THE FINE STRUCTURE OF *UROMYCES APPENDICULATUS* IN ITS HOST *PHASEOLUS VULGARIS*

by

Nigel Victor Hardwick, B.Sc., A.R.C.S.

A thesis presented in part fulfilment
of the requirements for the degree of

Ph.D. of the University of London

October, 1969

Department of Botany and Plant Technology,
Imperial College of Science and Technology,
London, S.W.7.

A B S T R A C T

A study of several aspects of Uromyces appendiculatus in Phaseolus vulgaris has been undertaken by the use of the light microscope, transmission electron microscope and scanning reflection electron microscope. Results are included of stages of the infection processes from the in vivo germination of uredospores to the colonisation and ultimate production and dispersal of the spores. Results of the interaction of the parasite with a resistant host and with a systemic fungicide are also included.

An examination of the fine structure of the haustorium has revealed a previously undescribed structure in the stem, the 'neckband'. This structure was associated with a comparatively inert part of the host plasmalemma between two active and growing regions covering the sheath and expanded body of the haustorium. The wall of the body of the young haustorium consisted of a single layer but mature haustoria possessed a two layered body wall, the outer layer of which was thicker in a resistant reaction. Evidence is presented to support the view that this outer layer, or 'encapsulation', was a deposit of the host. Penetration of the host wall by the fungus appeared to be a chemical rather than a mechanical process. Use of a systemic fungicide in a susceptible variety indicated that the death of an established parasite also resulted in the death of the host; an induced 'hypersensitivity'.

Spines of uredospores were formed within the spores and were elevated to their surface on maturation. On detachment from their stalks the uredospores assumed a characteristic 'dough-nut' shape.

Results using standard histochemical procedures for the identification of cell wall components were inconclusive, but did reveal several features of interest concerning the interaction between the two organisms.

The results obtained have also provided additional information about the infection processes of obligate parasites generally.

A C K N O W L E D G M E N T S

I am very grateful to my joint supervisors, Professor R.K.S. Wood and Mr. A.D. Greenwood, for their valuable guidance during the course of the work presented in this thesis and for their constructive criticism of the manuscript.

I would like to thank the Cambridge Instrument Company and its staff for the use of the 'Stereoscan';
Mr. R.H. Harris of the British Museum (Natural History) for help with specimen preparation for the Stereoscan;
Mr. J. Whittick for fitting the lights to the growth cabinet;
Miss A.G. Tomkins for help with the manuscript.

I am especially indebted to all the members of the Botany Department who have helped me during my stay at Imperial College and to my colleagues Dr. I.B Heath and Mr. P.C. Mercer. Finally Professor A.J. Rutter, my former tutor, for his continued interest in my welfare.

This work was supported by a Studentship granted by the Science Research Council.

C O N T E N T S

ABSTRACT 3

ACKNOWLEDGEMENTS 5

CONTENTS 6
ABBREVIATIONS USED IN TEXT 11

INTRODUCTION AND LITERATURE REVIEW 12
1. INTRODUCTION 12
2. LITERATURE REVIEW 14
(a) The Disease 14
(i) Specificity and Classification 14
(ii) Symptoms 15
(iii) Distribution and Economic Importance 15
(iv) Control 16
(b) Cytology of Infection 17
(i) Infection 17
(ii) Haustoria 18
1. Penetration by Haustoria 19
2. Composition of Haustorial Walls 20
3. Effect of Haustoria on Host Cells 21
4. The Sheath 22
(c) Histological Resistance Mechanisms 24
(i) Anatomical Features 24
(ii) The Sheath as a Defence Mechanism 26
(d) Uredospore Formation 27
(e) Fine Structure 29
(i) Infection 32
1. The Uredospore Wall 32
2. The in vitro Germination of Uredospores 32
(ii) General Fungal Cytology 33
(iii) Haustoria 35
1. Penetration by Haustoria 35
2. Composition of Haustorial Walls 35
3. The Effect of Haustoria on Host Cells 37
4. The Sheath 37
(iv) Uredospore Echinulation 38
(f) Conclusions 38

MATERIALS AND METHODS 40
1. MATERIALS 40
(a) Host and Pathogen 40
(b) Growth Cabinet 41
(c) Microscopy 42
(i) Microscopes 42
(ii) Ultramicrotomes 42
Figures 1-2 43
2. METHODS 44
(a) Growth and Preparation of Plant Material 44

(i) Routine Method for Infecting Plants 44
(ii) Germination of Uredospores on Agar Plates 45
(iii) Leaf Disk Cultures 45
(iv) Growth and Treatment of other Cultivars 46
(b) Preparation for Transmission Electron Microscopy 47
(i) Fixation 47
Schedules for Routine Fixation and Dehydration 48
1. Fixation with Glutaraldehyde followed by Osmium Tetroxide 48
2. Fixation with Potassium Permanganate 50
3. Dehydration 50
(ii) Embedding 50
(iii) Sectioning 52
(iv) Staining Grid Mounted Sections 52
(c) Scanning Reflection Electron Microscopy 54
Specimen Preparation 55
(d) Histochemistry 57
(i) Without Prior Embedding 57
(ii) Fixed Material Embedded in Epon 57
(iii) Fixed Material Embedded in Glycol Methacrylate 58
(iv) Staining 59
1. Toluidine Blue O 59
2. Aniline Blue 59
3. Resorcinol Blue 59
4. IKI/H_2SO_4 60
5. Ruthenium Red 60
(v) Extraction Procedures 60
1. Schweitzer's Reagent 60
2. Concentrated Ammonia Solution 61
3. KOH/Acetic Acid 61
EXPLANATION OF THE PLATES 63

RESULTS 65
1. GENERAL FEATURES OF THE DISEASE 65
Figures 3-8 67
2. GENERAL FINE STRUCTURE OF THE HOST AND FUNGUS 70
(a) Host 70
(i) Uninfected Cells 70
(ii) Invaded Cells 73
(b) Pathogen 75
Figures 9-33 79
3. HAUSTORIA 89
(a) Occurrence 89
(b) Morphology 89
(c) Host Cytoplasm 90
(d) Haustorial Cytoplasm 91
(e) Wall Structure 92
(i) Haustorial Mother Cell 92
(ii) The Base 94
(iii) The Sheath 94
(iv) The Stem and Neck 95

(v) The Body 97
(f) Anomalies 97
Figures 34-66 99
4. THE STRUCTURE AND DEVELOPMENT OF UREDOSPORES 117
(a) The Mature Spore 117
(b) Fine Structure of the Cytoplasm and Internal Organelles 118
(i) Nucleus 118
(ii) Mitochondria 119
(iii) Microtubules 119
(iv) Microbodies 120
(v) Membranes 120
(vi) Other Cytoplasmic Inclusions 121
(c) Spore Development 122
Figures 67-106 127
5. HISTOCHEMISTRY OF CELL WALLS 146
(a) Light Microscopy 146
(i) Staining with Toluidine Blue O 146
(ii) Fluorescent staining with Aniline Blue 146
(iii) Staining with Resorcinol Blue 147
(iv) Reactions to IKI/H_2SO_4 147
(v) Staining with Ruthenium Red 148
(vi) Examination in Polarised Light 148
(vii) Reactions after extraction with KOH/Acetic Acid 148
(b) Electron Microscopy of Epon Embedded Sections 149
(i) Material extracted with Schweitzer's Reagent 149
1. Host Walls 150
2. Fungal Walls 150
3. Haustorial Mother Cell 151
4. The Sheath 151
5. The Stem Wall 152
6. The Body of the Haustorium 152
7. Uredospores 153
(ii) Treatment of Glut/Os fixed material with Concentrated Ammonia Solution 153
1. Structure of the Host Walls 154
2. Structure of the Fungal Walls 154
(iii) Unfixed and Glut/Os fixed material treated with KOH/Acetic Acid 155
1. Host Walls 155
2. Fungal Walls 156
Figures 107-140 158
6. UREDOSPORE GERMINATION AND STOMATAL PENETRATION 172
(a) Germination 172
(b) Stomatal Penetration 175
Figures 141-160 179
7. YOUNG INFECTIONS 187
(a) Fine Structure of the Cytoplasm 187
(i) Host Cells 187
(ii) Fungal Cells 188

(b) Fine Structure of the Cell Walls 189
Figures 161-166 191
8. OLD INFECTIONS 194
(a) Host Cytoplasm 194
(b) Haustoria 195
(c) Teleutospores 196
Figures 167-178 198
9. RESISTANCE 204
(a) The Cytoplasm of Invaded Cells 205
(b) Fine Structure of Hyphae and Haustoria 207
Figures 179-194 210
10. THE EFFECT OF A SYSTEMIC FUNGICIDE ON FRENCH BEAN RUST 217
(a) To determine the effect of the compound on the germination of uredospores 218
(b) Determination of the concentration of Plantvax which would prevent infection and colonisation by the rust 219
(c) To determine the effectiveness of Plantvax as a curative agent 222
(d) Fine Structure of rust infected French bean plants with a post inoculative application of Plantvax 223
(i) Control Material 223
(ii) Infected Material treated with Plantvax 223
Figures 195-204 227
11. FINE STRUCTURE OF THE HAUSTORIAL APPARATUS OF UROMYCES VICIAE-FABAE 232
(a) Host and Fungal Cytoplasm 232
(b) The Haustorium 232
(c) The Haustorial Mother Cell 233
(d) The Sheath 234
(e) The Neckband 235
(f) The Body Wall 236
Figures 205-209 237

DISCUSSION 239
1. SPECIMEN PREPARATION 239
(a) Fixing and Embedding 239
(b) Embedding and Sectioning 239
(c) Staining of Sections 240
2. GENERAL FINE STRUCTURE 242
(a) Host Cytoplasm 242
(b) Fungal Cytoplasm 247
3. FINE STRUCTURE OF HAUSTORIA 255
Figure 210 257
(a) Use of the term 'Sheath' 258
(b) Use of the term 'Encapsulation' 260
(c) Fine Structure of the Haustorial Mother Cell and Penetration 262

(d) The Sheath 267
(i) Composition of the Sheath 269
(ii) Formation of the Sheath 271
(e) The Body Wall of the Haustorium 273
(f) The Neck and Neckband 275
(g) Host and Fungal Plamalemmas 277
4. FINE STRUCTURE OF UREDOSPORES 280
(a) Spore Wall and Spine Formation 280
(b) Dissemination and Germination 282
5. HISTOCHEMISTRY 285
(a) General Considerations 285
(b) Treatment with Schweitzer's Reagent 287
(c) Treatment with KOH/Acetic Acid 288
6. EFFECT OF A SYSTEMIC FUNGICIDE ON FRENCH BEAN RUST 290
7. CONCLUSIONS 291
(a) The Importance of Fine Structure in relation to Disease Resistance 291
(i) Isolation of Haustoria within the Sheath 293
(ii) Hypersensitive Reaction 295
(b) Movement of Materials between Host and Parasite 296
(c) In vitro Culture 298

APPENDIX 300
1. SCANNING REFLECTION ELECTRON MICROSCOPY 300
Results and Discussion 300
(i) Freeze-drying 300
(ii) Specimens 301
Figures 211-220 304
2. RACE IDENTIFICATION 309
3. LIST OF MAJOR SUPPLIERS 312
4. BUFFER 313
5. EPON MIXTURES 314
6. REYNOLDS' LEAD CITRATE 315
7. SCHEDULE FOR CLEANING GRIDS 316
8. FILMING GRIDS 317
9. PHOTOGRAPHIC MATERIALS 318
10. LONG ASHTON NUTRIENT SOLUTION 319

REFERENCES 321

ABBREVIATIONS USED IN THE TEXT

A/G - Material fixed in acrolein and embedded in glycol methacrylate.

DNA - Deoxyribonucleic acid

E.R. - Endoplasmic reticulum

F.A.A. - Formol-acetic-alcohol

G.P. - General purpose reagent

Glut/Os - Material fixed in glutaraldehyde and post-fixed in osmic acid.

h.m.c. - Haustorial mother cell

RNA - Ribonucleic acid

t.g. - Technical grade reagent

U/Pb - Stained in uranyl acetate followed by lead citrate

w.p. - Wettable powder

The chemical formula of a compound is used instead of its full name where convenient and where the formula is generally recognised.

Where more than two authors are associated with the same paper the senior author is quoted followed by *et al*. The full list of authors responsible appears in the References (p. 321).

INTRODUCTION AND LITERATURE REVIEW

1. INTRODUCTION

During the last hundred years numerous cytological and histochemical studies have been made on the invasion of higher plants by pathogenic fungi. The information produced by these studies, detailed though it was for many diseases, has been limited by the fact that the light microscope has a resolving power of only about 500nm.

The advent of electron optics and the commercial availability of the electron microscope during the last two decades with a resolving power now routinely available down to 0.5nm, combined with the introduction of many techniques for exploiting this resolution, has lead to major advances in the field of plant fine structure. The new methods for preparing specimens for examination and the sophistication with which the results can now be interpreted have rejuvenated cytology and the still rapidly developing field of histochemistry.

Not unexpectedly it was the tissues of higher plants that were examined first with the new techniques and it was several years before diseased plants were studied by electron microscopy.

When the work presented in this thesis was started in

October 1966, there were few published papers on the fine structure of plants infected by fungi and at that time it was still difficult to assess how our understanding of disease in plants would gain from an electron microscopic investigation. But it did seem likely that most profit would be derived from the study of disease caused by obligate parasites, in which there must be an intimate and compatible relation between parasite and host if the latter is to be colonised.

It was decided to study a rust disease and for a variety of reasons, largely of convenience, the disease of French beans caused by Uromyces appendiculatus (Pers.) Unger was chosen. The primary objective of the work was to be how haustoria develop in cells of susceptible plants and the nature of the relationship between haustoria and host cytoplasm and organelles.

The beginning and end of infection and colonisation of the plant has also been studied by electron microscopy of germinating spores and the way in which they develop from vegetative hyphae.

Work is also included on the identification of specific products of interaction between host and parasite and the nature of their walls. Material resistant to attack by U. appendiculatus has also been studied and some preliminary work on the effect of a systemic fungicide on the control of the disease is also presented.

Use has been made of the new scanning reflection electron

microscope, commercially available since 1965, for what it could reveal about the morphology and behaviour of the parasite on and in the plant.

The results obtained will be considered in the light of work with other obligate parasites published before and during the course of this work.

There now follows a general account of French bean rust and a survey of the literature relating to obligate parasitism; including the work of light microscopists where their work has direct bearing on the structures revealed and the concepts elucidated by electron microscopy.

2. LITERATURE REVIEW

(a) The Disease

(i) Specificity and Classification

Uromyces appendiculatus (Pers.) Unger, an autoecious rust having a total of thirty three synonyms, is recorded as a parasite on thirty five species of Phaseolus, and of forty three species in ten other genera of the Leguminosae: all except two genera are of the tribe Phaseoleae (Guyot, 1957). On Phaseolus vulgaris a number of physiologic races of the fungus have been differentiated (Harter & Zaumeyer, 1941): the number now totals at least thirty five (Laundon & Waterson, 1965).

The uredial stage of this rust was first recorded as

Uredo appendiculata by Persoon (1796) and later reclassified by Unger (1836) as Uromyces appendiculata, which, after a change in gender of the specific epithet to U. appendiculatus is its present name.

(ii) Symptoms

Symptoms first appear as minute chlorotic raised spots on the leaves, pods and occasionally the petioles. In a susceptible reaction these spots enlarge and the epidermis is ruptured to form reddish-brown circular sori, about 2mm in diameter. These sori may be surrounded by chlorotic haloes and in highly susceptible reactions eventually by rings of smaller sori. As the infection ages and the leaf becomes debilitated and the chlorotic areas surrounding the sori become confluent, the tissue ramified by the fungus remains green; which gives a distinct appearance described as 'green islands' (Wang, 1961; Wingard, 1935). The red-brown colour of the sori, due to the pigmentation of the uredospores (Shaw, 1963), turns a deeper brown as the darker pigmented and thicker walled, single-celled teleutospores are produced as the leaf gradually dies. Under favourable conditions for the development of the parasite complete defoliation and total loss of the crop can result.

(iii) Distribution and Economic Importance

Distribution of the disease is world-wide (see map No. 290, Commonwealth Mycological Institute). It is particularly

prevalent in the United States of America where French (snap) beans are grown in great quantities.

In England French bean rust has been regarded as of little economic importance. The Pea Growing Research Organisation, which also undertakes research on French beans for the processing industry in this country, does no work on the disease (Gane, 1968: personal communication) and little is done at Government research stations, although they do record outbreaks.

The disease is becoming important, mainly in the east and south east of England where increasing quantities of French beans are being grown under contract to large processing firms. Severe outbreaks were reported in Norfolk and Kent in 1959, Suffolk and Kent in 1960 and in 1962 it was wide-spread in Essex where some crops had to be ploughed-in. Several outbreaks have been recorded in the Isle of Wight and Glamorganshire. No cases were reported in 1968 (Dickens, 1969: personal communication).

(iv) Control

Control measures include the use of fungicides, mainly sulphur dusting, and resistant varieties (Zaumeyer & Thomas, 1957). However, in areas where there is frequent rainfall control with standard protectant fungicides fail, because they are removed before the fungus is killed (Zaumeyer, 1968: personal communication). Systemic fungicides may provide an

answer to this problem. Among the many products recently developed 'Plantvax' (2,3-Dihydro-5-carboxanilido-6-methyl-1, 4-oxathiin-4,4-dioxide)(Schmeling & Kulka, 1966) has been used successfully, both as a preventative and a curative systemic fungicide, on an experimental scale against rusts. Little information is available as to its affect on disease development.

(b) Cytology of Infection

(i) Infection

Germination of basidiospores, aecidiospores and uredospores of U. appendiculatus and the subsequent penetration of their germ tubes into the host was first described by de Bary (1863). He contrasted the direct method of penetration via the cuticle from the monokaryotic cell of the basidiospores with the stomatal penetration from the dikaryotic aecidiospores and uredospores. This difference is not universal in the rusts as examples of direct penetration from germ tubes of uredospores have been reported for Puccinia psidii and Ravenelia humphreyana (Hunt, 1968).

Germ tubes of P. psidii penetrated the cuticle at the junction of the epidermal cells and entered the intercellular spaces before producing haustoria. The mycelium of R. humphreyana never became intercellular and penetration was directly into the lumen of the epidermal cell and from

there to an adjacent epidermal or mesophyll cell. This is in contrast to the direct penetration by basidiospores of other rusts where the mycelium subsequently produced does become intercellular.

In Puccinia graminis an appressorium was produced over a stoma from the tip of a germ tube, an infection peg was produced which penetrated between the guard cells and expanded within the sub-stomatal cavity to produce a sub-stomatal vesicle. From the sub-stomatal vesicle infection hyphae emerged and ramified within the intercellular spaces of the host (Allen, 1923a).

(ii) Haustoria

Haustoria arise from terminal cells of advancing hyphae and penetrate cells of the epidermis and mesophyll. A haustorium is a specialised hyphal branch that has penetrated a host cell, its function has been assumed to be that of absorbing nutrients from the host.

The first record of haustoria was credited to Visiani (1851: cited G. Smith, 1900; Rice, 1927) who described root-like organs in cells of the grape vine arising from the mycelium of one of the grape mildews.

The first adequate descriptions of haustoria were made by de Bary (1863) and later in his Beiträge (1870) he distinguished three types of haustoria: (i) haustoria ex-appendiculata - haustoria arising directly from a mycelial filament, (ii) haustoria appendiculata - haustoria arising

from an appressorium and (iii) haustoria lobulata - haustoria arising from a lobed appressorium.

Various shapes of haustoria have been described for rusts, Sappin-Trouffy (1896) described four main shapes: simple, spiral, lobed and branched. In the Peronosporales, Fraymouth (1956) suggested that the shape of the haustorium may be related to the food supply: where plenty of nutrients were available haustoria were simple, but where the supply was limited elaborate haustoria were produced, presumably to increase the absorptive surface.

Haustoria are produced by certain species in each of the major groups of fungi (Rice, 1927). Haustoria of the Oomycetes, Ascomycetes and Basidiomycetes are almost entirely parasitic on higher plants, whereas analogous structures produced by the Zygomycetes (Piptocephalis sp., Dispira sp., Tieghemiomyces sp., Syncephalis sp.) are parasitic on other members of the Zygomycetes (Madelin, 1968).

1. Penetration by Haustoria

In Puccinia graminis, Allen (1923a) suggested that a small pore, of diameter below the resolution of the light microscope, was formed in both fungal and host cell walls, possibly by enzymes secreted by the fungus. She noted that the staining characteristics of the host wall were altered around the pore in its wall and attributed this to a local spreading of enzymes.

