted asymmetrically.

Ferdinandsen and Buchwald may have been influenced in their decision to regard the fungus on Juncus filiformis as a different species because a different host family was infected. However, an examination of Juncus pusillus shows that E. caricicola does occur on Juncaceous as well as Cyperaceous hosts. Entorrhiza caricicola thus becomes the first species of Entorrhiza to be reported on both the Cyperaceae and the Juncaceae.

E. caricicola has also been found with Entorrhiza casparyana in mixed infections on one species of Juncus.

The species description for Entorrhiza caricicola is based on the 11 collections examined. When all collections are considered there is only a narrow zone of overlap with E. cypericola in spore shape and size (Figs. 136 & 137).

Entorrhiza caricicola is referred to in various publications in addition to those mentioned in the above discussion. However, these contribute very little new information: Magnus (1895 a, b, 1902), Rostrup (1899), Zundel (1953), Talbot (1956), Fischer and Holton (1957), Buhr (1965).

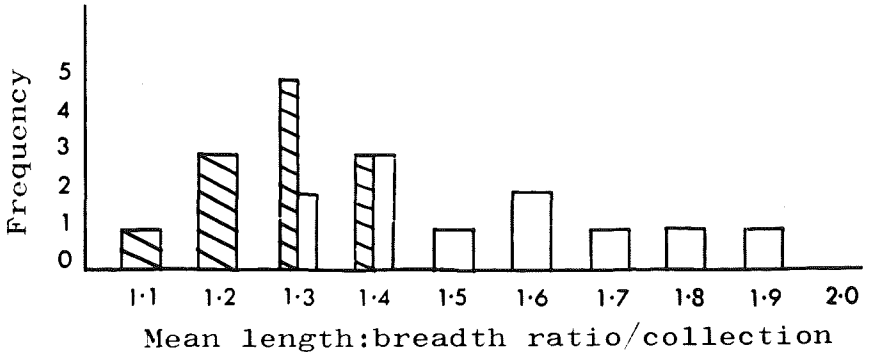


Fig. 136 Histogram to show range in mean length:breadth ratio for collections of Entorrhiza caricicola, □, and Entorrhiza cypericola (Magn.) de Toni, ▨.