In plants infected by members of the Erysiphaceae,

G. Smith (1900) noted differences in staining properties of the host cell wall around the point of penetration and also that the outer surface of the host wall around the penetration pore was usually depressed and deepest at this point, he suggested that the cellulose had been dissolved away. Fraymouth (1956), in her examination of the Peronosporales, found no evidence of a chemical alteration of the host cell wall in the region of penetration.

Allen (1923a) suggested that if the host and fungal cell walls were removed the two naked protoplasts would come into contact and the fungal protoplast would enter the host automatically. This was proposed on the observation that strong Bouin's fluid plasmolysed the cells of the host and not the fungus during fixation. It was possible that the fungus had a higher osmotic pressure than the host and, therefore, the fungal protoplast entered the host because of the difference. Thatcher (1939) gave support to this suggestion when he found that in all plant pathogens he examined the osmotic pressure of the fungus was greater than its host (e.g. the osmotic pressure of Uromyces viciae-fabae was approximately twice that of the invaded host cells).

2. Composition of Haustorial Walls

The composition of the haustorial wall has, in most cases, been assumed to be the same as the wall of the fungal hyphae as their staining reactions did not differ (Mangin, 1895).

Hutchins and Lutman (1938) noted differences in the

staining reaction of haustoria of Ustilago zeae to orseilline BB and aniline blue. They found that the mycelium stained blue to violet and the host tissues red, whereas the haustoria stained a deep red.

The presence of chitin has been reported in the walls of hyphae and haustoria of P. graminis by Thatcher (1943).

3. Effect of Haustoria on Host Cells

Haustoria have two main effects on cells (a) the stimulation of the host to produce new wall materials (see next section p. 22) and (b) changes in organelles and cytoplasm.

Abundant indirect evidence has suggested that the plasmalemma of the host was invaginated but not penetrated by haustoria. This was true for the many members of the Erysiphaceae (G. Smith, 1900) and for the Peronosporales (Fraymouth, 1956). Similar results have been obtained for the rusts Puccinia sorghi and Uromyces caladii (Rice, 1927, 1934) and for several other species by Thatcher (1939).

Certain changes in host organelles were noted by Allen (1926a) in wheat infected by Puccinia triticina (P. recondita f.sp. tritici). The chloroplasts decreased in size in the cells at the centre of the lesion, the nuclei increased in size and eventually collapsed. Similar observations were recorded by Whitney et al. (1962) for P. graminis. They also noted that the increase in size of the nuclei was accompanied by an increase in nuclear RNA, no increase in DNA was detected

but there was a loss of 60% DNA when the nuclei collapsed. They suggested that these results indicated that the fungus acted specifically on the metabolism of the host cells to the benefit of the parasite, though they add that these nuclear changes may have only constituted a non-specific response to stress.

Reports of haustoria-host nucleus associations were frequent and have been listed by Rice (1927). G. Smith (1900) found no association between the host nuclei and haustoria of Erysiphe communis, but O.F. Smith (1938) reported movement of nuclei of red clover cells towards the haustoria of E. polygoni. In some members of the Uredinales, Sappin-Trouffy (1896) found that haustoria entwined the host nuclei, occasionally effecting deformations. Colley (1918) described nuclei dented by the tips of haustoria of Cronartium ribicola. Allen (1928) recorded that 82% of nuclei of invaded cells of Bromus marginatus were closely associated with haustoria of Puccinia glumarum (P. striiformis) and that in P. triticina in wheat (Allen, 1926a) the nuclei appeared flattened against the haustoria.

4. The Sheath

In many host-parasite combinations a thickening has been described in the host wall at the point of penetration of a haustorium. This thickening has been variously described from its morphological and chemical characteristics.

G. Smith (1900) described a thickening in the host wall

at the site of the penetration of a haustorium as a 'sheath', as it initially totally ensheathed the haustorium. Corner (1935) used the term 'papilla' for other powdery mildews, as this described a projection into the host cell containing the penetrating haustorium. Young (1926) identified callose in a similar structure found in association with the entrance of a variety of facultative parasites and used the term 'callosity' and Fellows (1928) found lignin in a similar position around the penetrating hyphae of Ophiobolus graminis and called the structure a 'ligni-tuber'.

These different terms are used for what, although appearing in different host-parasite combinations, are morphologically similar. As G. Smith (1900) was the first to give a detailed account of this structure it is proposed to use his term sheath, as it is a general and descriptive term, in this review (for a detailed discussion see p. 258).

Colley (1918) described sheaths around the haustoria of C. ribicola. The sheaths were thick at the base of the haustoria and extended to envelop them, as if they were set in cup-like holders. At the tips of the haustoria the sheaths may be as thick or thicker than at their bases.

Sheathed haustoria have also been reported for P. graminis (Allen, 1923b), P. triticina (Allen, 1927) and P. sorghi (Rice, 1927). Moss (1926) described sheaths around haustoria of a number of the Pucciniastreae, formation starting at the base of the haustorium and proceeding towards

their tips, ultimately enclosing the haustoria.

The material comprising the sheaths in different host-parasite combinations varies. De Bary (1863) described a sheath around the penetrating germ tubes of Peronospora radii apparently consisting of cellulose. G. Smith (1900) believed the sheaths around the haustoria of E. communis to be cellulose ingrowths of the host cell wall. Recently Bracker (1968) failed to get reactions in the sheaths around the stems of haustoria of E. graminis in cytochemical tests for cellulose, chitin, pectin and callose.

Cellulose has been identified in the sheaths around some of the rusts (Colley, 1918; Moss, 1926), callose around haustoria of the Peronosporales (Mangin, 1895; Fraymouth, 1956) and various penetrating organs of the Deuteromycetes (Young, 1926) and lignin in the sheaths around the penetrating hyphae of O. graminis (Fellows, 1928).

(c) Histological Resistance Mechanisms

Reviews by Akai (1959) and by Wood (1967) have covered many of the aspects relating to the histology of resistance of plants to fungal attack. For the rusts there are many detailed accounts of possible mechanisms of resistance.

(i) Anatomical Features

Ward (1902) could find no relationship between anatomical features, such as thick cell walls and small stomata, and

resistance to Puccinia dispersa (P. recondita f.sp. bromina) in Bromus spp. Gibson (1904) found that almost any species of rust would penetrate the stomata of a plant which was not its normal host. The rusts did not however produce haustoria and the fungus died out. She suggested that the ability of the rust to establish haustoria should be taken as an index of 'infective capacity'. Maryat (1907) found that P. glumarum would enter some varieties of wheat but was unable to develop normally and spores were rarely produced. Stakman (1915) working with plants highly resistant to attack by P. graminis found that penetration occurred in the usual way, that there was an attempt to form haustoria, but this usually brought about the death of the host cell concerned. The more resistant the host, the quicker were a few host cells killed in the immediate neighbourhood of the invading hyphae and the fungus, unable to utilise the dead cells for food, died out.

Allen (1926b, 1927) described in detail the reactions of resistant varieties of wheat to invasion by P. graminis and P. triticina. In resistant plants the guard cells were often killed, haustoria were not successfully established, host cells were rapidly killed and ultimately fewer spores were produced. The number of uredospores produced, in gross terms, has provided a method for distinguishing races of French bean rust which differed in their 'infective capacity' (Davison & Vaughan, 1963; Harter & Zaumeyer, 1941).

Corner (1935) found that given the right conditions conidia of powdery mildews would, like the uredospores of the rusts, begin to infect plants on which they alighted. Penetration of the cuticle and cell appeared to be mechanical and depended on the penetrating organ having a greater turgor pressure than the host cell. Plants resistant to attack either had a thicker cuticle, or cells with a greater turgor pressure than the invading organ; so that the fungus could not penetrate at all, or did so by piercing the host cytoplasm and killing the cell which then prevented further action by the fungus.

The rôle of the cuticle in the general defence of the plant against disease has been reviewed by Martin (1964) who concluded that its contribution was not great as it could easily be by-passed, e.g. via stomata, it was often feebly developed and was of such composition that it could not provide a serious barrier to invasion.

(ii) The Sheath as a Defence Mechanism

The sheath was usually regarded as a deposition of material by the host that may check the invasion of a haustorium (Butler, 1907; G. Smith, 1900).

In the rusts Allen (1923b) suggested that the sheath was deposited by the host, but she did not exclude the possibility that the haustorium contributed to it, or that both host and fungus secreted more or less of a cell wall

substance around the haustorium.

Colley (1918) also thought it likely that the sheath in some of the rusts was laid down by the host as a response to the parasite. Rice (1927) stated that if the sheath was a result of resistance offered by the host, then its absence in certain host-parasite combinations might indicate the presence of a well established mutual existence. She further suggested that the capacity of a plant for sheath formation may be a factor in the expression of immunity.

Allen (1923a) on the infection of 'Kanred' wheat resistant to P. graminis noted that the walls of hypersensitive cells bordering onto healthy cells thickened after infection, there was evidence to suppose that these thickenings were impervious to substances from the diseased cells.

How much stimulation of the host to produce deposits of sheathing material depended on the fungus was uncertain, as similar structures have been induced by mechanical pricking of epidermal cell walls of sweet potato stem (Ito, 1949). Other Japanese workers (Kusamo, 1939: Iwata, 1940: cited Ito, 1949) have suggested that the formation of a sheath was purely a wound response.

(d) Uredospore Formation

In a susceptible bean it was found that the uredospores of U. appendiculatus matured seven to eight days after the plant was inoculated and spore liberation began two days after

maturation (Yarwood, 1961).

Colley (1918) has described in detail the production of uredospores of C. ribicola. Allen (1928) described the production of uredospores in P. glumarum. A wave of septation, beginning at the centre where the mycelium was oldest, advanced along the lines of growth, always some distance behind the growing tips of runners (long stoloniferous non-septate hyphae). A wave of spore formation followed behind septation and chains of uredosori were formed, which gave stripe rust its name. The cytoplasm moved into the spore bearing layer. An apical cell divided to produce a basal cell and a uredospore initial, the uredospore initial divided again to form a large upper cell which developed into the uredospore and a smaller lower cell which became the stalk cell. The same basal cell could give rise to several uredospore initials in succession but the stalk cell of a dispersed uredospore eventually degenerated.

Saville (1954) described a mechanism by which spine formation of uredospores in Puccinia and Uromyces spp. occurred. When the spore was less than full size the primary wall, and at that stage the only wall, became gelatinised and swelled to several times its original thickness. Through violent activity of the cytoplasm conical pits were formed in the gelatinised primary wall and secondary wall material was laid down against this template in a manner analogous to centrifugal casting. The conical pits were thus

filled with material and then a substantial amount of secondary wall was laid down behind them. The gelatinous layer shrank to its original thickness taking the shape of the spines as it did so. The secondary wall was now the major component of the mature spore. The spines were formed in a regular hexagonal pattern on the surface of the spore.

Hutchins and Lutman (1938) described the formation of spines on the brand spores of Ustilgo zeae. A thick gelatinous wall surrounded the cytoplasm of the spore; the spines, which stained differently from the wall, were formed within the gelatinous matrix. They surmised from drawings of rust uredospores made by Sappin-Trouffy (1896) that the spine formation of brand spores of U. zeae was analogous to that of the uredospores of the rusts.

(e) Fine Structure

Only during the last eight years have plant parasitic fungi been studied by electron microscopy, the first electron micrograph of a haustorium appeared in a paper by Moore and McAlear (1961b) on the aecidial stage of Uromyces caladii.

The resolving power of electron optics now routinely available is about a thousand times that of the light microscope and although current observations have often only confirmed the critical work and inspired hypotheses of the

early light microscopists, the new techniques have revealed more detailed information about the host reaction and the penetration processes of plant parasitic fungi.

Below is a list of plant pathogenic fungi and their hosts so far examined (August, 1969).

PATHOGEN	HOST AND REFERENCE
Olpidium brassicae	Lactuca sativa (Temmink & Campbell, 1969)
Phytophthora infestans	Solanum tuberosum (Ehrlich & Ehrlich, 1966)
Peronospora parasitica	Wallflower (Davison, 1968)
P. manshurica	Glycine max (Peyton & Bowen, 1963)
Albugo candida	Raphanus sativus (Berlin & Bowen, 1964) Brassica juncea (Harding et al., 1968)
Plasmodiophora brassicae	Brassica oleracea (Williams & Yakawa, 1967; Williams & McNabola, 1967)
Erysiphe cichoracearum	Helianthus annuus (McKeen et al., 1966, 1969)
E. graminis	Wheat var. (Ehrlich & Ehrlich, 1963b) Hordeum vulgare (Bracker, 1964, 1968; McKeen et al., 1969)
E. polygoni	Trifolium pratense (McKeen et al., 1969)
Sphaerotheca fuliginea	Cucumber (Dekhuijzen & Scheer, 1967, 1969)

PATHOGEN	HOST AND REFERENCE
Sphaerotheca macularis	*Fragaria virginiana* (McKeen *et al.*, 1969)
Cronartium ribicola	*Pinus strobus* (Boyer & Isaac, 1964)
Melampsora lini	*Linum usitatissimum* (Manocha & Shaw, 1967)
Puccinia graminis	Wheat var. (Ehrlich & Ehrlich, 1963a; Ehrlich *et al.*, 1968a,b; Shaw & Manocha, 1965b)
P. hordei	*Hordeum vulgare* (Rubio-Huertos & Calonge, 1965)
P. podophylli	*Podophyllum peltatum* (Moore, 1963a)
P. recondita	Wheat var. (Ehrlich *et al.*, 1968a,b)
Uromyces caladii	*Arisaema triphyllum* (Moore & McAlear, 1961b)

The literature before 1966 has been summarised in tabular form by Ehrlich and Ehrlich (1966). Bracker (1967) has reviewed the fine structure of the fungi in general terms and in a later paper (Bracker, 1968) has discussed in more detail the literature on haustoria.

The results so far obtained, although based on a small number of examples, relate to fungi from most of the important groups of plant parasites. At the fine structural level, common and specific features of organisation can readily be distinguished, although they have been obscured more often than clarified by variations in the terminology

adopted by different workers (see Bracker, 1968).

A review and discussion of the general concepts which are now emerging will be deferred until the present work has been described since it will include new facts that will enable a more reasoned and balanced argument to be presented. However, at this stage investigations on rust fungi so far published will be reviewed.

(i) Infection

There was no information available on the primary infection process, i.e. stomatal penetration, though the fine structure of the in vitro germination of uredospores has been investigated (Manocha & Shaw, 1967; Williams & Ledingham, 1964).

1. The Uredospore Wall

Williams and Ledingham (1964) described the walls of uredospores of P. graminis. They consisted of three layers, an outer, thin, electron dense and hydrophobic layer; a middle, thin, electron-lucent layer and an inner, thick electron dense layer. The uredospore wall of Melampsora lini differed from the above as it consisted of a wall of several layers, delimited at its inner and outer sufaces by a thin dense layer (Manocha & Shaw, 1967).

2. The in vitro Germination of Uredospores

The cytoplasmic contents of uredospores of P. graminis differed between the resting and germinating states. On

germination oil bodies decreased in size and there was increased proliferation of endoplasmic reticulum (E.R.) (Williams & Ledingham, 1964). On hydration the walls of the uredospores of M. lini (Manocha & Shaw, 1967) thinned, which suggested a stretching of the wall. The germ tubes of M. lini emerged through one or more germ pores; the site of each germ pore was marked by a pre-existing thinner part of the spore wall, however, a germ tube was not necessarily produced at every pore. An outer dense layer of the germ tube was continuous with the outer, thin, electron dense layer of the uredospore wall and was covered by material which may be polysaccharide in nature and secreted by the fungus. Nucleoli appeared to be absent from the nuclei of mature uredospores and of germ tubes.

(ii) General Fungal Cytology

Lomasomes (border-bodies) (Moore & McAlear, 1961a), aggregations of membrane between the plasmalemma and the cell wall, often continuous with the plasmalemma and in a variety of configurations and assumed functions, have been reported in hyphae and haustoria of all rusts so far examined.

Golgi-like bodies have been reported in hyphal cells of Puccinia podophylli (Moore, 1963b) and in haustoria of M. lini (Manocha & Shaw, 1967).

All cells of M. lini were dikaryotic (Manocha & Shaw, 1967) and also the haustoria of P. podophylli (Moore, 1963a). The mitochondria in all stages of the rusts so far examined possessed plate-like cristae.

In P. podophylli thallus formation was described as being of the arthrogenous type by Moore (1963a), which distinguishes it from other Basidiomycetes which have an incomplete type of cell division (Moore, 1965). The complete division of cells described for P. podophylli has not been reported for other rusts.

Ehrlich et al. (1968a) described the formation of septa in the intercellular hyphae of P. graminis and P. recondita. They distinguished two types of septa, (i) formed by centripetal growth of lateral walls and (ii) pseudosepta, formed by a partial infolding of one side or opposite sides of lateral walls. It was not known whether the septal wall was incompletely formed or if it was formed as a complete plate and then ruptured to produce a pore (see also Moore, 1962, 1965).

Septal pores in the rusts were similar to those of the Ascomycetes, the septum tapering to the pore (Bracker, 1967). In the young hyphae of P. graminis and P. recondita (Ehrlich et al., 1968a) the septa around the pores resembled a state mid-way between those of the typical Ascomycete and the typical Basidiomycete. In the Basidiomycetes in general a swelling or thickening of the septum around the pore has been

described and termed the 'dolipore', it was covered by a dome-shaped double membrane structure, the 'parenthesome' (Moore & McAlear, 1962).

A homogeneous matrix, devoid of organelles occurred around septal pores (Ehrlich et al., 1968a). However, nuclear migration and cytoplasmic streaming does not appear to be prevented by any specific structure associated with the septal pores in fungi generally (Bracker, 1967).

(iii) Haustoria

Manocha and Shaw (1967) confirmed the view of Allen (1923a) that a haustorium was formed from an intercellular hypha in contact with the host wall.

1. Penetration by Haustoria

Ehrlich and Ehrlich (1963a) described an early penetration stage of a haustorium of P. graminis. They suggested that the host and fungal walls were absent and that naked host and fungal plasmalemmas came initially into brief contact. They suggested that this was indicative of a chemical rather than a mechanical method of penetration. This was surmised by Allen in 1923(a).

2. Composition of Haustorial Walls

The wall of the haustorium of Uromyces caladii (Moore & McAlear, 1961b) consisted of two layers, an inner electron-lucent layer and an outer layer which was electron dense. Ehrlich and Ehrlich (1963a) described a wall of a similar

structure in the haustorium of P. graminis. They termed the outer layer of the haustorial wall an 'encapsulation', a volume of material between the inner layer of the haustorial wall and host plasmalemma. The encapsulation was connected to the inside of the haustorium by small channel-like areas, or inter-fibrillar capillary spaces (not found by Shaw & Manocha, 1965b), which they assumed to be for the passage of cytoplasm and metabolic products.

Ehrlich and Ehrlich (1963a) suggested that the membrane around the haustorium may be produced in part by the pathogen and that there were possibly two membranes, one the original invaginated host plasmalemma and the other produced by the encapsulation. They further suggested that the encapsulation consisted of fungal or host metabolic products, or wholly or partly of fungal cytoplasm.

Ehrlich and Ehrlich (1963a) noted vesicles in the host cytoplasm around the haustorium and suggested that they originated from the boundary of the encapsulation and that they contained material similar to that found in the encapsulation. They thought that these vesicles may represent material being taken into the host cytoplasm and because of the size of the vesicles (32-170nm in diam.) they were capable of passing through plasmodesmata (42-74nm in diam.) into neighbouring cells which may not be invaded. However, Shaw and Manocha (1965b) stated that the movement of vesicles was more likely to be from the host cytoplasm to the encapsulation. They also reported that an encapsulation was not

present around young haustoria, but was a later development always associated with a stimulated production of host E.R.

3. The Effect of Haustoria on Host Cells

Haustoria of rusts appeared to invaginate the host protoplast (Shaw & Manocha, 1965b). The break-down of cells of wheat infected by P. graminis was similar to that described for scenescing wheat cells (Shaw & Manocha, 1965a). Mitochondria swelled and the cristae degenerated, the grana of the chloroplasts collapsed, structural abnormalities appeared in the nuclei and the vacuole contracted and fragmented. These various processes culminated in the final disorganisation of the invaded cell.

Fragmentation of the host vacuole was reported as being the most pronounced response to invasion by Cronartium ribicola (Boyer & Isaac, 1964).

Host produced lomasomes have been reported around haustoria in cells of susceptible and resistant wheat leaves infected by P. graminis and P. recondita (Ehrlich et al., 1968b). Although they appeared to occur in response to the pathogens they were not associated with either host resistance or susceptibility.