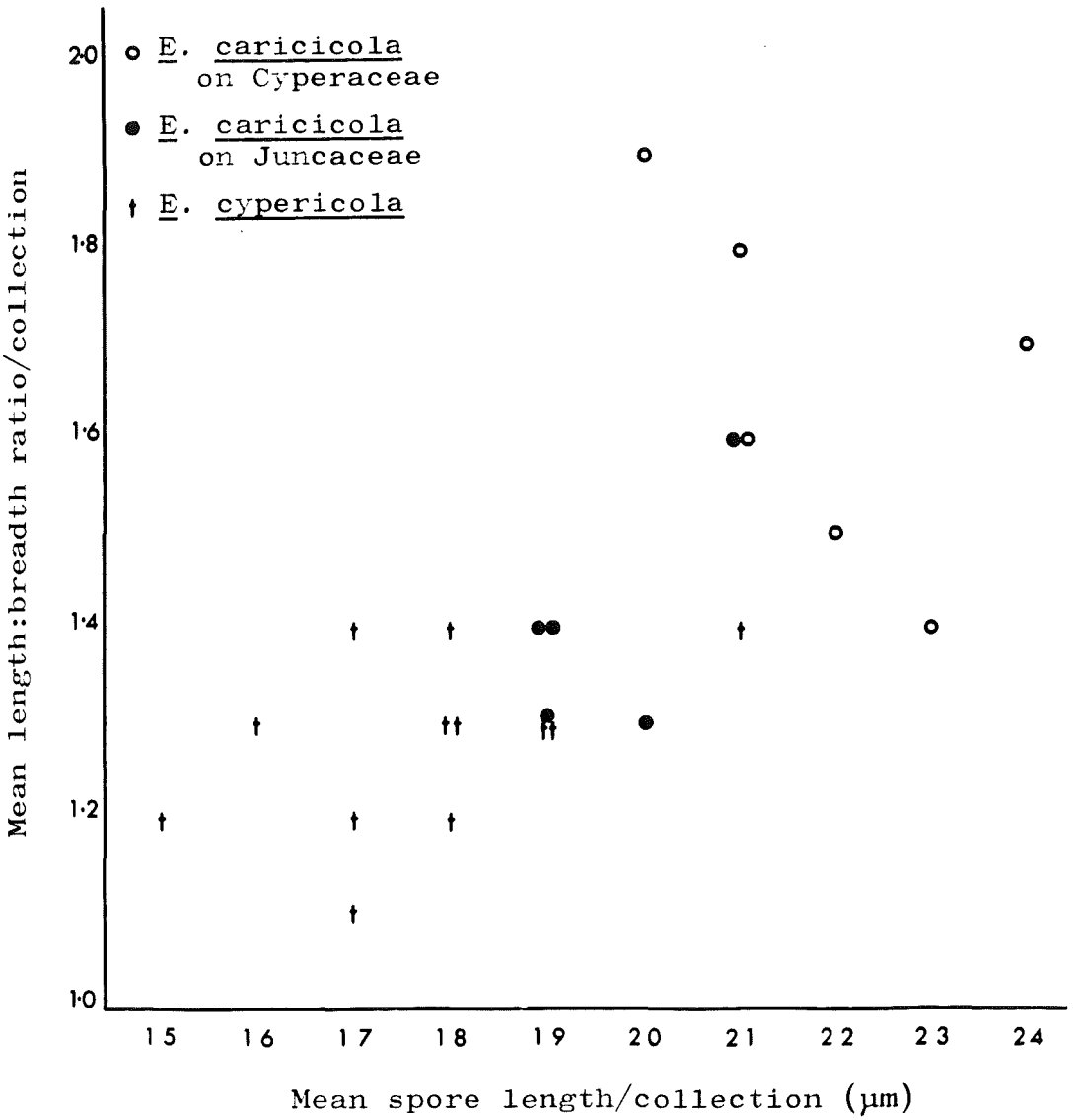


Fig. 137 Scatter diagram of mean spore length and mean spore shape (length:breadth ratio) for collections of Entorrhiza caricicola and Entorrhiza cypericola.

Entorrhiza cypericola (Magn.) de Toni

Sacc. Syll. Fung. 7(2) : 498 (1888)

Figs. 136-140

Schinzia cypericola Magnus - Verh. bot. Ver. Prov. Brandenb., 20:53 (1878)

Entorrhiza cypericola (Magn.) Weber p.p. - Bot. Ztg., 42 : 378 (1884)

Melanotaenium cypericola (Magn.) Thirum. & Whitehead - Am. J. Bot., 55(2) : 185 (1968)

Entorrhiza cyperi Brefeld - Unters. Gesammt. Myk., 15 : 80 (1912)- nom. nud.

Holotype: on Cyperus flavescens L., Germany, Berlin, Halensee, Grünwald, August 1876, C. Müller (HBG 48)

Sori in galls, usually elongate, often branched, the branches usually lying parallel, up to 14 mm long and spreading to 10 mm wide. Teliospores ellipsoidal, (12.5 -) 14.5 - 21.0 (- 28.0) x (11.0 -) 12.5 - 15.5 (- 19.0) µm, light yellow; spore wall rugulose to broadly reticulate often overlaid by a thick, smooth layer.

Illustrations: Magnus (1888, 1893); Lindau (1912); Ferdinandsen and Winge (1914), Fig. 8 (after Magnus); Zambettakis (1970), Fig. 179.

Host plants: Cyperus flavescens L., *Cyperus amabilis Vahl.

Geographical distribution: Algeria, Corsica (Maire et al., 1903), *Czechoslovakia (Zundel, 1953), *Denmark (Ciferri, 1938, 1963; Thirumalachar & Whitehead, 1968), *Erythraea (Ethiopia), France, Germany, Italy, Poland (Kochman, 1960), Roumania, *Switzerland (Schellenberg, 1911), U.S.S.R. (Ciferri, 1963; Ulyanischev, 1968).

Collections examined: on Cyperus flavescens

ex Herb. Magnus, Tour Castelvieil pr. Bagnères de Luchon, Dép. Haute Garonnes, Frankreich, Aug. 1851, J. Lange (HBG); ex Herb. Magnus, Fl. march, Halensee, Grünwald, Berlin, (Germany), Aug. 1876,

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Fig. 138 Entorrhiza cypericola (Magn.) de Toni on Cyperus flavescens L.; HOLOTYPE.

Fig. 139 Galls of the above at higher magnification.

C. Müller, HOLOTYPE (HBG); ex Herb. J.I. Liro, Rhanandieres, Sept. 1881, collector unknown (H); bei Röttenbach bei Schweinau, südlich von Nürnberg, (Germany), Oct. 1884, Fr. Schultheiss, (HBG); ex Herb. Syd. Myc. march. 1138, Halensee, Berlin, (Germany), Aug. 1886, P. Sydow, (HBG); Halensee, (Berlin, Germany), Oct. 1889, J. Hennings (C); ex Herb. J.I. Liro, Grünwald, Germany, Oct. 1889, P. Hennings (H); ex Herb. Magnus, Meran, Sudtirol, (Italy), Sept. 1890, P. Magnus (HBG); ex Herb. Magnus, Dichsendorfer bei Erlangen, (Germany), Aug. 1892, P. Ascherson & A. Schwarz (HBG); ex Herb. Magnus, près de Michelet (Grande-Kabylie), Algérie, Sept. 1902, B. Maire, (HBG); K Vánky Ustilag. 47, Transsilvania, pr. Brasov, Rétyi-Nyir, ca 500 m.s.m., Roumania, Sept. 1960, K. Vánky (ZT); ex Herb. J.I.Liro, (Herb. Firenze), collection details unknown (H).