4. The Sheath

Ehrlich and Ehrlich (1963a) did not report the presence of a sheath around the stem of the haustorium of P. graminis. Shaw and Manocha (1965b) working with the same disease noted that a sheath was occasionally present.

Sheaths have not been reported in association with haustoria of C. ribicola (Boyer & Isaac, 1964), P. hordei (Rubio-Huertos & Calonge, 1965), P. podophylli (Moore, 1963a), P. recondita (Ehrlich et al., 1968b) or U. caladii (Moore & McAlear, 1961b).

A thickening of the host wall which formed a collar around the stem of the haustorium of M. lini has been observed by Manocha and Shaw (1967).

(iv) Uredospore Echinulation

Thomas and Isaac (1967a) described the development of spines of uredospores of P. graminis. The spine started as an electron-lucent, globose body beneath the wall of the spore and developed centrifugally. They suggested that increased metabolic activity within the spore, accompanied by an expanding and stretching of the wall and a local autolysis of the outer layer of the spore wall immediately above the mature spine, was responsible for pushing the spine to its surface. A thick inner layer of wall material was laid down after the spine had reached the surface. The final dimensions of the uredospore were as described by Williams and Ledingham (1964).

(f) Conclusions

Much has been written about obligate parasitism from

an anatomical view point. The formation of haustoria, their affect on the host; the synthesis of new materials, formed either as a specific defence mechanism or merely as a normal response to wounding.

There is apparent a delicate balance between host and parasite in the initial stages of invasion by rust fungi, later to overbalance into a pathological phase at the onset of sporulation when excessive demands are being made upon the host by the fungus.

Little is known about the nature of obligate parasitism and the studies on the structure of the haustorial apparatus of the rusts has so far failed to provide any clues as to the rôle of haustoria in establishing obligate parasites within their respective hosts.

It is with this background in mind that the results of this thesis are presented. They seek to illustrate further the gross morphology and fine structure of several aspects of a specific rust in an attempt to find out more information about the special relationship which exists between the rust fungi and their hosts.

MATERIALS AND METHODS

1. MATERIALS

Unless otherwise stated all chemicals were of the 'Analar' grade and the water used was double glass distilled. A list of suppliers is included in the Appendix (p. 312).

(a) Host and Pathogen

At the beginning of the research presented in this thesis a limited amount of information was available on the fine structure of diseased plants. It was decided to work on a disease related to, but different from, one of the few that had already been studied by electron microscopy.

The rust disease of French beans was selected on this basis and because it satisfied two important considerations.

(i) The rust as an obligate parasite would be expected to react with the host in complex ways, well suited to a fine structural study.

(ii) The host plant is good, well tried experimental material, relatively easy to grow in a greenhouse and in growth cabinets, quickly producing two large ~~unifoliate~~ simple leaves which can conveniently be inoculated with the rust.

Phaseolus vulgaris L. cv. Pinto was selected because it has been extensively used in physiological and biochemical

work with the rust.

Uromyces appendiculatus (Pers.) Unger, isolated originally from field grown beans in the Evesham area, was kept in culture exclusively on Pinto and also stored as uredospores without prior treatment in corked glass specimen tubes at -20°C, where they would remain viable for at least twelve months.

(b) Growth Cabinet

This was a refrigerated display cabinet (Fig.1) (Frigidaire, R.A. Bott, Ltd.), modified as follows:

(i) rear doors replaced by double glazed sliding doors.

(ii) thermostat range 7°-24°C.

(iii) forced air cooling unit. The down-draft caused by the air circulation fan tended to damage the plants, the pitch of the blades was thus altered to reduce the force of air without affecting the efficiency of the cooling unit.

(iv) outside the cabinet, on each side, were placed five 5ft, 65-80W 'cool white' and one 5ft, 65-80W 'warm white' fluorescent tubes (Philips).

The light intensity of the tubes fell off during the first two months of use and then remained fairly constant until replacements were required. At plant level the light intensity started at 750 lumens/sq.ft and dropped finally to

540 lumens/sq.ft.

The lights were connected to the mains via a timer to give a daylength of 16h; a temperature of $20^{\circ}C \pm 1.5^{\circ}C$ was maintained.

(c) Microscopy

(i) Microscopes

A Reichert Zetopan with Binolux twin lamp unit, equiped with phase contrast, anoptoral contrast, interference (Nomarski) contrast, polarising and fluorescence systems.

An Olympus SZ III zoom dissecting microscope.

AEI EM6 and EM6B transmission electron microscopes.

Cambridge Stereoscan scanning reflection electron microscope.

(ii) Ultramicrotomes

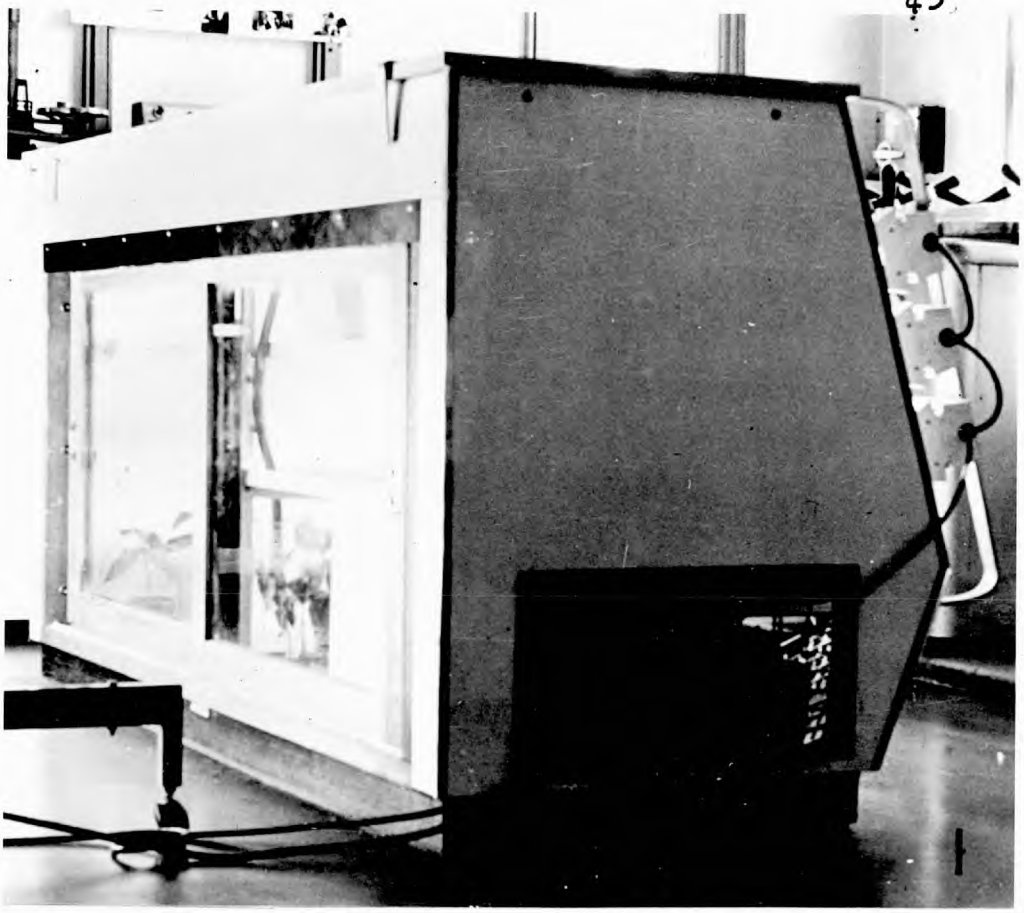
Reichert OmU2.

Sorvall Porter-Blum MT1.

Photographic materials used are listed in the Appendix (p. 318).

1. Growth cabinet, viewed from the rear right hand corner with the rear bank of lights drawn back and used to illuminate the cabinet.

2. Pinto beans on a greenhouse bench covered by polythene bags supported on split canes to provide a humid atmosphere after inoculation.





2. METHODS

(a) Growth and Preparation of Plant Material

(i) Routine Method for Infecting Plants

Seeds were placed for 5min in a solution of sodium hypochlorite (G.P.) containing 2% available chlorine, washed free of sterilant, soaked in tap water for 4-8h and then left on moist paper towels in opaque plastic boxes with transparent lids in the laboratory.

When the hypocotyls were about 2.5-5.0cm long the testas were removed and the plants transferred to horticultural vermiculite (No. 2) soaked in tap water in 10cm diam. plastic pots (one plant per pot), on saucers in the growth cabinet or a greenhouse. The vermiculite was kept moist with tap water until the cotyledons had abscissed, then watered with a modified Long Ashton Nutrient Solution (Hewitt, 1952: see Appendix p.319).

About fourteen days after soaking the seed the undersurfaces of two-thirds expanded primary leaves were inoculated by applying uredospores in talc with a sable-haired brush, to give approximately 10 sori/cm^2. The inoculated plants were covered with polythene bags for 24h to provide the saturated atmosphere necessary for germination of uredospores (Fig. 2).

The average time sequence from soaking the seed to

collection of the uredospores was as follows:

Days from soaking seed to planting out	- 7
" " " " " inoculation	- 14
" " " " " appearance of raised chlorotic spots	- 19
" " " " " collection of uredospores	- 26

The uredospores were collected by shaking the leaves over a sheet of paper, funnelling into glass specimen tubes with cork stoppers and stored at -20°C.

(ii) Germination of Uredospores on Agar Plates

Freshly collected uredospores were blown across the surface of 2% tap water agar in a Petri dish and incubated in the dark at 20°C±1.5°C for 4h. Molten 2% agar at approx. 45°C was poured over the uredospores, to prevent them being dislodged during fixation. 2mm cubes of agar were cut from areas bearing a large number and high proportion of germinating uredospores and fixed, dehydrated and embedded as described p. 48.

(iii) Leaf-disk Cultures

Six 12.5mm diam. leaf disks were cut from leaves of different ages of Pinto beans with a cork-borer and floated lower surface uppermost on Long Ashton Nutrient Solution

in 57x37x23mm transparent plastic boxes. Uninoculated leaf disks remained green and apparently healthy for 21 days under these conditions.

Three 0.001ml drops of a suspension of uredospores (10^6 spores/ml) in 0.001% Tween 20 were place on the lower surface of each leaf disk and incubated in the growth cabinet for 24h. 2mm squares were cut from the inoculated area and fixed, dehydrated and embedded as described on p.48.

The infection of inoculated disks left for longer periods of time progressed to the production of uredosori in about eight days.

(iv) Growth and Treatment of other Cultivars

Several cultivars of French beans were tested for resistance to this unknown race of rust, so that the fine structure of resistant reactions could be examined.

Plants were prepared and inoculated as described (p.45). To ensure germination of some old stocks of seed the testa was broken at some distance from the embryo to facilitate absorption of water.

Inoculated plants, three replicates per cultivar, were left on the greenhouse bench for fourteen days. The size of the sori, ten per replicate, were then measured using the scale (1-5) of Davison and Vaughan (1963).

The identification of the race of U. appendiculatus was determined by using the American differential varieties as described in the Appendix (p. 309).

(b) Preparation for Transmission Electron Microscopy

(i) Fixation

The use of glutaraldehyde followed by osmium tetroxide at pH 7.0 gave the best general preservation of the tissues, although potassium permanganate, osmium tetroxide alone, acrolein followed by osmium tetroxide and mixtures of acrolein and glutaraldehyde followed by osmium tetroxide were also tried but were found to be inadequate for most purposes.

Mature plant cells are highly vacuolated and it was important to use concentrations of fixatives that would not cause plasmolysis. Tests with epidermal strips of leaves of P. vulgaris showed that 0.2M sucrose, or a concentration of glutaraldehyde in excess of 2.5%, would plasmolyse about 50% of the cells.

The concentration of glutaraldehyde finally chosen was 2.5% with 0.1M sodium phosphate buffer (see Appendix p. 313). The glutaraldehyde was removed with several washes in buffer prior to osmic fixation. The buffered washes and the osmic fixative were made approximately isotonic with the glutaraldehyde mixture by the addition of sucrose to a concentration of 0.2M.

It was thought that granularity observed in sections at high magnifications, following the standard fixation procedure, might be caused by the use of sodium phosphate

buffer. Consequently, a batch of material was fixed in the usual manner but using 0.1M sodium cacodylate buffer at pH 7.2 (Sabatini et al., 1963). As there was no reduction in the granularity observed the phosphate buffer was used routinely.

The length of time the specimens were in a given reagent was varied and when found to give satisfactory results that time was adopted.

Schedules for Routine Fixation and Dehydration

1. Fixation with glutaraldehyde followed by osmium tetroxide (Glut/Os)

All the following stages were carried out at room temperature and at 0°-$4^{\circ}C$, but no detectable differences were observed in the resulting fine structure.

2mm squares of leaf tissue were placed in small specimen tubes containing 2.5% glutaraldehyde in 0.1M NaH_2PO_4/Na_2HPO_4 buffer, pH 7.0, for 4h.

The glutaraldehyde was obtained as a 25% aqueous stock solution, either specially purified (from TAAB Laboratories), or as the supernatant from general purpose grade glutaraldehyde (L. Light & Co.Ltd.), treated with excess powdered barium carbonate and centrifuged.

Specimens were infiltrated with fixative by repeated evacuation, until on releasing the vacuum they sank in the fluid. The specimens were then transferred to covered embryo cups.

Washing The glutaraldehyde was replaced by 0.1M buffer in 0.2M sucrose and left for 30min. This was repeated twice whilst keeping the specimens just covered at all times by reagent.

During the first wash the original edges were cut from the 2mm squares of tissue and discarded, to remove the outer tissue damaged prior to fixation.

Post-fixation The buffered sucrose was drawn off and replaced by 1% osmium tetroxide in buffered sucrose and left for 2h.

The osmium tetroxide was prepared from a 4% (w/v) aqueous stock solution and mixed with buffered sucrose prior to use (1 part 4% OsO_4 : 2 parts 0.2M buffer : 1 part 0.8M sucrose). The mixture could be kept for several days at $4^{o}C$, but was discarded when it became pink.

The osmium tetroxide solution, withdrawn after 2h, was discarded into waste alcohol for safety reasons.

2. Fixation with potassium permanganate

A 2% (w/v) aqueous potassium permanganate solution was freshly prepared and material fixed for 2h at room temperature, using the infiltration procedure described on p.49.

Material was washed in several changes of buffered sucrose for 5min each, until all excess fixative was removed.

3. Dehydration

In all cases the final reagent was replaced at once by 20% ethanol and dehydration completed by increasing the concentration of ethanol in steps as follows:

20%, 30%, 40%, 50%; allowing 5min in each concentration (Material fixed at 0°-4°C was brought to room temperature at this stage.); 75%, 95%; for 20min in each, followed by three changes of absolute ethanol for 30min each. The material was then left overnight in a further replacement of absolute ethanol in the covered embryo cups.

(ii) Embedding

The Epon 812 resin mixtures used throughout were based on those originally devised by Luft (1961), but with the substitution of 1% benzyldimethylamine (BDMA) as the catalyst in place of 2,4,6-tridimethyl amino-methyl phenol (DMP 30). For the mixtures used see the Appendix p. 314.

Procedure The absolute ethanol was replaced, left 20min,

and replaced again before commencing the following treatments:

One third of the volume of ethanol was removed and replaced slowly, drop by drop, with epoxy-propane and left for 10min.

One half of the above mixture was replaced by epoxy-propane and left for 10min. This was repeated.

The above mixture was replaced by pure epoxy-propane, changed three times at 10min intervals.

Replaced again by epoxy-propane and finally one third the volume of fully catalysed Epon 812 resin mixture was added.

The embryo cups containing the specimens and epoxy-propane/resin mixtures were left covered for 1-2h, to allow the epon mixture to diffuse through the material. The lids of the embryo cups were then left slightly open overnight to allow the epoxy-propane to evaporate.

The following day the specimens were transferred to fresh resin and left overnight at room temperature; after which they were transferred to fresh resin in flat polythene trays (40mm diam. x 7mm deep, of the type used as finger grips in the sliding doors of glass cabinets), with the inclusion of a slip of paper bearing the specimen number. The trays of resin were left overnight at room temperature and finally cured at 60°C for 48h.

The hard blocks were readily removed from the trays and the flat shape facilitated the subsequent cutting out and orientation of the specimen in any direction required.

(iii) Sectioning

Small rectangular blocks containing specimens were cut from the resin disks and stuck to small lengths of 8mm diam. sharpened wooden dowel with 'Durofix' in the orientation required and set at 60°C for 20min.

Each block was trimmed under a microscope to leave the specimen exposed at the apex of a small trapezoidal pyramid, before being sectioned with a diamond knife (Ge-Fe-Ri or I.V.I.C.) on either a Reichert OmU2 or a Sorvall Porter-Blum MT1 ultramicrotome.

Sections in the silver-grey range, about 60nm thick (Peachey, 1958), were picked up on formvar filmed copper grids (100 mesh or 'Robertson' types) (see Appendix p. 317) and dried.

(iv) Staining grid-mounted sections

The Glut/Os fixed material was stained as follows:

Grids were immersed singly, each in one of a number of drops of 2% aqueous uranyl acetate (U) arranged in grooves cut in 3mm thick polythene sheet in a covered Petri dish and stained for 20min at 60°C.

It has been found in this laboratory that staining with uranyl acetate at 60°C reduces considerably the length of time required to stain adequately, compared with the period of 12-24h found necessary at room temperatures.

The grids were washed in a stream of water from a Pasteur pipette and released into a large Petri dish of water, where they were left for 5min, supported in the grooves of a piece of reeded glass.

The grids were removed and, whilst still gripped at the extreme edge, the excess water between the prongs of the tweezers was carefully drawn off with the point of a triangle of filter paper, to prevent water being drawn across the grid or introduced into the lead stain when the tweezers were released.

Grids were immersed for 10min in a large volume of lead citrate (Pb) (Reynolds, 1963: see Appendix p.315) contained in channels cut in polythene sheet placed on filter paper soaked in concentrated NaOH solution in a closed plastic Petri dish to maintain a low CO_2 concentration in the surrounding air. Access to the grids was gained through a small slit cut in the lid which was kept covered whenever possible with a microscope slide.

The grids were washed as described above, removed from the Petri dish and washed again in a stream of water from a Pasteur pipette, dried as described above and each placed in a size 4 gelatine capsule. For convenience of storing and maintaining the sequence of the grids the capsules, in groups of about twelve, were previously glued between two pieces of card and ensheathed in a 35mm film negative envelope.

Material was viewed either in an AEI EM6 at 75KV, or an EM6B at 60KV. The magnifications of the microscopes were calibrated with an AEI diffraction grating replica with 2,160 lines/mm.

(c) Scanning Reflection Electron Microscopy

The scanning reflection electron microscope differs from the transmission electron microscope in that it displays information detected from the action of an ultra-fine electron probe, scanning a specimen on a square raster. A final image is produced on a high resolution television monitor. Although only capable of viewing surfaces it has the advantages of:

A useful magnification range from 20x to 50,000x.

An image appearing as though shadowed by illumination from one direction (dependant on the orientation of the specimen to the collector), which gives a readily interpretable three dimensional impression of the surface contours. The depth of focus is, at comparable magnifications, better than the light microscope by a factor of 500.

The ability to turn the specimen, whilst under observation, in the electron beam.

The large size of the specimen, 12mm diam. x 3mm high, that can be accommodated.

Simpler preparation of specimens compared with the

replica techniques needed for the transmission electron microscope and its applicability to surfaces with deep and re-entrant features which defy replication.

Resolution is limited by the diameter of the electron probe, but is better than 30nm with most types of specimen (about ten times greater than the light microscope), and can be as good as 15nm.

Specimen Preparation

Metallic specimens, which are electrical conductors, require no preparation and can be viewed directly. Non-conducting specimens require a coating of conducting materials, such as gold/palladium alloy, aluminium or carbon. Such a coating earths the charges built up in the specimens during bombardment with electrons, which otherwise results in areas of the specimen glowing brightly and causing a loss of image resolution.

Botanical material with low water content and rigid cell walls present few problems because they are unaffected by the high vacuum in the microscope. The retention of the original morphology of material with a high water content, as in mesophyll cells of leaves, fungal hyphae and some fungal spores, presents serious difficulties.

The only technique so far employed with any success in preserving cell morphology is that of freeze-drying under high vacuum.

Material was prepared either by:

(i) thick hand-sections for examining the internal structure of tissues.

or (ii) 2mm squares of leaf for surface views and, if sectioned after freeze-drying, also for interior views.

Material was freeze-dried by the following two methods:

(i) specimens were placed onto specimen stubs with a minimal amount of water. The stubs were then placed on a large brass block (76mm diam. x 20mm thick), drilled to take the stubs and previously cooled in liquid nitrogen. The block with the specimens was lowered slowly into the liquid nitrogen, to avoid violent bubbling which would dislodge the specimens. When the bubbling had ceased, the block was transferred rapidly to the chamber of a vacuum coating unit which contained a Petri dish of P_2O_5. The block was insulated from the base of the coating unit by a thick piece of expanded polystyrene. The chamber was rapidly pumped down and left for 24h.