Discussion:

Entorrhiza cypericola (Magn.) de Toni is first known to have been collected in 1845 (Magnus, 1888) from the Grünwald near Berlin. The first published record appears to be that of Lange (1864) who described swellings on the roots of Cyperus flavescens collected in 1851 from France (see Magnus, 1893). However, it was not until 1878 that Magnus described this species as Schinzia cypericola based on material collected by Müller from the Grünwald, Halensee, Germany. Later, Magnus (1888, 1893) extended his description. This was the first species of the genus Entorrhiza to be described and is sometimes listed incorrectly as the type species of the genus (see earlier discussion).

Various collections were reported in the early literature (Magnus, 1888, 1893, 1902; Maire et al., 1903; Schellenberg, 1911) but recent collections appear to be limited to those of Kochman (1960) and Vánky (for Roumania, 1960). The records for Czechoslovakia (Zundel, 1953; Ciferri, 1963) and Denmark (Ciferri, 1938, 1963; Thirumalachar & Whitehead, 1968) are probably based erroneously on Bubák (1916) and Rostrup (1894) respectively. Scholz (1968) states that since 1912 E. cypericola

has not been reported from Brandenburg and Berlin.

Cyperus amabilis has been reported as a host plant for Entorrhiza cypericola (Ciferri, 1963) but this is based on an unconfirmed report in Liro (1938). A collection of C. amabilis from Erythraea, in the Helsinki herbarium, has been examined but spores of Entorrhiza could not be detected; swellings resembling those produced by Entorrhiza were present but may not have been induced by fungi (see Davies et al., 1973). The records for both host and locality may therefore be regarded as doubtful.

There has been almost no work on Entorrhiza cypericola since Magnus (1878, 1888, 1893) first studied the species. Brefeld (1912) attempted to germinate the spores and was apparently successful although most of his observations seem to have been carried out on Entorrhiza aschersoniana.

The present study is based on an examination of 12 collections from 5 countries extending from Algeria in the south to Germany in the north.

The spores are variable in their sculpturing, as first pointed out by Magnus (1888, 1893). The smaller spores with a narrow spore wall are delicately sculptured and superficially resemble young spores of E. aschersoniana in size and shape. However, the sculpturing is rugulose to broadly reticulate rather than verrucose; in cross section the troughs between the broad reticulating ridges show as indentations or pits in the wall. In the majority of spores this sculptured wall layer is covered by a smooth wall layer which is often extremely thick; the reticulate inner wall layer is often still apparent. The smooth wall layer may be comparable to the sheath on some E. casparyana spores (see Chapt. 3). In both species spore

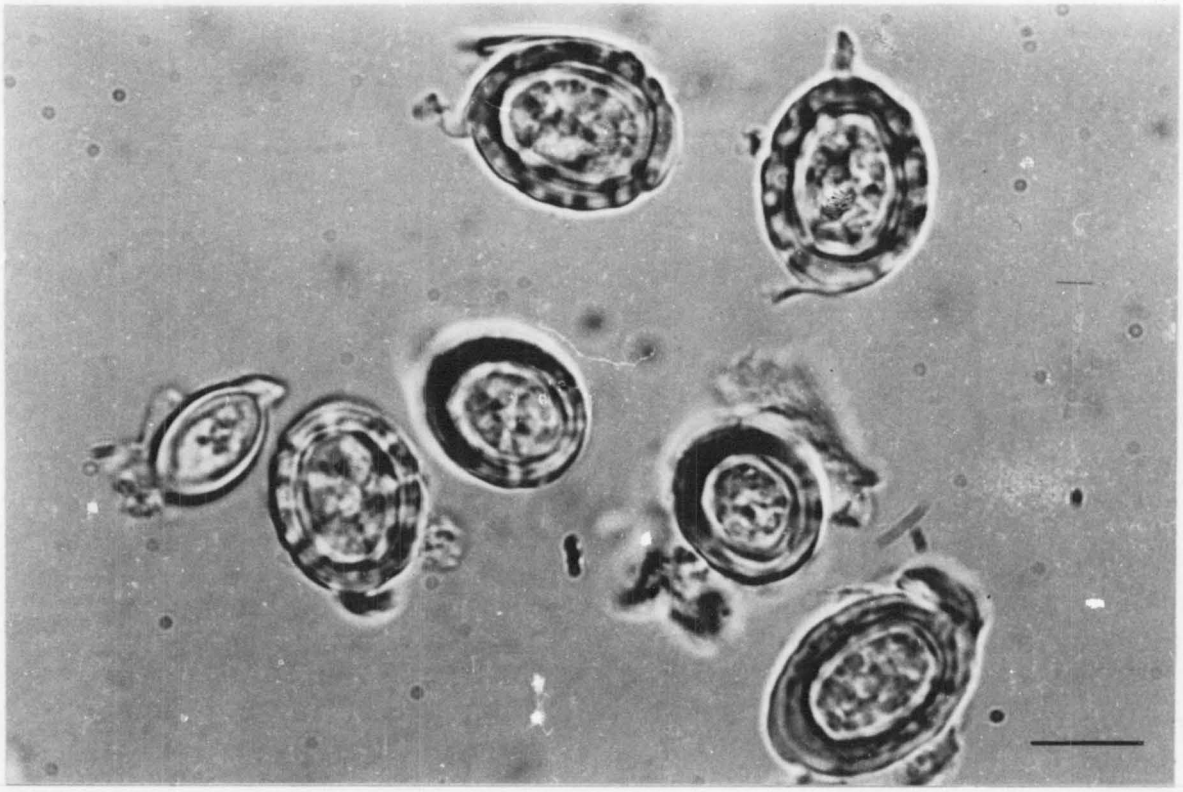

Fig. 140 Spores of Entorrhiza cypericola from Cyperus flavescens; TYPE locality, Hennings, 1889.

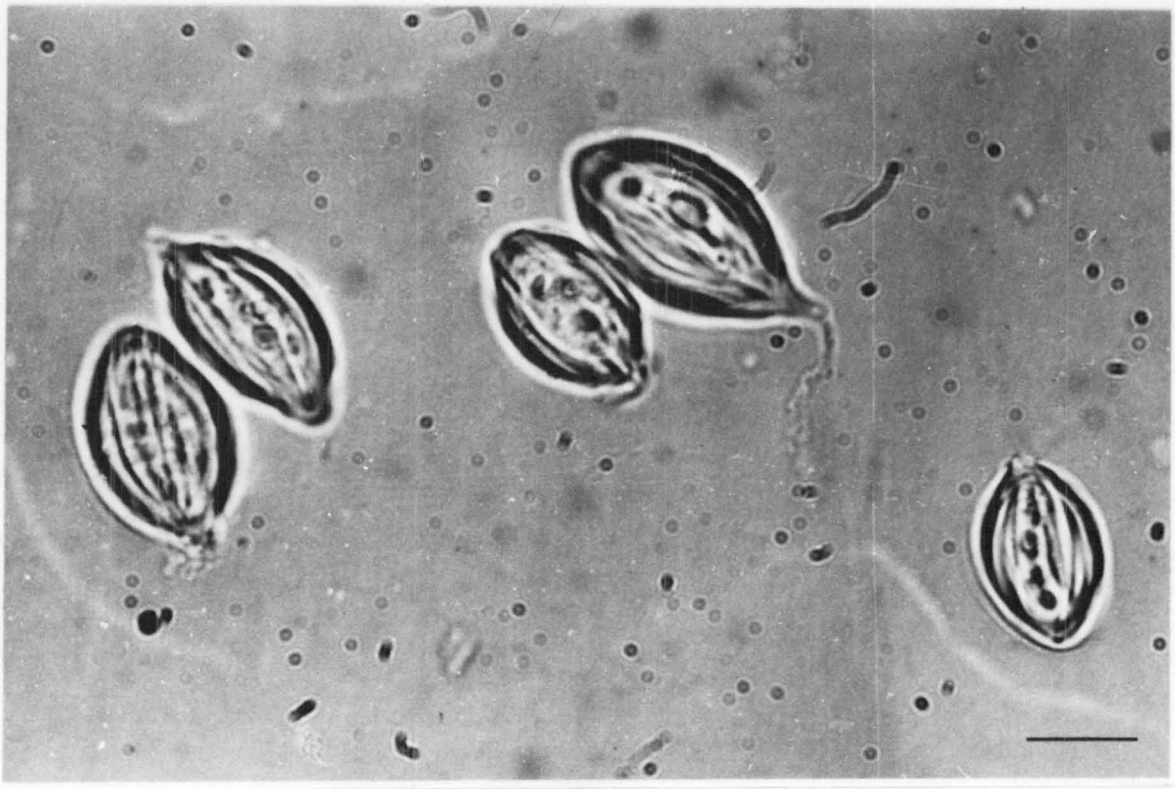

Fig. 141 Spores of Entorrhiza scirpicola (Correns) Sacc. & Syd. from Scirpus cernuus L.; Horning, 1971, Snares Is., N.Z.

size and sculpturing are variable and double spores, united by wall material, are often present.

The variability in sculpturing has caused some confusion in species descriptions. Magnus (1888, 1893) tended to underemphasize the importance of the smooth wall, believing that it was possibly only present in immature spores and corresponded to the gelatinous sheath of the spores of many smut fungi. Subsequent authors, relying on Magnus's description have described the spore wall as verrucose or truncate verrucose (see Zundel, 1953; Ciferri, 1963) omitting to mention that most spores are smooth walled. Although smooth walled, E. cypericola is distinct from E. caricicola (see discussion under that species).

E. cypericola has been mentioned by a number of authors without the addition of any new information: Magnus (1895 a, b, c, 1905), Tubeuf (1895), Frank (1896), Correns (1897), Trail (1903), Lindau (1908), Schwartz (1910), Migula (1910, 1912), Ferdinandsen and Winge (1914), Dietel (1928), Ciferri (1938), Ainsworth and Sampson (1950), Kelley (1950), Fischer (1951), Talbot (1956), Viennot-Bourgin (1956), Fischer and Holton (1957), Lindeberg (1959), Buhr (1965). Further references are listed by Liro (1938).