(ii) The method of Harris (1964, 1968) was followed. Specimens were placed in embryo cups in a vacuum desiccator contained in a deep-freeze at -20°C, using concentrated H_2SO_4 as the desiccant. After two hours the desiccator was evacuated with a rotary pump giving a vacuum greater than 10^{-2}torr, and left for 24h to complete the sublimation.

After drying, the specimens were fixed in position on stubs coated with 'Araldite', 'Durofix' or double-sided 'Sellotape'.

The material was finally cone-shadowed with an approximately 20nm coating of gold/palladium alloy in a vacuum coating unit.

(d) Histochemistry

Material was prepared for light microscopy in the following ways.

(i) Without Prior Embedding

Specimens, fixed or unfixed and before or following the extraction procedures described on p. 60 , were mounted in stain, water or lactophenol.

(ii) Fixed Material Embedded in Epon (as used for electron microscopy)

Sections were cut at 2μm on a Reichert OmU2 ultramicrotome with glass knives and picked up on a needle, transferred to drops of water on a microscope slide and allowed to dry. The sections adhered to the slides during subsequent staining and washing procedures.

Epon embedded sections have only limited usefulness for histochemical investigations with the light microscope because the embedding medium is impermeable to many of the

usual dyes. Toluidine blue O can be used with some success if at pH 11.0 (0.1% toluidine blue O in 1% borax: Pease,1964).

(iii) Fixed Material Embedded in Glycol Methacrylate

For the reasons given above glycol methacrylate, a water permeable embedding medium,was also used. Material was fixed and embedded by the methods of Feder and O'Brien (1968).

2mm squares of leaf tissue were fixed in 10% aqueous acrolein for 24h at 0°-4°C. To ensure rapid infiltration of the fixative specimens were subjected to repeated evacuations until they sank in the fixative. They were then dehydrated at 0°-4°C by replacing the fixative first with 2-methoxyethanol, using two changes during 24h, followed by one change to absolute ethanol for 24h, n-propanol for 24h, and by n-butanol for a further 24h. After bringing to room temperature the n-butanol was replaced with three changes of purified glycol methacrylate for 24h each. Finally each batch of specimens was placed in a thin layer of embedding medium in an aluminium weighing dish. To enable polymerisation to occur air was excluded by resting a second dish inside the first one, with its base resting on the layer of resin, and the assembly placed at 60°C for 48h.

Specimens were trimmed and 2μm sections prepared as described in (ii) above.

(iv) Staining

1. Toluidine Blue O

Sections of acrolein fixed and glycol methacrylate (A/G) material were stained in 0.05% toluidine blue O in benzoate buffer, pH 4.4. (Sidman et al.,1961) for 1-5min, rinsed in tap water to remove excess stain, dried and mounted in immersion oil beneath a cover glass. Staining reactions are described by O'Brien et al.(1964) and Feder and Wolf (1965).

2. Aniline Blue

A/G sections were mounted in water-soluble aniline blue, 0.005% in 0.15M K_2HPO_4 buffer, pH 8.2 (Currier & Strugger, 1956) and viewed in a fluorescence microscope for callose fluorescence (yellow). Fresh sections of Vitis vinifera stems were examined as controls to confirm that sieve plate callose would react to the stain.

3. Resorcinol Blue

A/G sections were mounted in a fresh solution of the stain prepared as follows.

To 3g of resorcinol (fresh white) made up to 200ml with water, 3ml of concentrated ammonia solution were added. This mixture was heated for 10min on a steam bath without boiling. The solution was kept in a bottle plugged with cotton wool for approximately 6h, until it became blue. The solution was reheated on a steam bath for 30min, filtered into an evaporating dish and heated, without boiling, until all traces of ammonia were removed (after Eschrich & Currier,

1964).

The stain was used undiluted and stains callose blue. Fresh sections of V. vinifera stem were again used as a test of the efficiency of the stain.

4. IKI/H_2SO_4

Fresh uredospores were examined for the presence of cellulose by mounting in a solution of 0.2% iodine in 2% potassium iodide, 63% (by weight, 50% by volume) and concentrated H_2SO_4 was drawn under the cover glass whilst observing the specimens (Roelofsen, 1959). With the 63% H_2SO_4 cellulose swells and stains blue.

5. Ruthenium Red

A/G sections and fresh uredospores were mounted in 0.002% aqueous ruthenium red. Pectin stains red.

(v) Extraction Procedures

1. Schweitzer's Reagent

Schweitzer's reagent (cuoxam, cupram, cuprammonium), used for the removal of cellulose, was prepared by dissolving cupric hydroxide in a minimal amount of concentrated ammonia solution (Cross et al., 1918). The preparation totally dissolved filter paper and cotton wool, whether unfixed or fixed in formol-acetic-alcohol (F.A.A.) (formalin, 13ml; glacial acetic acid, 5ml; 50% ethanol, 200ml).

F.A.A. and Glut/Os fixed leaf material was transferred to 20% ethanol as described on p. 50 and then to concentrated ammonia solution for 10min before replacing the latter by

Schweitzer's reagent for 10min. The specimens were then transferred to fresh Schweitzer's reagent in small stoppered conical flasks and rotated on a blood cell mixer for 10 days, changing the reagent every two days. The specimens were then washed repeatedly in concentrated ammonia solution until the blue colour of the solution had disappeared. All remaining traces of blue colour from the specimens was removed by rotating for a further 24h in fresh ammonia solution. The ammonia was then removed by gradual dilution followed by several changes of water until it was no longer detected by smell or litmus paper.

2. Concentrated Ammonia Solution

Glut/Os fixed leaf material was placed in concentrated ammonia solution as 1. above, and rotated in stoppered conical flasks on a blood cell mixer for 10 days. The ammonia was washed out with water as described above.

3. KOH/Acetic Acid

Fresh uredospores, 2mm squares of fresh or Glut/Os fixed leaf and untreated chitin (BDH) were autoclaved in 60% (w/v) aqueous KOH for 3h at 15p.s.i. (Fuller & Barshad, 1960) to convert chitin to chitosan. Afterwards specimens were thoroughly rinsed in several changes of water until all the alkali was removed and then either stained in 1% H_2SO_4 in IKI, or treated with two changes, 6h each, of 2% aqueous acetic acid for dissolution of chitosan.

The leaf material treated with Schweitzer's reagent, concentrated ammonia solution or KOH/acetic acid was taken into 20% ethanol, dehydrated and embedded as described on p.50 and used for electron microscopy.

EXPLANATION OF THE PLATES

To reduce the number of plates required several figures have been grouped onto one plate. This was achieved by mounting the photographs on card and rephotographing onto Ilford N30 plates with the aid of an enlarger. A ground glass screen was placed in the negative carrier of an enlarger, the mounted photographs were placed on the base-board and focussed onto the screen, the latter was replaced by an N30 plate and the photographs illuminated briefly with light from strategically placed reading lamps. The N30 plates were developed in Ilford ID 11. All photographic paper used was Agfa Rapidoprint, developed in an Agfa automatic processor and fixed in Ilfofix. Some loss of tonal quality and definition has resulted from this process but essential details have been preserved.

All dimension lines are equivalent to 1μm unless otherwise stated, those on the scanning reflection electron micrographs merely serve as an indication of the magnification.

All material was fixed in Glut/Os followed by staining in U/Pb and taken from uredospore bearing sori unless otherwise indicated. All specimens for scanning reflection electron microscopy were prepared by method (ii) p. 56 and photographed at an angle of 45° unless otherwise stated.

The following abbreviations are used:

A - appressorium
B - neckband
Ch - chloroplast
Ep - epidermis
ER - endoplasmic reticulum
e - encapsulation
fp - fungal plasmalemma
fw - fungal cell wall
G - Golgi apparatus
g - granular material
gn - granum
gp - germ pore
gt - germ tube
H - haustorium
Hl - hilum
hmc - haustorium mother cell
hp - host plasmalemma
hw - host cell wall
I - intercellular hypha
l - lipid droplet
lo - lomasome
m - mitochondrion
mb - microbody
mc - membrane complex
mt - microtubule
n - nucleus
np - nuclear pore
nu - nucleolus
nv - nucleolar vacuole
os - osmiophilic droplet
p - septal pore
pd - plasmodesma
pl - pellicle
R - runner
r - ring
S - sheath
Sc - sub-stomatal cavity
Sk - stalk cell
Sn - spine
Sp - spore
St - starch
t - tonoplast
v - vacuole
vc - vesicle
x - intercellular material
z - pale zone

In the legends to the figures the specimen number is followed by the negative number.

RESULTS

1. GENERAL FEATURES OF THE DISEASE

Under the experimental conditions of this investigation the first symptoms, in a susceptible host (Pinto), appeared as slightly raised chlorotic spots on the dorsal and ventral surfaces of the leaves about five days after inoculation with uredospores. The chlorotic spots enlarged and about eight days after inoculation the epidermis was ruptured as a result of the production of uredospores beneath it (Fig. 6). This exposure of the pigmented uredospores gave the red-brown colour to the sorus and a 'rusted' appearance to the leaves.

A halo of chlorotic leaf tissue, about 3mm in diameter, surrounded the sori (Fig. 4). This chlorotic area marked the extent to which the hyphae of the rust had ramified the intercellular spaces. About ten days after the primary sori had appeared a ring of smaller sori developed around the central sorus (Fig. 4), followed a few days later by a second ring of sori around the first (Fig. 5). The smaller sori were also surrounded by chlorotic haloes, but as the host tissue between the infection centres became generally chlorotic the haloes around the sori became greener again until eventually they were darker than the leaf tissue between the infection centres, giving the familiar 'green island' phenomenon. The epidermis was not ruptured beyond the ring

of tertiary sori and the extent of the intercellular hyphae was now marked by the green islands.

Depending on the severity of infection and the conditions of growth the leaves abscissed prematurely after about twenty five days. The green islands often remained visible after the leaf was detached from the plant and the whole of the tissue was dead. The final diameter of the sori around a centre of infection, including the satdlite rings of secondary and tertiary sori, may be 5mm with the green islands extending for a further 2mm each side.

Uredosori from a single infection centre were produced on both dorsal and ventral surfaces of the leaves (Fig. 3, 7).

The mature, detached uredospores seen in surface view (Fig. 7) and in section (Fig. 8) were 'dough-nut' in shape. The morphology and development of the uredospores are described in detail in Section 4 (p. 117).

3. A thirty day old French bean plant cv. Pinto, with a sixteen day rust infection. Note the sori on both upper and lower surfaces of the leaves.

3

47

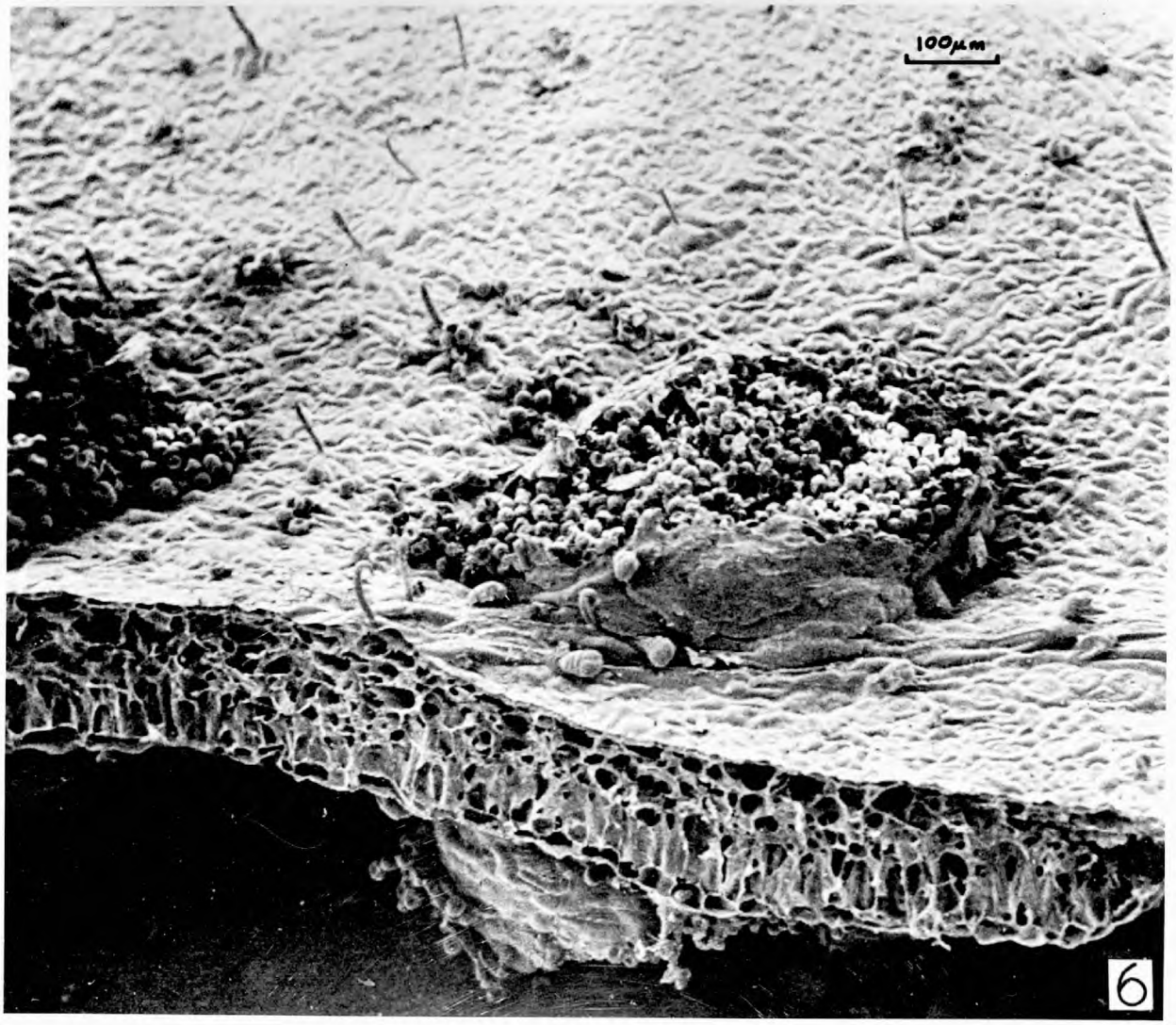
4. Uredosori on the upper surface of a leaf. Note the chlorotic halo around the ring of secondary sori.

5. A later stage in the development of the disease just prior to premature leaf abscission. Note the ring of tertiary sori, green islands of tissue around the sori while the remainder of the leaf is chlorotic. Teleutospores have been produced at this stage.

6. Scanning reflection electron micrograph of a 32 day old plant 11 days after inoculation viewed from the lower epidermis. Some 'charging' of the detached uredospores has occurred. Note position of the veins, marked by long cells crossing the leaf surface and two types of trichome, a hair-type surmounting a rosette of larger epidermal cells and a multicelled gland-type, visible near the veins. 1790/1. D6.

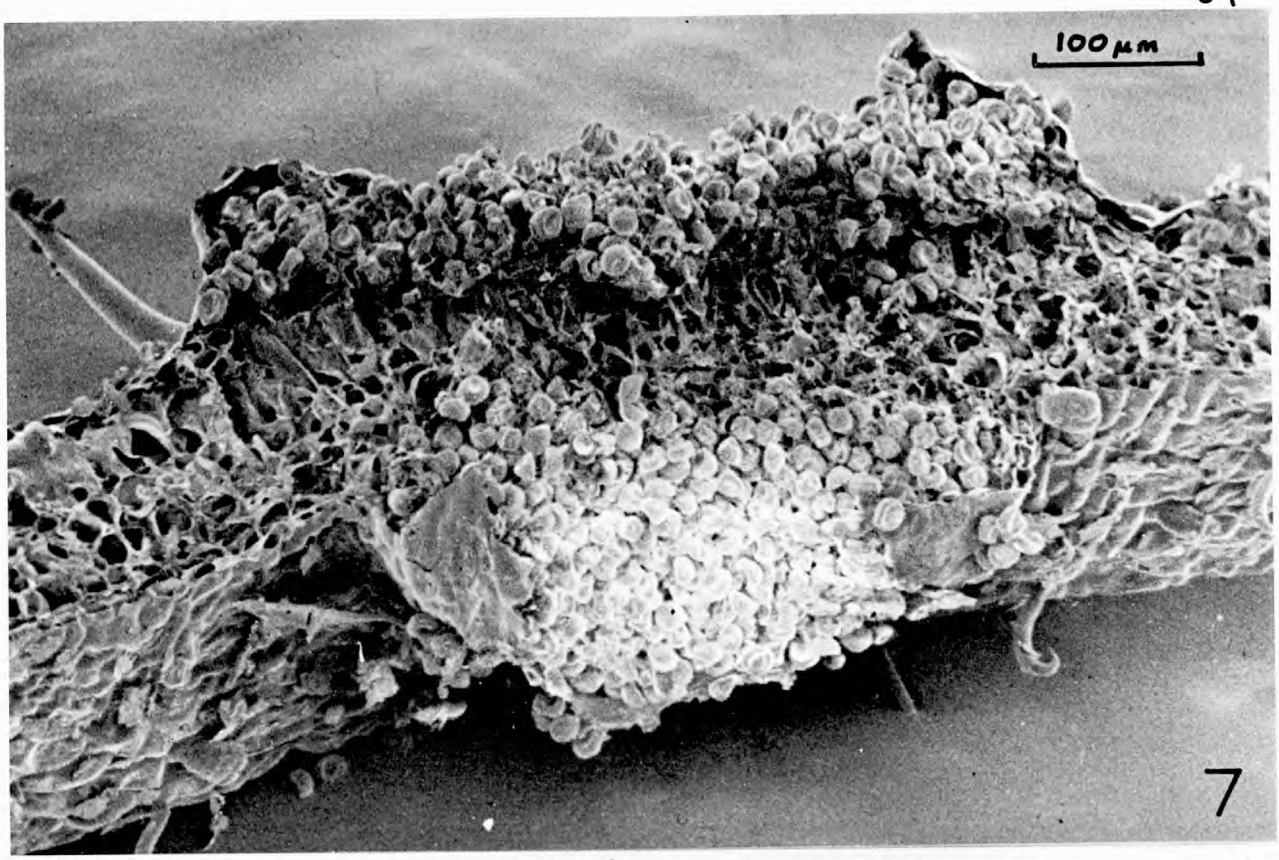


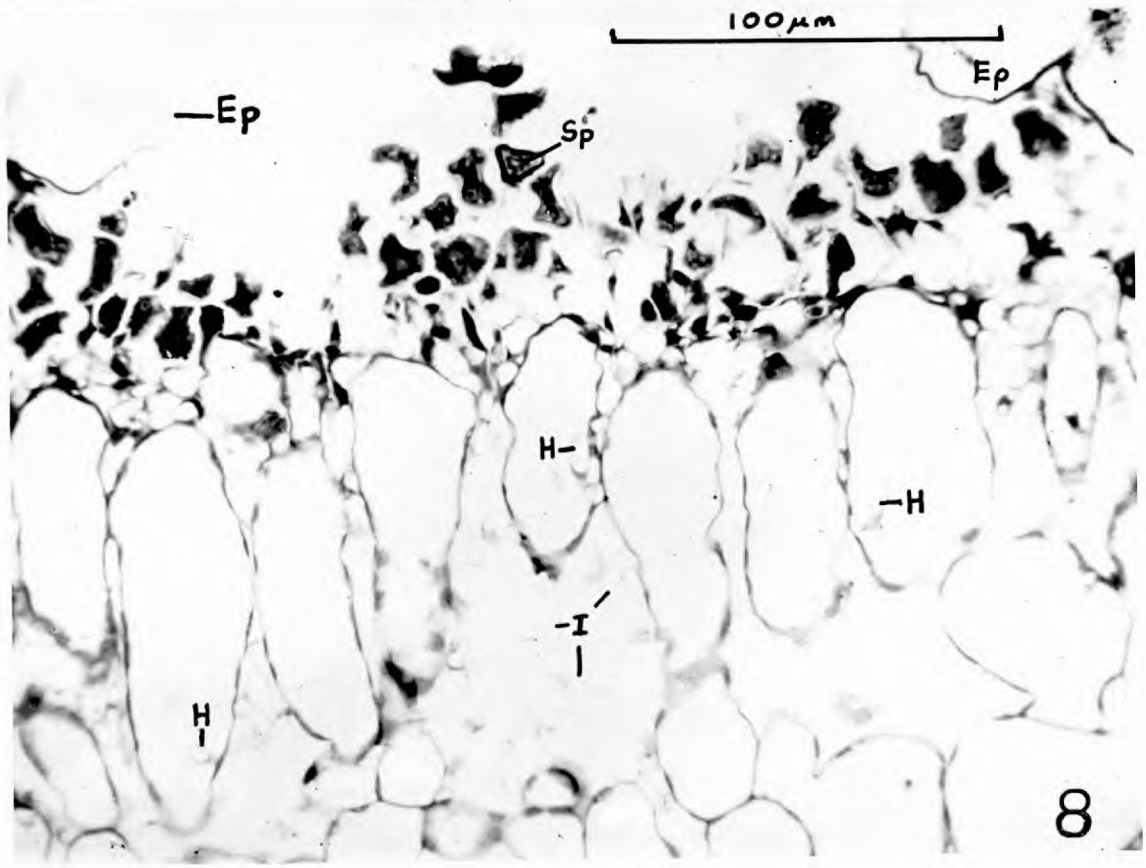

5


100µm
6

7. Scanning E.M. of a thick section of a uredosorus. Note sorus erumpent through upper and lower leaf surfaces and the 'dough-nut' shape of the detached uredospores. 'Charging' of the specimen has occurred in the uredsorus on the lower epidermis, this was probably because of a break in conductivity between the uredospores and the rest of the specimen. 1790/1. D33A.