Entorrhiza scirpicola (Correns) Sacc. & Syd.

Sacc. Syll. Fung., 14 : 425 (1899)

Fig. 141

Schinzia scirpicola Correns - Hedwigia 36 : 40 (1897)

Melanotaenium scirpicola (Correns) Thirum. & Whitehead - Am. J. Bot., 55 (2) : 185 (1968)

Entorrhiza raunkiaeriana Ferd. & Winge - Dansk bot. Ark. 2(1) : 8 (1914)

Melanotaenium raunkiaerianum (Ferd. & Winge) Thirum. & Whitehead - Am. J. Bot., 55 (2) : 185 (1968)

Entorrhiza cypericola auct. non de Toni : Rostrup, Botany of the

Faeroes : 306 (1901).

Holotype: on Scirpus pauciflorus Lightf., Switzerland, Canton Tessin, Val Maggia above Fusio, 1350 m., Aug. 1895, C. Correns (HBG).

Sori in galls, usually ovoid to elongate, occasionally branching irregularly, up to 9.5 mm long and spreading to 6.5 mm wide. Teliospores ellipsoidal to oval citriform, (16.0 -) 18.0 - 28.5 (- 31.5) x (10.0 -) 13.0 - 20.0 (- 23.5) µm, yellow spore wall with longitudinal ridges, sometimes obliquely orientated, sometimes branching, about 1.5 - 2.0 µm apart, extending partly or completely along the length of the spore.

Illustrations: Correns (1897); Ferdinandsen and Winge (1914), Fig. 5 (after Correns), Figs. 2, 3, 4 and 6.

Host plants: (H) Eleocharis punctiflora (Ciferri, 1963), Scirpus basilaris (Hook. f.) C.B. Clarke, Scirpus cernuus Vahl, Scirpus fluitans L. (syn. (H) Eleogiton fluitans (L.) Link, Isolepis fluitans (L.) R. Br.) (Lind, 1913; Ferdinandsen & Winge, 1914), Scirpus pauciflorus Lightf. (syn. (H) Eleocharis pauciflora (Lightf.) Link), Scirpus setaceus L.

Geographical distribution: *Czechoslovakia (Bohemia) (Ciferri, 1938), Denmark (Lind, 1913; Ferdinandsen & Winge, 1914), *England (Liro, 1938), Faeroe Is., *Italy (Zundel, 1953), New Zealand, including Snares Is., Switzerland (Correns, 1897), *U.S.S.R. (Ciferri, 1963).

Collections examined:

1. on Scirpus basilaris
Turakina Beach, south of Wanganui, New Zealand, Nov. 1967, A.P. Druce (CANU).

2. on Scirpus cernuus
seepage area Snares Is., N.Z., Feb. 1961, B. A. Fineran

(CANU); Sink Hole Flat, Snares Is., N.Z., Mar. 1971, D.S. and C.J. Horning (CANU).

3. on Scirpus setaceus
Blowing Pt. Bridge, Canterbury, New Zealand, Dec. 1971, J.M. Fineran (CANU); Smiths Creek, Canterbury, New Zealand, Dec. 1971, J.M. Fineran (CANU).

4. on Scirpus pauciflorus
ex. Herb. J.I. Liro, Faroarna, Sando, Aug. 1897, Hartz and Ostenfeld (H).

Discussion:

Entorrhiza scirpicola was described first by Correns (1897) on Scirpus pauciflorus from Fusio, Val Maggia, Switzerland. This fungus was also collected on the same host species from the Faeroes by Ostenfeld; Rostrup (1901) identified it as E. cypericola (Magn.) de Toni. The affinity of this fungus to E. scirpicola was first inferred by Magnus (1902) presumably because he believed the Entorrhiza species were confined to single host species. Ferdinandsen and Winge (1914) re-examined the Faeroes material and confirmed that it was E. scirpicola.

E. scirpicola was also recorded by Lind (1913) for a collection by Raunkiaer, September, 1911, on Scirpus fluitans from Fano, Denmark (in the herbarium of E. Rostrup). This fungus had already been found in the same locality by Raunkiaer in 1896 and was collected again in 1912 (Ferdinandsen & Winge, 1914).

Ferdinandsen and Winge re-examined the fungus on Scirpus fluitans. Although they were unable to procure the type specimen of E. scirpicola on Scirpus pauciflorus for comparison they were able to examine E. scirpicola on the same host species from the Faeroes, then in the collection of the Botanical Museum of Copenhagen University (C). On the basis of differences in the shape of the galls and in spore form they erected a new

species, E. raunkiaeriana, for the fungus on Scirpus fluitans. In E. raunkiaeriana the spores were more slender (18 - 21 x 9 - 11 µm) than in E. scirpicola (16 - 20 x 11 - 14 µm), the proportion between length and breadth of the spores in the two species being respectively 1.9 : 1.0 and 1.5 : 1.0. In addition, the galls were ovoid or constricted as a siliqua whereas those of E. scirpicola were cylindrical.

Although Ferdinandsen and Winge recognised the close resemblance between E. raunkiaeriana and E. scirpicola they were strongly influenced in their decision by the belief that each species of Entorrhiza was confined to a single host species. E. raunkiaeriana has been reduced to synonymy with E. scirpicola by Ciferri (1963).

Although no material of Entorrhiza on Scirpus fluitans has been located for comparison, Ciferri's decision to unite the two species has been confirmed by the present study. Ferdinandsen and Winge (1914) erected their new species mainly on the basis of small differences in gall shape and spore form. Present studies have shown that these characters may vary considerably for one species of Entorrhiza. Ferdinandsen and Winge found that for the two collections they examined, one on Scirpus fluitans and the other on S. pauciflorus, the length: breadth ratio for the spores was 1.9 and 1.5 respectively. But, two collections of Entorrhiza scirpicola on one host species, Scirpus cernuus, showed almost as great a difference in length: breadth ratio (1.4 and 1.7) indicating that the differences observed by Ferdinandsen and Winge were probably not significant.

The collections of E. scirpicola examined during this study show a wide range in spore size. Further collections will need to be examined to determine whether there is continuous

variation or whether the variation in spore size is discontinuous and correlated with the host species. Among the collections studied so far E. scirpicola in Scirpus setaceus has conspicuously larger spores. The length:breadth ratio of spores is, however, less variable (1.2 - 1.7), most collections having means of 1.4.

New Zealand collections of E. scirpicola appear to differ slightly from European collections in the orientation of the ridges on the spore wall. Records from the literature (Correns, 1897; Ferdinandsen & Winge, 1914) indicate that the ridges spiral and this appears to be the situation in the only European material available for study (Faeroes, Hartz & Ostenfeld). The material is, however, rather fragmentary and it is difficult to examine a comprehensive selection of spores. In the New Zealand collections, the ridges are usually almost parallel to the longitudinal axis of the spores; but, some spores with obliquely orientated ridges are present indicating that this may not be a significant difference.

Until now very little has been known of the biology of this species. All the available information may be found in Correns (1897) and Ferdinandsen and Winge (1914).

Many of the distribution records in the literature cannot be substantiated. Most records appear to be due to the perpetuation of errors or the misinterpretation of information in smut floras. Thus Ciferri (1938, 1963) and Zundel (1953) list Czechoslovakia (Bohemia), probably a misinterpretation of Bubák (1916); Zundel (1953) and Thirumalachar and Whitehead (1968) list Italy, probably a geographical error (Canton Ticino) based on Ciferri (1938); and Liro (1938), Zundel (1953) and Ciferri (1963) list England, presumably on the basis of

"E. cypericola Plowright" and "Schinzia cypericola Swanton" cited by Liro. Ciferri (1963) records material from the U.S.S.R. but this is not confirmed by Ulyanischev (1968). In fact the only records from the literature which can be regarded as valid are those for Switzerland, Denmark and the Faeroes. E. scirpicola on Scirpus fluitans has been recorded only from one locality in Denmark (Lindeberg, 1959); E. scirpicola on Scirpus pauciflorus, from the type locality and from the Faeroes. Ciferri (1963) recorded (H) Eleocharis punctiflora as a host but this has not been confirmed .

A number of authors in addition to those mentioned in the above discussion have referred to E. scirpicola but add no new information: Trail (1903), Schellenberg (1911), Ross (1927), Dietel (1928), Möller (1945), Fischer (1951), Talbot (1956), Viennot-Bourgin (1956), Fischer and Holton (1957), Buhr (1965). Further references are listed by Liro (1938).

3.4. Unidentified Species

Magnus (1902) noted Entorrhiza in the root swellings of Juncus pygmaeus from the Island of Caprera(off Sardinia). No material has been located to substantiate this record.

There is a recent report (J. Walker, Department of Agriculture, N.S.W., Australia, pers. comm.) of Entorrhiza on Juncus planifolius R. Br. and Scirpus inundatus (R. Br.) Spreng. Both plants were found growing as weeds in a commercial nursery on the outskirts of Sydney. Walker proposes to publish these records in a forthcoming paper on some plant parasitic fungi of New South Wales.

3.5. Excluded Species

1. Entorrhiza calospora Talbot - Bothalia 6:453 (1956)

Collections examined: on Limeum glomeratum E & Z, Brummeria, Pretoria, J.J.O. Pazzi, 1943 (PREM 33770) (HOLOTYPE); Brummeria, Pretoria, J.J.O. Pazzi, 1944 (PREM 35291): on Trianthema pentandra, Mahale, P.O. Malati, Transvaal, B.N. Wolff, April 1945 (PREM 39031)

Discussion: This species is excluded from the genus Entorrhiza largely on the basis of soral position and structure. In Limeum and Trianthema the galls in which the sori are produced are consistently lateral, often surround the root, and are somewhat convoluted. They are larger than the galls of accepted species of Entorrhiza and according to Talbot (1956) the continuity of the conducting system may be interrupted.