8. 2μm section of a uredosorus stained in toluidine blue O. Note ruptured epidermis (Ep); distorted, often dough-nut shaped uredospores (Sp); numerous haustoria (H) in the palisade cells and the intercellular mycelium (I). A/G. 1523. 645.


100µm
7


100µm
Ep
Ep
Sp
H
H
I
H
8

2. GENERAL FINE STRUCTURE OF HOST AND FUNGUS

(a) Host

(i) Uninfected Cells

Where the fine structure does not differ between uninfected and infected cells the number of plates required has been reduced by referring text figures to details from the latter.

Cells of the epidermis, palisade and spongy mesophyll were highly vacuolated with a thin lining of cytoplasm only about 80nm thick (Fig. 19), except at intervals where it was expanded and enclosed cytoplasmic organelles (Fig. 9).

The chloroplasts in the mesophyll cells were approximately circular platelets with a diameter of about 12µm and a thickness varying from about 2 to 5µm (Fig. 11). The majority of the plastids were orientated flat against the cell wall and in the palisade cells formed usually a single layer with each plastid almost in contact with its neighbours. They sometimes contained conspicuous starch grains, often 3 to 5 were visible in a single section of a chloroplast, and usually contained osmiophilic droplets (Greenwood *etal.*, 1963), each about 150nm in diameter and numbering about 10 per section (Fig. 12). These droplets, in which lipids probably predominated, were more electron dense than cytoplasmic lipid droplets found in host (Fig. 179)

or fungus (Fig. 26).

The chloroplasts had an extensive system of lamellae arranged predominately parallel to the face of the plastid and usually, therefore, parallel to the cell walls. The lamellae were organised into granal stacks of 35 to 40 lamellae. The number of grana intersected in a single section of a chloroplast was frequently as many as 40.

In the epidermal cells chloroplasts were less frequent and were smaller than those of the palisade and spongy mesophyll cells, measuring only about 4.0μm in diameter and 0.8μm in thickness. The grana were less well developed, each stack containing no more than ten lamellae. Osmiophilic droplets were present but starch grains were not observed (Fig. 13).

In guard cells surface views with the scanning reflection electron microscope showed the presence of a number of chloroplasts, larger than those of other epidermal cells, arranged in single rows and almost filling the cells (Fig. 212). The grana system was poorly developed, with little more than ten lamellae in each granum, but in contrast to other epidermal chloroplasts they contained large starch grains which occupied most of their volume (Fig. 14, 15).

The granular stroma of the mesophyll chloroplasts often contained clear areas in which fine fibrils, resembling those identified in recent literature on chloroplasts as DNA (e.g. Kislev et al., 1965), were present (Fig. 13, 46, 47).

Similar fibrils were often observed in the immediate proximity of starch grains (Fig. 48). In some cases the clear areas appeared to take the place occupied by starch grains (Fig. 46).

Lamellae often appeared slightly distended, probably the result of changes in the osmotic balance of the cell components during fixation (Fig. 11).

Starch grains were frequently, but not always, surrounded by clear haloes, as if the starch had contracted away from the stroma or vice versa (Fig. 12), and often had a sculptured appearance caused by an uneven deposit of fine particles on the surface, often extending into the clear area surrounding the starch grain (Fig. 15). Large rosette-shaped crystals of stain were sometimes deposited on starch grains while the rest of the section was free of such a deposit (Fig. 14).

Nuclei were rarely seen in sections of mesophyll cells but were often found in sections of cells adjacent to veins which being smaller, offered a greater chance of cutting through a nucleus (Fig. 10). Nucleoli were situated against the nuclear membrane (Fig. 10). but aggregations of chromatin within the nucleus were not observed.

Mitochondria, ovoid to spherical in shape, were numerous in all cells and possessed finger-like cristae (microvillae) projecting into a uniform granular matrix (Fig. 10).

Rough and smooth E.R. (Fig. 19) and Golgi bodies (Fig. 10) were frequent in the thin lining of cytoplasm.

(ii) Invaded Cells

The cytoplasmic contents of cells invaded by well-developed haustoria were similar in general appearance to those of the uninvaded cells of infected plants and those of uninfected plants.

There were no obvious signs of degeneration in the chloroplast lamellae, osmiophilic droplets or starch grains (Fig. 12). Although fixation of different samples of infected tissue was usually carried out at the same time of day the size of the starch grains in the chloroplasts varied considerably. The condition of the starch appeared to be independant of the origin of the tissue whether from green-house or growth cabinet grown plants, or from non-invaded or invaded cells within the area ramified by the fungus (Fig. 12, 21, 22, 25). Chloroplasts possessing large starch grains were without extensive granal systems and the starch tended to occupy the lamellae free areas (Fig. 21, 22).

Nuclei were more frequently found in sections of invaded than non-invaded cells (Fig. 22, 25). The contents of the host nuclei did not appear to be significantly different from those of normal plants. The special relationship of the host nucleus to the haustorium is described in Section 3 (p. 91).

The lining of cytoplasm to the cells was generally thin but was considerably thicker around the haustoria ~~which~~ and also included a large number of organelles (Fig. 22).

Lomasome-like structures were present, appearing as infolds of the plasmalemma. Such folds often extended through the layer of cytoplasm into the vacuole (Fig. 17); evaginations of the chloroplast membrane into the vacuole were also observed (Fig. 18), but both these features were probably artefacts of fixation.

Single membrane-enclosed bodies with a uniform granular matrix similar to microbodies, a term used by Frederick et al. (1968) to include crystal-containing bodies, lysosomes, peroxisomes and spherosomes, were frequently observed in the cytoplasm (Fig. 16).

Further details of host organelles are described in connection with the haustoria in Section 3 (p.91).

One example of a plasmodesma in a section through an infected cell was observed (Fig. 20). It was delimited in the normal manner by the joined plasmalemmas lining the wall of the channel connecting the two cells, but contained a tube of E.R. which passed from one cell into the other and appeared to have an open lumen, although the continuity of the latter was somewhat obscured in the region of the middle lamella.

(b) Pathogen

Hyphae which ramified the intercellular spaces of the host were much branched and septate, although some non-septate runners, at least six times as long as the normal cells, were also observed (Fig. 21).

The cell walls of the fungus were much more electron dense than those of the host cells, which were often barely detectable (Fig. 19, 21, 22). The characteristic electron dense staining of the fungal cell walls, including those of the haustoria and spores, made it easy to distinguish between host and parasite in sections.

Dividing nuclei were not seen. Interphase nuclei contained large nucleoli usually situated against the nuclear envelope (Fig. 101) and large amounts of dense staining chromatin-like material, present as a reticulum (Fig. 68). Nuclei were observed constricted within the pores of 'pseudo-septa' occurring across the hyphae (Fig. 26).

Mitochondria had plate-like cristae (Fig. 61) and were round (Fig. 61), sausage (Fig. 42, 43) or dimpled (Fig. 159, 163) in shape. All three types could be found within the same cell and there was no evidence to suggest that the particular shape of mitochondria was associated with any specific state of the fungus.

Smooth E.R. (Fig. 89, 93, 98) was present in the fungus generally, but no typical 'rough' areas were seen.

Dictyosomes or other structures characteristic of Golgi formations were not observed.

Numerous lomasome-like bodies, of varied configurations, were found against the cell walls of intercellular hyphae but the plasmalemma could not always be traced around them (Fig. 30-33). The outer layer of the membrane triplet (e.g. Robertson, 1964) of the lomasome was often thicker than the inner, caused by a deposit of material on its outer face, although the corresponding layer of the adjacent plasmalemma was unthickened (Fig. 31). The tubular infolds of the lomasome often contained a granular matrix, similar in appearance to the layer of adjacent cytoplasm associated with the walls (Fig. 31).

Numerous lipid droplets were present in the cytoplasm of the intercellular hyphae (Fig. 26), haustoria (Fig. 45) and uredospores (Fig. 72). They appeared electron dense and granular after pemanganate fixation, often with clear centres and a thin electron dense boundary somewhat resembling a membrane though without a triplet structure (Fig. 99).

Ribosomes not greatly different in size from those of the host cells were numerous (Fig. 26, 61), generally aggregated into small clusters, but were not found in polysomal chains or spirals or obviously associated with cell membranes.

Electron-lucent granular material was also present, aggregated in large amounts to the exclusion of other cyto-

plasmic components (Fig. 26). This material appeared electron dense after permanganate fixation, often clustered in the form of rosettes (Fig. 27).

Two types of septa were apparent in the hyphae: a cross-wall composed of two layers, one associated with each cell, with a clear zone between them. Each half-layer of the septum was bonded to the lateral wall of the hypha and was about half its thickness (Fig. 27, 28). It could not be determined whether the septum was formed when the hyphal wall generally was thinner and thickened simultaneously with the adjacent lateral walls or if it was deposited independantly (cf. septum formed during uredospore formation Fig. 85, 86, 97, 98). The septum tapered gradually towards the centre of the hypha where it formed an acute edge to a central pore about 0.1µm in diameter (Fig. 27-29). The cytoplasm immediately around the pore was devoid of cytoplasmic organelles, apart from a number of microbodies; these were electron dense after Glut/Os fixation (Fig. 28, 29) but after permanganate fixation were of similar density to the general matrix of the cytoplasm (Fig. 27). These microbodies frequently possessed crystalline inclusions with a repeating unit of approximately 7nm (Fig. 28, 87, 88) and were similar in general appearance to Woronin bodies described for the Ascomycetes and Deuteromycetes (Bracker, 1967; Moore &McAlear, 1962; Reichle & Alexander, 1965).

In the second type of septum the two half-layers were

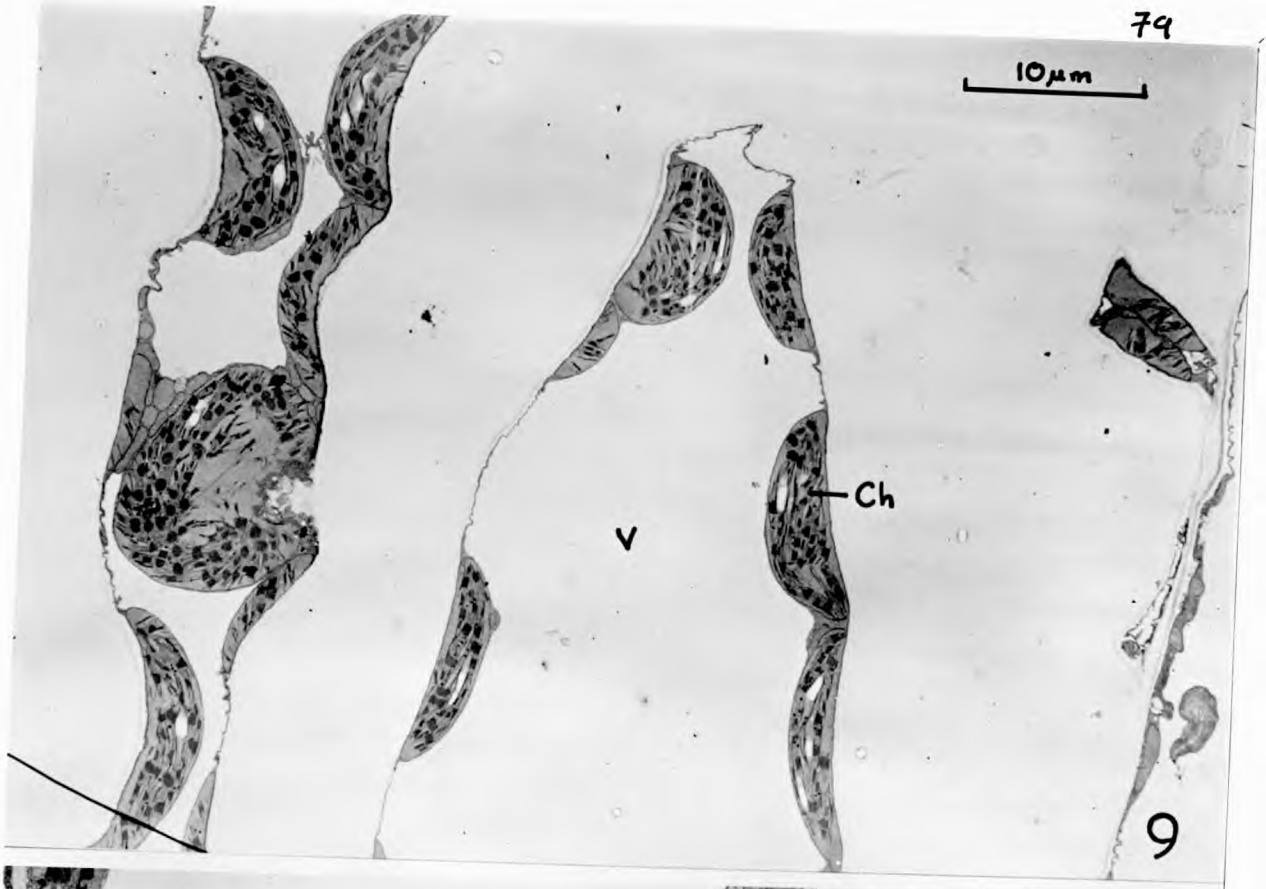
apparently formed by an infolding of the original lateral walls (Fig. 26). The septum was, therefore, about twice as thick as the lateral walls. The pore so formed was frequently eccentric and often contained the larger organelles, such as nuclei and mitochondria. Woronin bodies were not detected in association with these pores. This second type of septum has been termed a 'pseudoseptum' by Ehrlich et al. (1968a).

Intercellular hyphae were often highly vacuolated and contained only a few broken membranes (Fig. 36). Cells of the stroma where uredospores were being produced were well filled with cytoplasm (Fig. 67, 68).

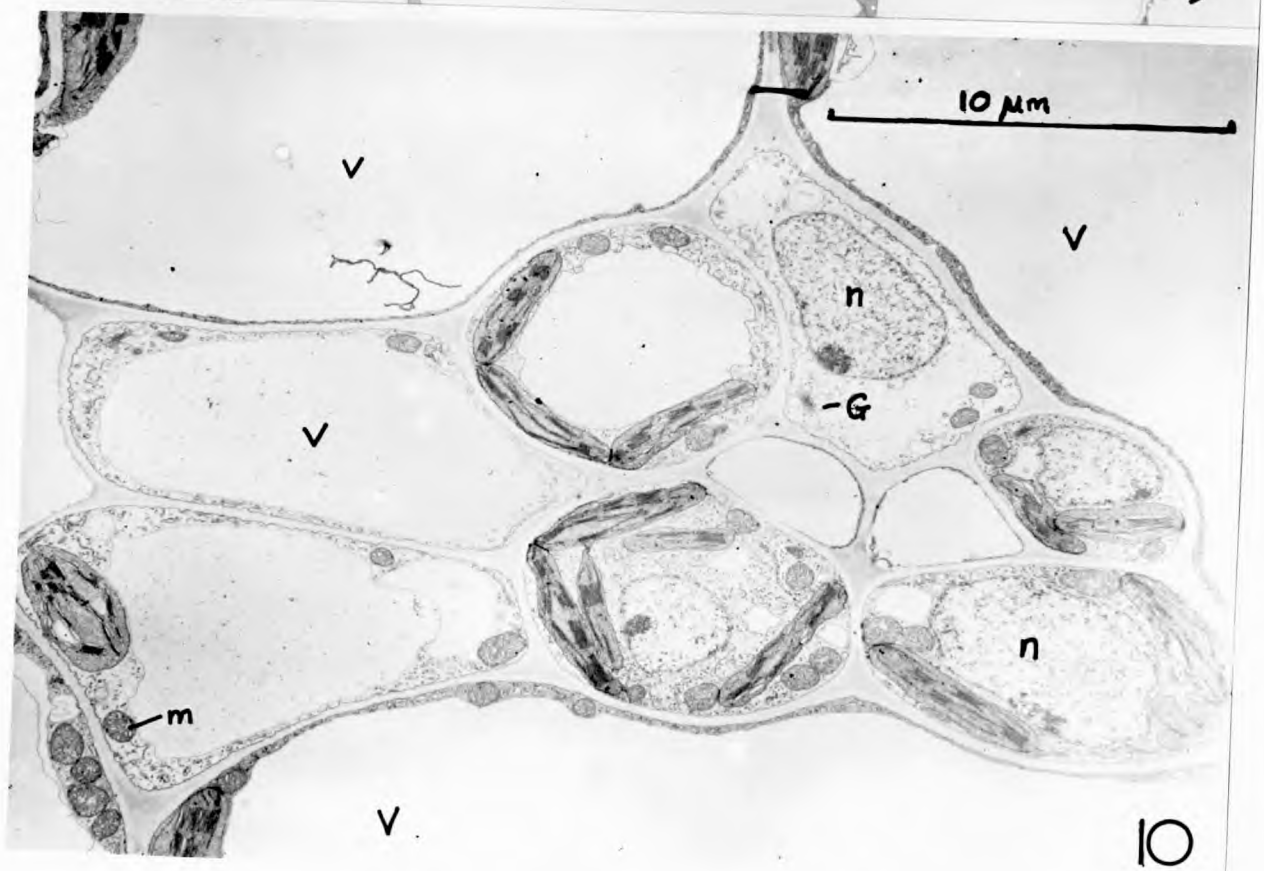
Angles of contact between adjacent cells, whether host-host, fungal-fungal or fungal-host, were often filled by material of a loose fibrous nature, appearing as a meniscus between the cells (Fig. 23, 24).

9. Palisade cells of an uninfected plant showing large central vacuoles (v) and numerous chloroplasts (Ch). 1381D1/9. 9073.

10. Cells from the vein region of an uninfected plant showing nuclei (n), mitochondria (m) and Golgi bodies (G). 1381D1/11. 14509.



9

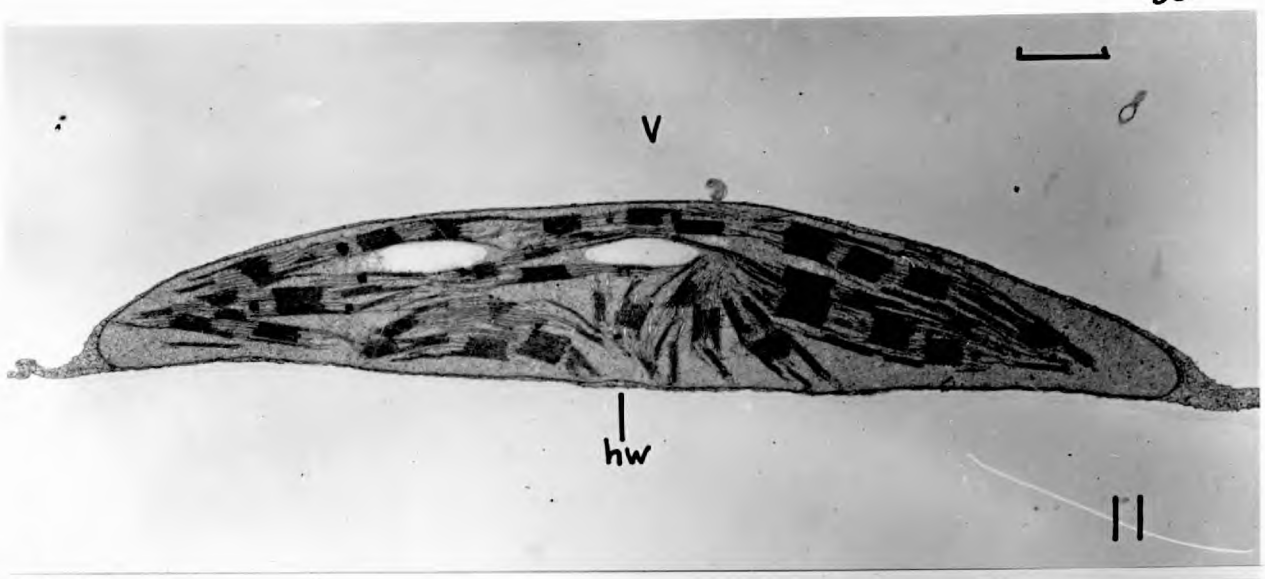


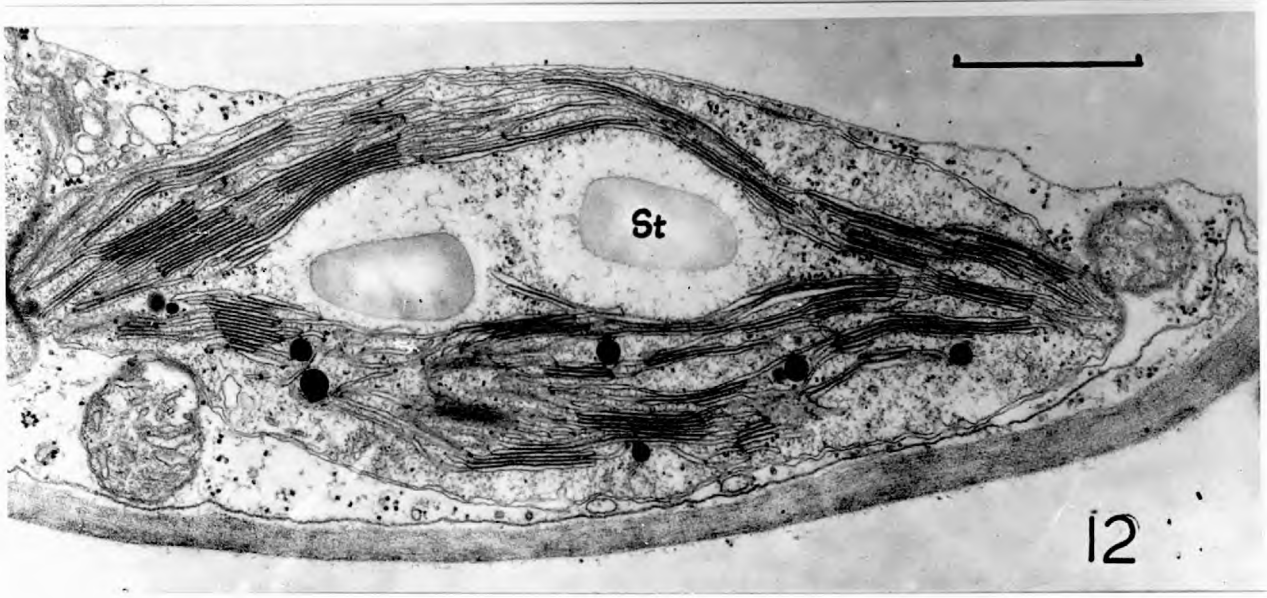
10

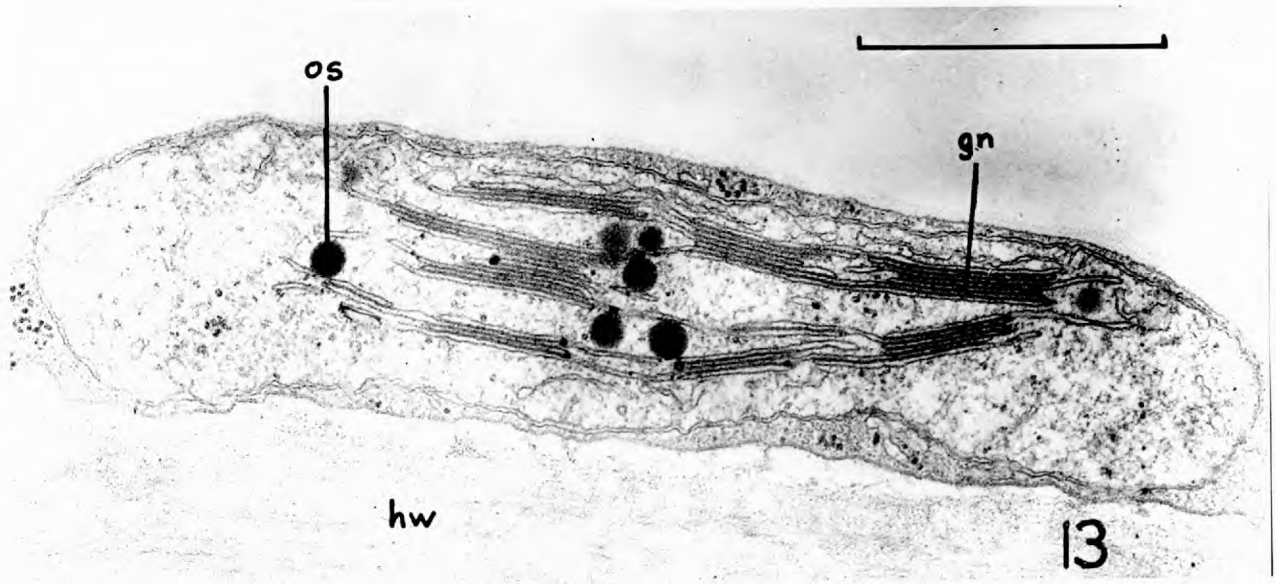
11. Chloroplast from the palisade of an uninfected plant. Host cell wall (hw) barely distinguishable against the background. 1381D1/9. 14498.

12. Chloroplast from a non-invaded cell of an infected 25 day old plant, 9 days after inoculation. Starch grains (St) surrounded by an area devoid of stroma but containing fibrillar material. Some staining of the host wall has occurred. 1382B/25. 14233.

13. Chloroplast of a non-invaded epidermal cell of an infected plant. Note the absence of starch, reduced number of lamellae in the grana (gn) and the presence of osmiophilic droplets (os). 1382/B34. 6276.

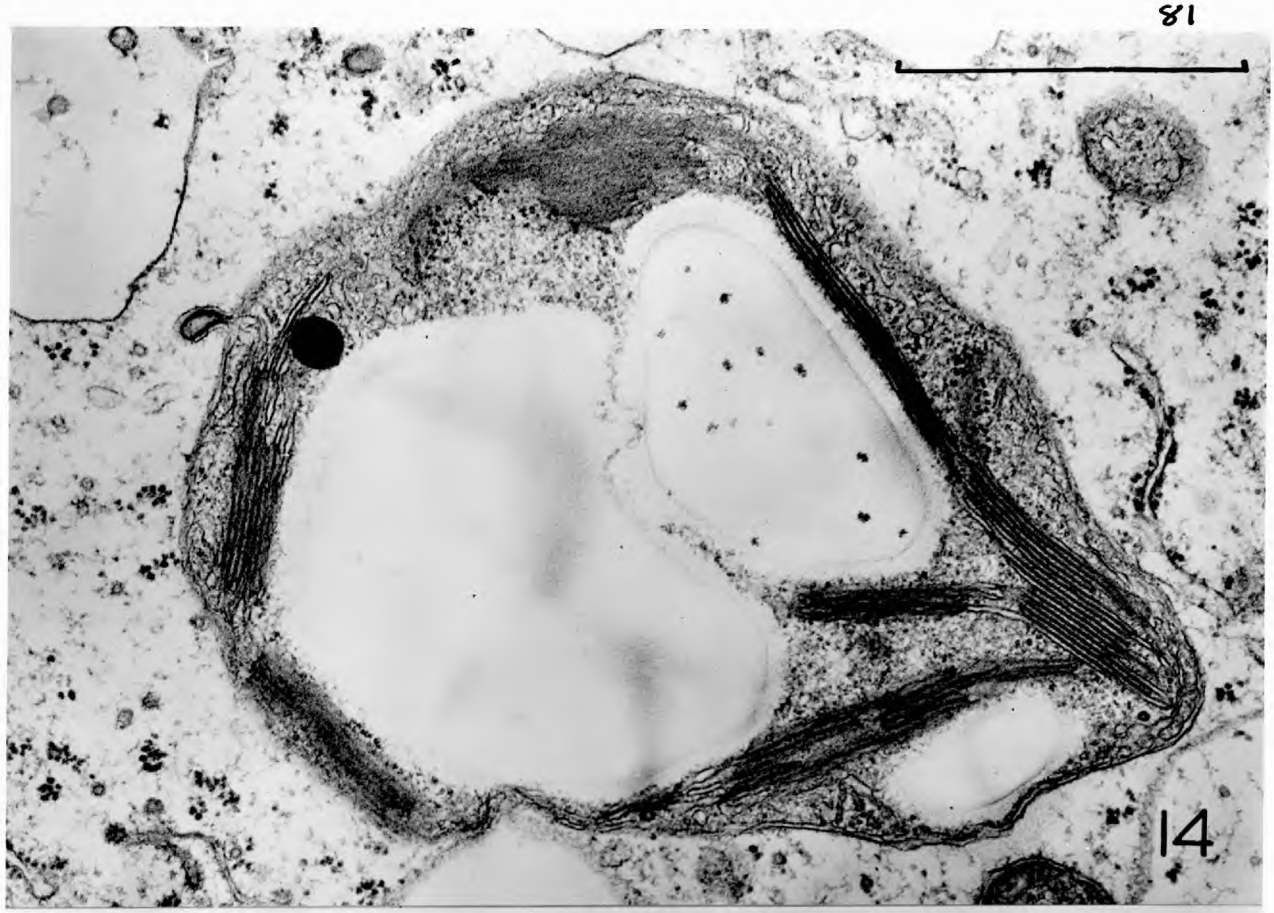

V
hw
11


St
12

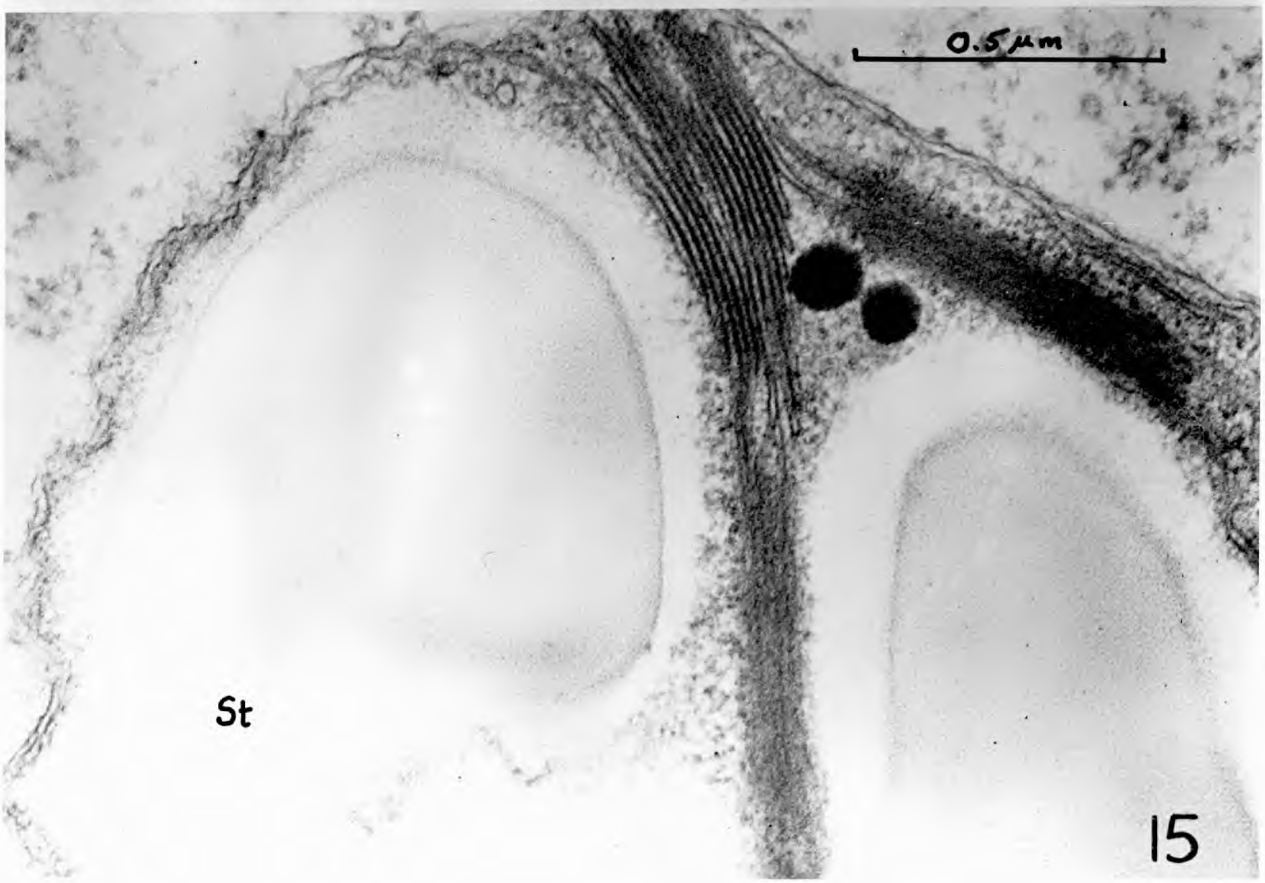

os
gn
hw
13

14. Chloroplast from a guard cell of an infected plant. Note the large starch grains and reduced grana. The upper starch grain has a deposit of large crystals of stain not found on the neighbouring grain. 1382B/33. 6264.

15. Starch grain (St) from a guard cell chloroplast showing a deposit of small particles extending into the clear area surrounding the grain. 1382/B33. 6265.



14

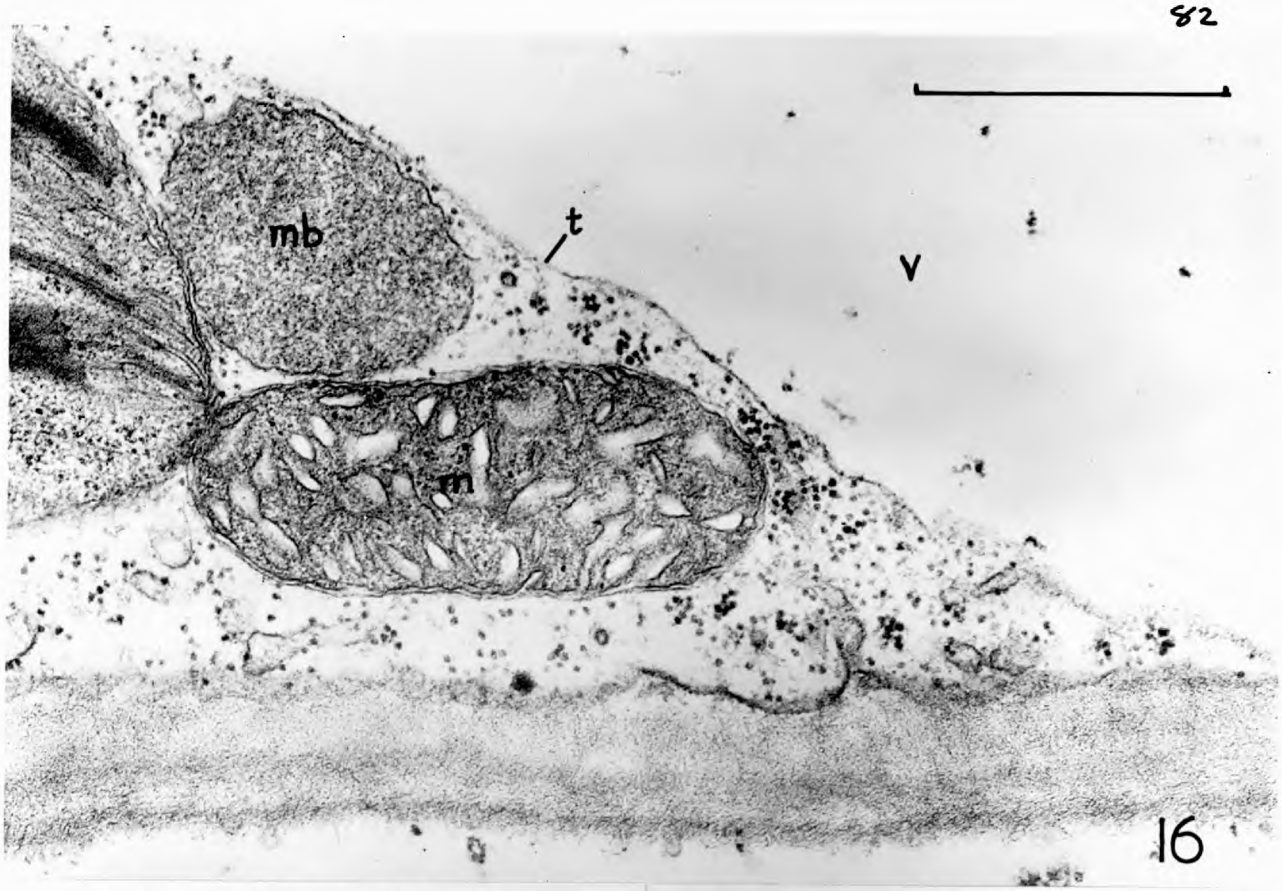


15

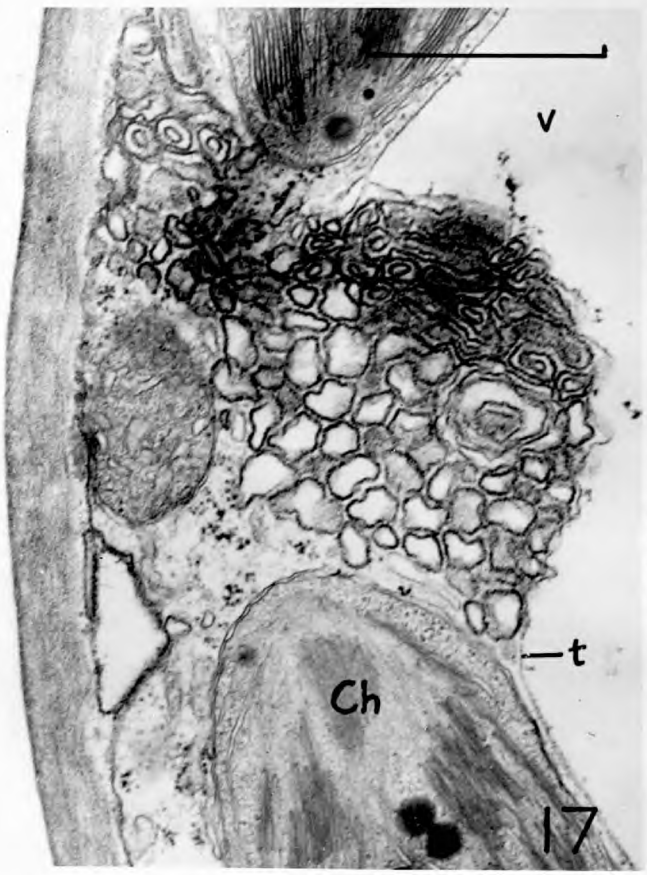
16. A microbody (mb), bounded by a single membrane and a mitochondrion (m), bounded by a double membrane. 1382B/34. 6279.

17. A lomasome-type body from a non-invaded cell of infected material, extending from the plasmalemma through the lining of cytoplasm into the vacuole (v). The tonoplast (t) is continuous over the lomasome. 1382B/31. 5880.

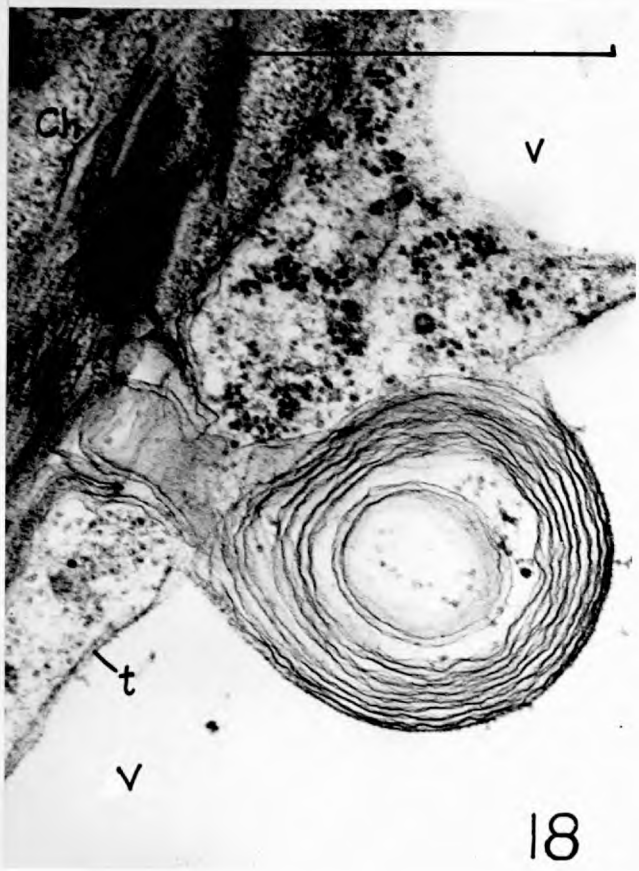
18. A coiled membrane extending from the bounding membrane of the chloroplast (Ch) into the vacuole (v), remaining covered by the tonoplast (t). 1382B/30. 6280.