Ciferri (1963) has retained this species in the genus Entorrhiza. However, Thirumalachar and Whitehead (1968) on the basis of an examination of type material listed E. calospora as a "doubtful or excluded species". They believed their observations and Talbot's illustrations indicated the fungus was a species of Protomycopsis. Nevertheless, it is difficult to identify the fungus correctly in the absence of germination studies.

2. Entorrhiza cellulicola (Naegeli) de Toni - Sacc. Syll Fung. 7 (2) :498 (1888)
Schinzia cellulicola Naegeli - Linnaea 16:281 (1842)

No material has been located for examination.

Discussion: This species is excluded from the genus because many features characteristic of Entorrhiza infections are not mentioned in Naegeli's description eg. swelling of the roots, coiling of the hyphae, predominance of spores.

Entorrhiza cellulicola has always been regarded as a doubtful species (Correns, 1897; Trail, 1903; Schellenberg, 1911; Ferdinandsen & Winge, 1914; Talbot, 1956). Thirumalachar and Whitehead (1968) listed it under their "doubtful or excluded species" and Ciferri (1963) excluded it although, probably, on the wrong grounds. He noted "cecidia bacterica radicalia" after de Toni (1888) in Schellenberg (1911). However, these authors were incorrectly cited as they do not mention bacteria in their discussion.

Although the material on which Naegeli based his description has been lost (Correns, 1897) and the fungus has not been found since (Schellenberg, 1911; Liro, 1938) a number of authors have put forward suggestions as to its identity. Schellenberg believed that Schinzia cellulicola was definitely a member of the smut fungi and possibly belonged to the genus Neovossia as had already been emphasized by Magnus. Liro stated that the fungus was almost certainly an endotrophic mycorrhizal fungus but pointed out, in contrast, that Brunchorst (1885, p. 250) was of the opinion that it was definitely a member of the Ustilagineae. According to Thirumalachar and Whitehead (1968) the fungus incites gall formation and "is stated to be a member of the Plasmodiophoraceae". However, the source of their information is unknown.

3. Entorrhiza isoetes (Rostrup) Liro - Luonnon Ystävä 38:110 (1934)

Ustilago isoetis Rostrup - Bot. Tidsskr. 26:306 (1905)

No material has been located for examination.

Discussion: This species is excluded from the genus Entorrhiza largely on the basis of soral position and structure. The

sori are between and in the microspores at the base of the leaves of Isoetes lacustris and the spore mass is powdery (Rostrup, 1905; Liro, 1938).

The species was regarded as doubtful by both Ciferri (1963) and Thirumalachar and Whitehead (1968). It has been found only once (Denmark, J.E. Lange, 1900) according to Liro (1938). Ciferri (1963) lists E. isoetes from Russia but this is not confirmed by Ulyanischev (1968). In the absence of material the fungus on Isoetes should be retained as Ustilago isoetis.

4. Entorrhiza solani Fautrey - Rev. Mycol. Toulouse 18:12 (1896)

Collections examined: 3 collections on Solanum tuberosum (UPS). Material is also housed at BPI.

Discussion: This species bears little resemblance to members of the genus Entorrhiza. The spores germinate readily and the mode of germination is unlike that of Entorrhiza (Fautrey, plate 159, fig. 2).

E. solani has always been regarded as a doubtful member of the genus (Correns, 1897; Ferdinandsen & Winge, 1914; Talbot, 1956). Even Fautrey regarded his placing of the fungus in Entorrhiza as provisional. Ciferri (1963) excluded E. solani suggesting that it was probably Spongospora solani (Wallroth) Lagerheim or a similar species. Thirumalachar and Whitehead (1968) listed E. solani under their "doubtful or excluded species" but were unable to locate material for examination.

5. Entorrhiza vaccinii Rostrup - in Hartz, Danmarks geologiske Undersögelse, R. 2, No. 20, Table 7, Fig. 4-6 (1909) - nom. nud.

No material has been located for examination.

Discussion: This species was delineated by Rostrup in Hartz (1909) but because no description accompanied the illustrations, the species is not validly published.

From the illustrations provided in Hartz (1909) there seems little reason to believe that this is a species of Entorrhiza. The spores figured come within the size range of Entorrhiza spores but the swellings on the roots appear to be lateral rather than terminal. Furthermore, the host plant (Vaccinium uliginosum) is not a member of the Cyperaceae or Juncaceae. Because of the inadequacy of the illustrations one must agree with Liro (1938) that it is uncertain what E. vaccinii really is. Root swellings of a similar kind have been described on other species of Vaccinium (Dop & Marquès-Trochain, 1931) but these swellings tended to be larger and the endophyte did not possess large spores.

E. vaccinii was regarded as doubtful but was not excluded by Ciferri (1963). It was not considered by Thirumalachar and Whitehead (1968).