16



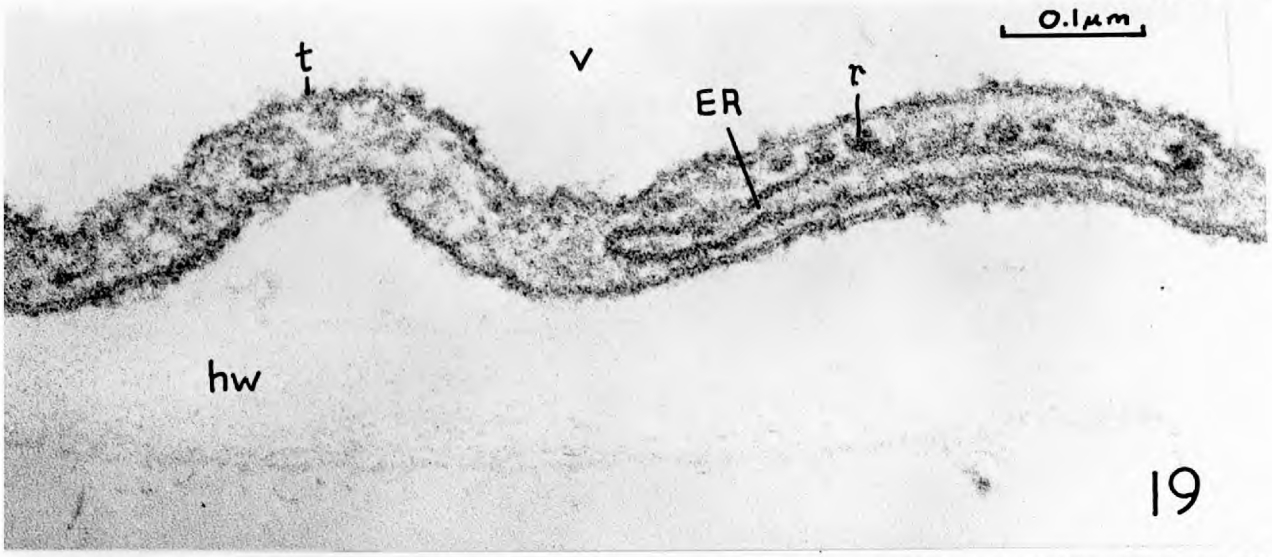
17



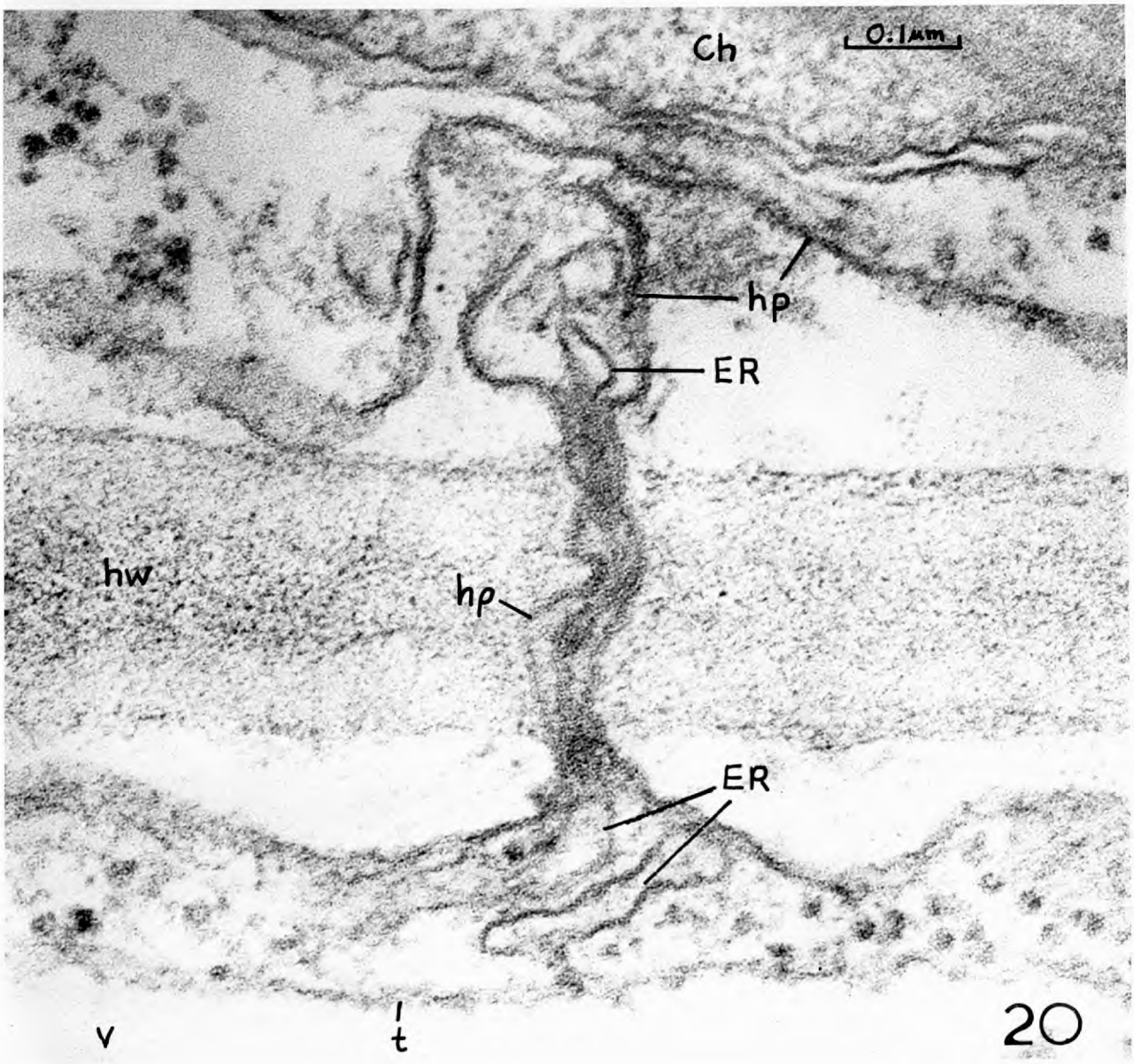
18

19. Thin lining of cytoplasm to a cell of the palisade containing elements of the E.R. The host wall (hw) is barely visible against the background. 1381D1/10. 14507.

20. A plasmodesma from a non-invaded cell of an infected plant. Note the continuity of the plasmalemma through the cell walls (hw) and the cisternae of E.R. entering even if they cannot be traced unequivocally between the cells. 1382B/31. 6282.



19

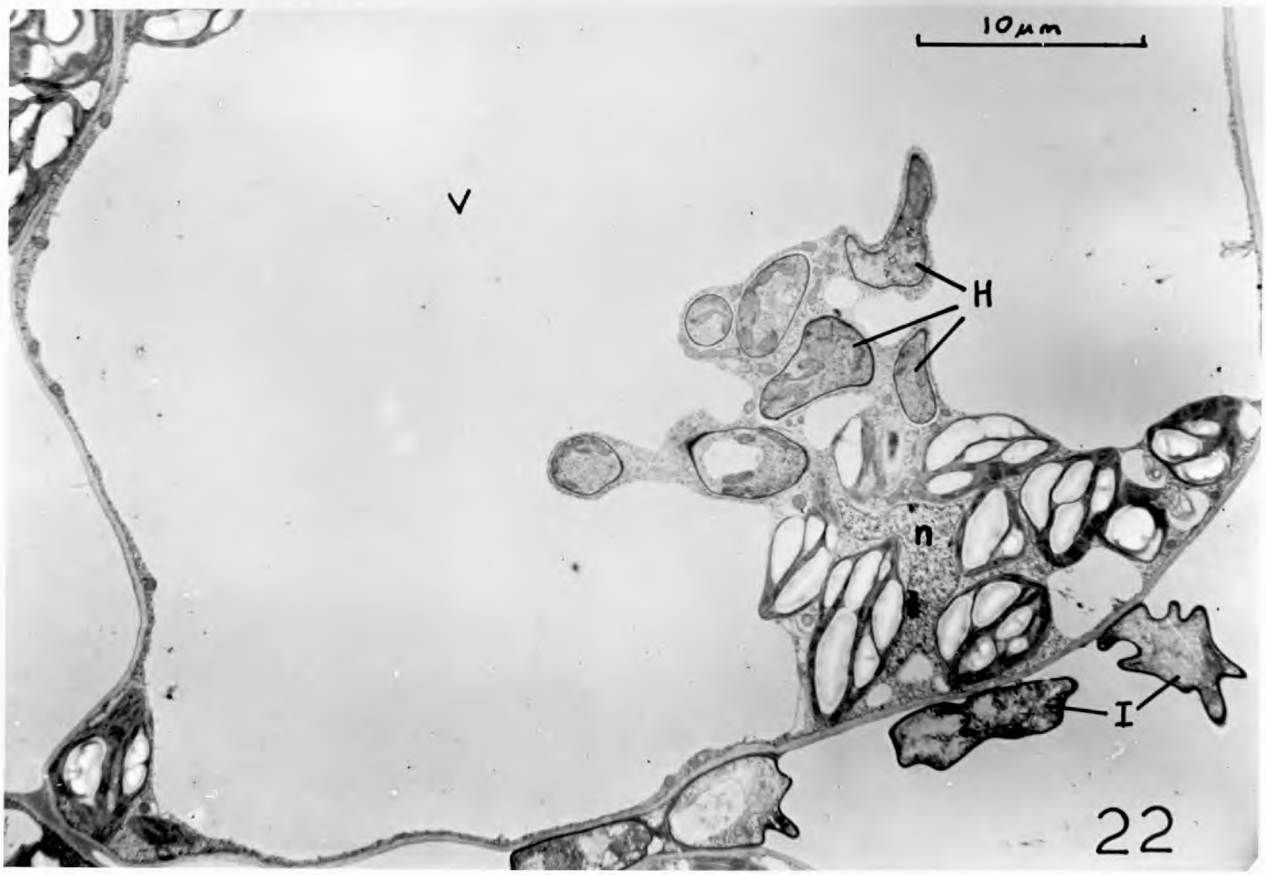


20

21. Low power of invaded leaf tissue showing the intercellular mycelium (I) of the rust consisting of many branched septate hyphae and long non-septate runners (R). Note the difference in electron density between host and parasite cell walls. 1540B/5. 8301.

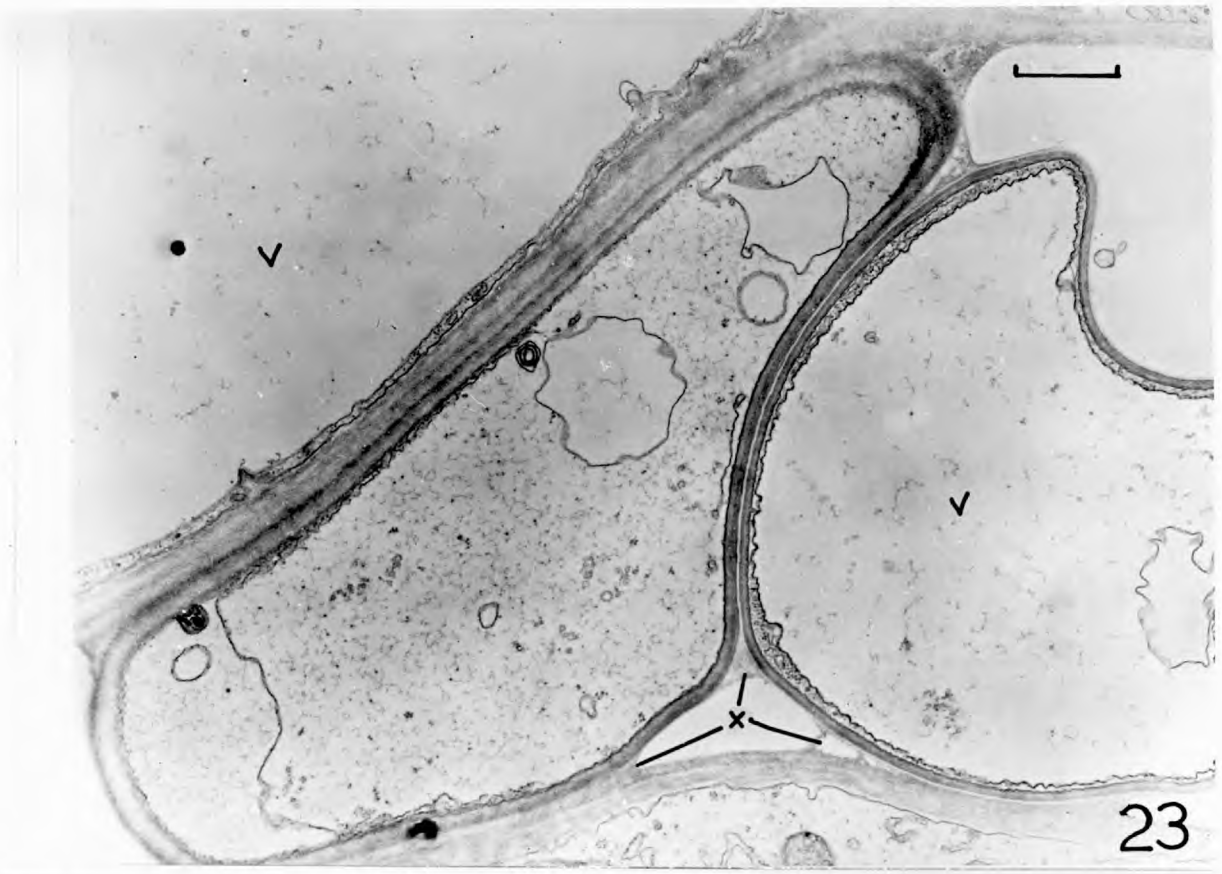
22. Low power of a spongy mesophyll cell containing haustoria (H) which are surrounded by host cytoplasm. A host nucleus (n) can be seen distorted between surrounding chloroplasts. 1540B/1. 8291.


10µm
V
R
Ch
I
St
V
21

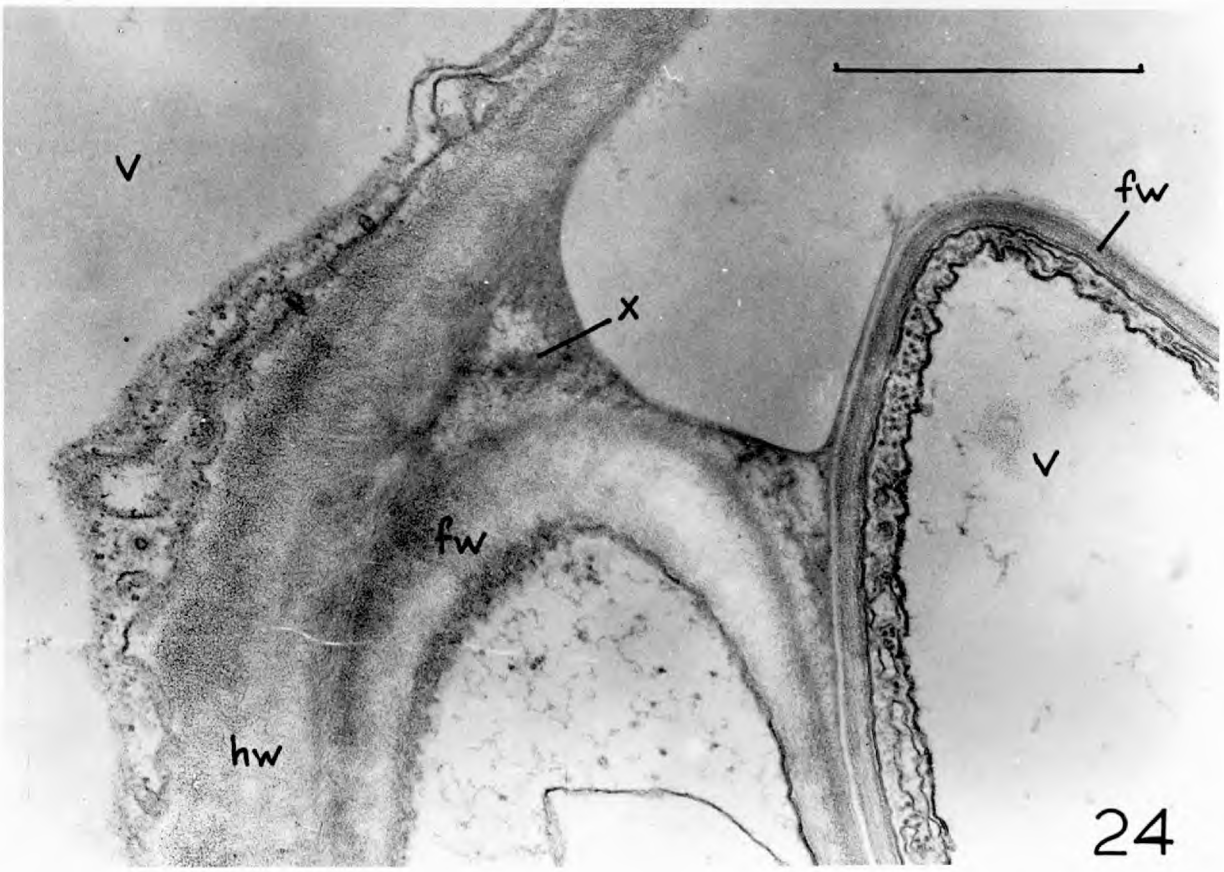

10µm
V
H
n
I
22

23. Two fungal cells between two host cells showing an accretion of intercellular material (x) in the angle of contact between the cells. 1382B/72. 6524.

24. Detail of Fig. 23 showing material (x) in the angle of contact between the cells and its continuity around the fungal cell wall (fw). The gap apparent between the fungal wall of the right hand cell and its neighbour may be due to movement of the cells during fixing and embedding. 1382B/72. 6525.