4. SUMMARY

1. The genus Entorrhiza is characterised by having sori in terminal, occasionally lateral, galls on roots. Infection is restricted to the galls. Spores are intracellular in the cortex of the gall, terminal and usually·

single. Occasionally they are surrounded by a sheath (apparently of host origin). The spores are released passively by decay of the gall. Spore germination, where known, is by the production of up to 4 aseptate promycelia bearing 1-4 terminal sporidia. The sporidia are aseptate, slender, falcate and often distinctively looped.

2. Species of Entorrhiza are delimited mainly on the basis of spore shape and ornamentation.

3. For the revision most of the collections of Entorrhiza in European herbaria were examined as well as fresh material of several species.

4. Five species of Entorrhiza are recognised, viz.

Entorrhiza aschersoniana (Magn.) Lagerh.

Entorrhiza caricicola Ferd. & Winge

syn. E. juncicola Ferd. & Buchw. in Lindeberg (nom. nud.)

Entorrhiza casparyana (Magn.) Lagerh.

syn. E. digitata Lagerh.

Entorrhiza cypericola (Magn.) de Toni

Entorrhiza scirpicola (Correns) Sacc. & Syd.

syn. E. raunkiaeriana Ferd. & Winge

Each species is described and its host range and geographical distribution listed; this is followed by a discussion and literature review.

5. The following species are excluded from the genus Entorrhiza:

Entorrhiza calospora Talbot

Entorrhiza cellulicola (Naegeli) de Toni

Entorrhiza isoetes (Rostrup) Liro

Entorrhiza solani Fautrey

Entorrhiza vaccinii Rostrup

6. The genus is known to infect only members of the Cyperaceae and Juncaceae.

7. The genus has so far been recorded only from Europe, (including the Faeroes), Algeria, New Zealand (including the Snares Is.) and Australia (J. Walker, pers. comm.).

GENERAL DISCUSSION

From the studies outlined it is clear that Entorrhiza is an atypical member of the Ustilaginales in many respects. Even the common name, smut fungus, has little relevance as the spore mass is light brown rather than black and is enclosed in host tissue rather than being powdery. Furthermore, it is one of the few members of the Ustilaginales infecting roots and one of the few where infection is restricted to galls. The most distinctive feature of the genus appears to be the mode of germination of the spores. Although other smut fungi occasionally produce several germ tubes when germinating, there is no other group which consistently produces up to four germ tubes after cleavage of the spore into up to four sectors.

Although several aspects of the biology of Entorrhiza have been studied, knowledge of the genus is still incomplete. Firstly, the life history is only partially known. We need to discover what happens to the sporidia after their formation and how infection of the host plant is brought about. If sporidia can be induced to develop on culture media experimental work on the genetics of the fungus is then possible and infection studies using pure cultures can be carried out.

This work has shown how relatively new techniques such as transmission electron microscopy can be used to advantage in elucidating details in the life history of a fungus. Although many details are revealed by light microscopy they are given added perspective when they are correlated with electron microscope studies. This has been particularly evident when elucidating changes in spores undergoing germination.

Further work on spores at a variety of stages during germination would provide a complete picture of the germination process for one species.

The electron microscope has also been invaluable in determining the cytology of the host-parasite relationship. A knowledge of this relationship helps to explain the effect of the fungus on the host at the macro-morphological level. EM studies confirm that Entorrhiza is a highly specialised parasite which does not invade the protoplast of the host cell and disturb the host metabolism, at least until after teliospore production. As a specialised parasite, Entorrhiza is not unlike many other smut fungi in its effect on the host plant; the growth of the fungus is so restrained that it is often impossible to decide by casual inspection whether the plant is infected or not. The difference between other smut fungi of this type, eg. Ustilago nuda, and Entorrhiza is that in the former the teliospores attack and disrupt the inflorescence of barley and wheat with considerable economic loss while in the latter the teliospores are produced locally in an extensive root system where loss in function of some portions is counteracted by the continued functioning of the major part of the root system.

One promising sphere of research which has not yet been investigated is host specificity. Once material of each species of Entorrhiza is readily available cross inoculation experiments can be carried out to determine to what extent specificity is real or assumed and delimits physiological forms (f. spp.). In addition host specificity can be a valuable aid in classification to the higher plant taxonomist as the host preferences of a fungal species may indicate taxonomic

relationships among host plants.

So far the extent of the genus throughout the world and within the Cyperaceae and Juncaceae (and possibly other families) has not been investigated. Even in New Zealand no extensive survey has been carried out to discover how widespread Entorrhiza is and to what extent it infects indigenous species of rushes and sedges. Much more information on the geographical distribution and host range of this genus is required before the question of its origin and age can be satisfactorily discussed. The absence of records in the past is probably due mainly to the subterranean position of infection, the small size of the galls and the fact that they are readily detached, and the absence of any obvious effects on the growth of the host plant. By bringing the notice of mycologists to this group it is hoped that more information on the distribution of the genus will become available. This has already happened to a limited extent with the recent report of Entorrhiza in Australia.

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