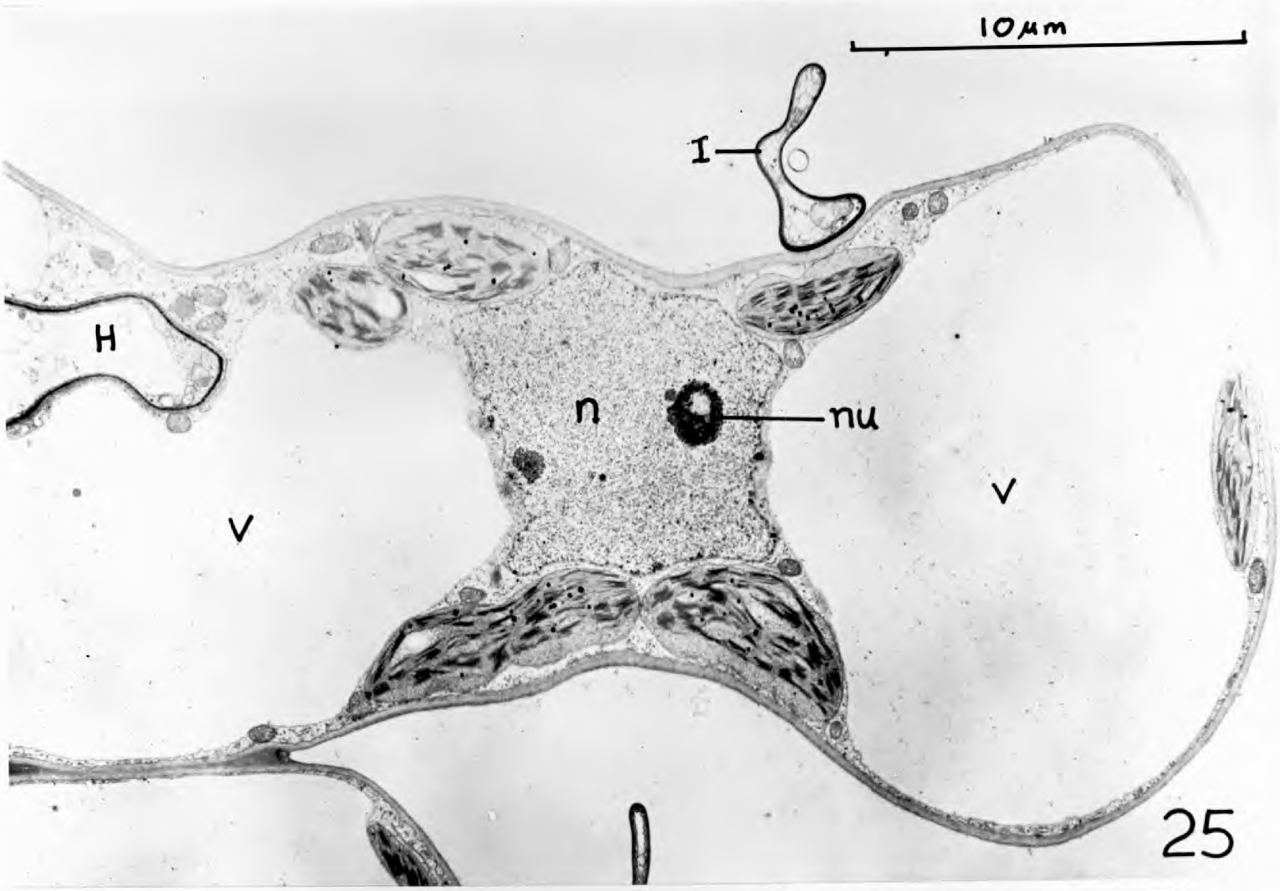
23



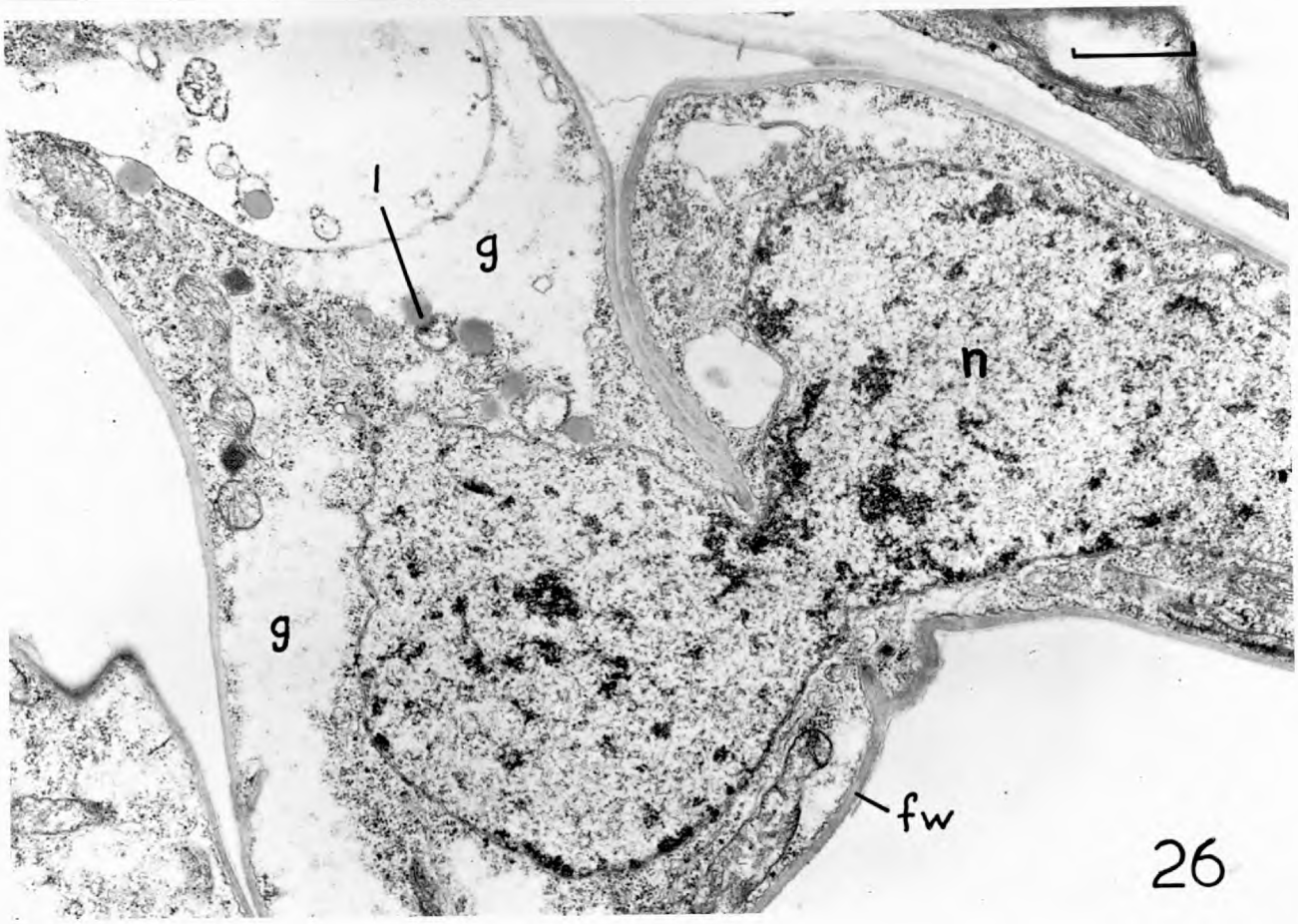
24

25. An invaded palisade cell with a nucleus (n) containing a nucleolus (nu) suspended in the vacuole. 1382B/31. 5876.

26. An intercellular hyphal cell with a nucleus (n) within the pore of a pseudo-septum. A large aggregation of electron-lucent granular material (g) is present in the cytoplasm, also numerous droplets of lipid (l). 1540B/9. 8301.



25

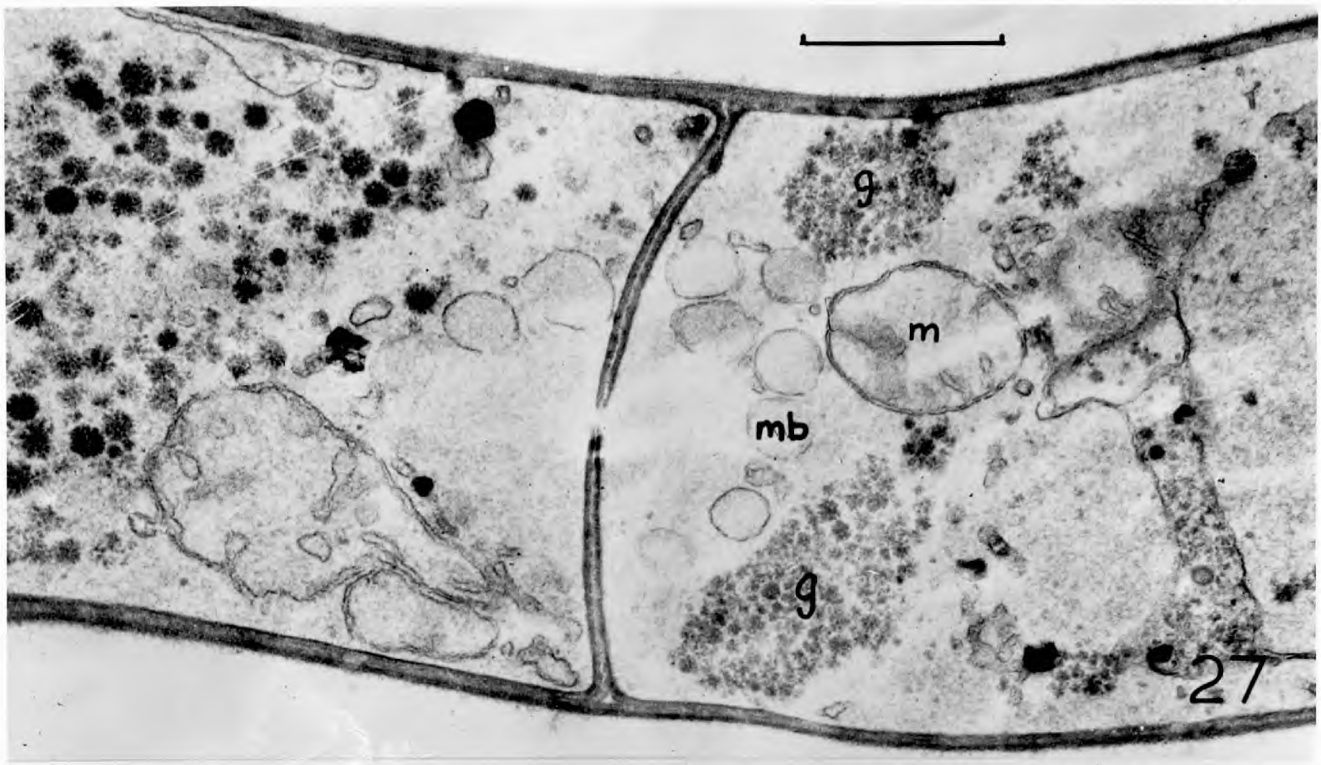


26

27. Section median to the pore in a septum of an intercellular hypha. The area around the pore is devoid of organelles and is surrounded by single membrane bound vesicles (mb). Granular material (g) is present in the hypha. Note the continuity of the hyphal wall across the end of the septum. $KMnO_4$. 40KV. 1529/16. 6610.

28. Septum showing tapering of the wall to the pore and the electron-lucent gap running between the two halves of the septum. Numerous microbodies (mb) are present in the vicinity of the pore. The hyphal wall is continuous across the end of the septum. 1851A/16. 14156.

29. A septum tapering more abrubtly to a pore, but as in Fig. 27, 28 an electron-lucent gap is continuous to the margin of the pore. 1870A/3. 12563.


g
m
mb
g
27

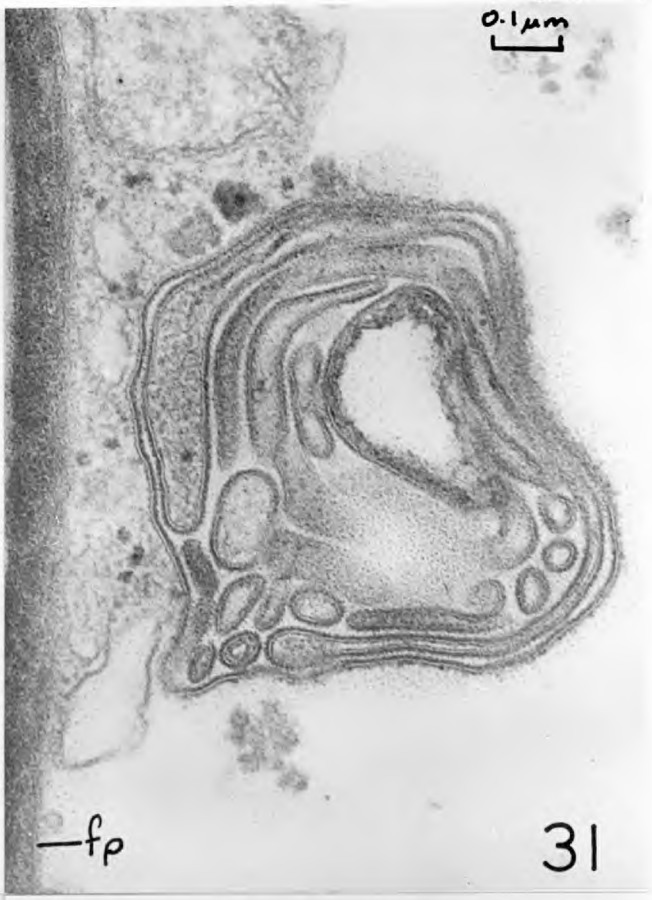

mb
28


29

30-33. Lomasome-like bodies in fungal hyphae showing the variety of configurations that can be formed. Only in Fig. 33 can the fungal plasmalemma be traced around the lomasome. 30. 1382B/32; 5886. 31. 1382B/32; 5881. 32. 1382B/31; 5878. 33. 1382B/33; 5866.



30



31



32



33

3. HAUSTORIA

This section describes in detail the structure of the haustorium and the penetrated host cell in a susceptible reaction with Pinto beans.

(a) Occurrence

Haustoria were observed, frequently more than one to a cell (Fig. 35), in the epidermis (Fig. 40), the palisade (Fig. 36) and the spongy mesophyll (Fig. 38).

(b) Morphology

The haustorium had three main parts; the base, contained within the original host wall; the stem, of cylindrical form and of limited length, which may be sub-divided into the neck, extending from the base to the 'neckband' (see p. 95); and an expanded distal portion, the body.

A mature haustorium projected from an area of its mother cell, which adhered to the host cell wall (Fig. 36-38), and penetrated the latter through a small and often slightly constricted pore (Fig. 48, 49). Within the host cell the stem of the haustorium, which was straight or slightly curved, expanded more or less abruptly into the tubular and usually unbranched body. The body was generally set at

right angles to the stem, characteristically in the form of a foot with a small heel (Fig. 34, 35). When expansion of the body into the host was restricted, as when the direction of entry was oblique, the haustoriun although bent and distorted still retained its basic foot-like shape (Fig. 36).

(c) Host Cytoplasm

A few broken membranes with free edges and other evidence of cytoplasmic disorder, which can be ascribed to damage and contraction caused by fixation, were sometimes found in host cell contents (Fig. 36) but in good preparations (Fig. 35) membranes and organelles were typically intact, even up to a late stage after invasion by haustoria.

The plasmalemma of the invaded cell was invaginated and extended to form a complete covering to the haustorium. The haustorium usually penetrated deeply into the lumen of the host cell, even so a layer of associated cytoplasm was retained between the tonoplast and the plasmalemma (Fig. 45).

Mitochondria (Fig. 44) were often accumulated in regions near to the haustorium but appeared to be structurally normal. Mature chloroplasts with median constrictions, seemingly in division, were present in invaded (Fig. 36) and adjacent non-invaded cells.

A single layer of E.R. often surrounded part or all of the stem of the haustorium (Fig. 47, 48, 63) and was

composed of large much flattened cisternae virtually in contact with the host plasmalemma. The membrane of the cisternae carried cytoplasmic ribosomes, arranged almost exclusively in spiral chains, on the side facing away from the stem (Fig. 51). Cisternae were often in contact at their margins and in some cases probably formed an almost uninterrupted covering to the sheath and stem.

Small, rounded vesicles were observed, widely spaced against the body of the haustorium. The vesicles did not possess coatings of polysomes and were mostly smooth apart from some which bore a few scattered ribosomes (Fig. 36, 61). Golgi bodies did not appear to be unusually abundant in the invaded cell nor particularly active judging by the non-proliferation of associated vesicles (Fig. 45).

The nucleus was frequently seen in close proximity to the haustorium and was usually irregular in shape, distorted to follow the contours of the invading organ (Fig. 41-43). Densely stained chromatin regions were absent but nucleoli, each with one or more nucleolar vacuoles, were present (Fig. 41).

(d) Haustorial Cytoplasm

In sections of haustoria one nucleus, several times the diameter of the penetration pore, were often observed (Fig. 39, 45, 46); occasionally two nuclei (Fig. 43) or

exceptionally three (Fig. 165), were visible. A large amount of densely staining chromatin was present in the form of a reticulum, and nucleoli when seen were small, round, without nucleolar vacuoles and of a similar opacity to the chromatin regions (Fig. 46).

Mitochondria were numerous, particularly in non-vacuolated parts of the haustoria (Fig. 61) and were rounded (Fig. 61), extended (Fig. 36, 45) or not infrequently ring-shaped (Fig. 41, 42) in sectional profiles.

Lipid droplets (Fig. 45, 46) and flattened cisternae of smooth E.R. (Fig. 45, 64) were present and occasionally microtubules were seen (Fig. 53).

Lomasomes, frequent in cells of the mycelium (Fig. 30-33), were not observed in mature haustoria. Conspicuous dense membranes of uncertain origin, single or paired around small vesicles, or in multilayered, concentric of coiled configurations often occurred in the base and stem of the haustorium (Fig. 45, 48, 53, 54).

Haustoria were vacuolated to varying degrees (Fig. 35-38, 45, 46).

(e) Wall Structure

(i) Haustorial Mother Cell

The haustorial mother cell (h.m.c.) in Glut/Os fixed and U/Pb stained material had an electron dense wall with a

thin and paler, but usually well defined, external layer which was continued over the surface of the supporting hypha (Fig. 36-38). This layer appeared to adhere to the host wall and often extended to the edge of the penetration pore without change in thickness or electron opacity (Fig. 47-50); this intercellular layer appeared dark after permanganate fixation (Fig. 64). The surface of the host wall sometimes retained a coating of intercellular material of its own or was delimited merely by a thin electron dense boundary (Fig. 49). In addition an accretion of intercellular material usually occupied the angle of contact between the walls of parasite and host (see also p. 78) which presented a smooth surface to the intercellular space shaped like a meniscus (Fig. 36, 47).

The h.m.c. wall contained one or more electron dense sub-layers (Fig. 48, 49), one of which was particularly prominent and was resistant to permanganate fixation which left the surrounding material pale (Fig. 64). These layers ended abruptly a short distance from the origin of the haustorium and the remainder of the dense wall tapered to a vanishing edge at the margin of the penetration pore (Fig. 36, 48-50, 64). Typically the wall of the h.m.c. was considerably inflated where it was in contact with the host wall, but the wider this area of contact the smaller the degree of thickening so that in many cases it was scarcely or not at all evident (cf. Fig. 48, 49).

(ii) The Base

There was no recognisable fungal wall deposited in the base of the haustorium, although there was instead an increase in electron density which sometimes extended a short distance into the host wall (Fig. 48-50). The penetration pore was a smooth surfaced aperture about 0.5µm in diameter. The internal structure of the original host wall surrounding the pore usually remained unaltered, but occasionally the superficial layers were torn and thrust aside particularly where the contacting area of the h.m.c. wall was greatly thickened (Fig. 48).

(iii) The Sheath

The sheath was a new deposit of material fused to the inside of the host wall around the penetration pore which extended for a varying distance along the stem of the haustorium (Fig. 34, 40, 49, 52-55). The base of the sheath was impregnated with electron dense material which was distinct next to the fungal plasmalemma and became more diffuse towards its outer limits (Fig. 49). Further along the stem this dense material became less diffused into the sheath and eventually consolidated into a dense, sharply defined layer of uniform thickness (about 23nm thick) adjacent to the fungal plasmalemma (Fig. 63, 64). This layer of material did not appear to be separated from the outer zone of the sheath but it was of a similar general electron density to the h.m.c. wall; it was, however, thinner than the

latter and lacked its characteristic sub-layers of varying density.

The remainder of the sheath consisted either entirely of fibrillar material (Fig. 49, 54) of a looser, more disordered texture than the host wall, or possessed a distinct inner region of a non-fibrous electron-lucent material (Fig. 52, 53, 55, 56).

The surface of the sheath on the host side was irregularly pocketed and loosely associated with the plasmalemma (Fig. 47). The outer fibrillar layer and the inner electron-lucent layer often included pockets of sequestered membranes which were probably isolated infolds of the host plasmalemma (Fig. 49, 52, 53).

(iv) The Stem and Neck

At a point at varying distances from the base of the haustorium, sheathing material, apart from the innermost electron dense layer, ceased to be present. At this point, for a short distance where the outer layers of the sheath were no longer in contact, the stem wall thickened slightly, became somewhat reduced in electron density and the gaps between it and the adjacent host and fungal plasmalemmas were filled with diffuse material of comparable density (Fig. 65, 66). This region of the stem wall extended for approximately 100 to 250nm, its termination being marked by a variable number of very opaque irregularly shaped areas. This narrow

zone of dark material formed a conspicuous 'ring' at this point in the stem which has been observed in all haustoria examined (Fig. 36-40, 45-48, 62-66) and is particularly well seen when viewed in glancing section (Fig. 62).

The stem of the haustorium between the base and the end of the electron dense ring was defined earlier (p. 89) as the neck region and the short terminal section, described in detail above, including the electron dense material will be termed the 'neckband'.

In the presence of deposits of sheathing material the projections of the outer zones of the sheath often extended beyond the neckband, but in such cases the covering host plasmalemma returned to the beginning of the neckband forming a re-entrant fold; the projecting part of the sheath sometimes stood as a complete collar encircling the neckband and part of the stem beyond (Fig. 52). These same essential features of the neckband were apparent after permanganate fixation (Fig. 64,65), although the stem was often broken just beyond the electron dense deposit of the ring (Fig. 64).

The electron dense 'blotches' comprising the terminal ring tended to obliterate detail in that region of the neckband (Fig. 65). In some instances clear gaps appeared to be present and it was not certain if the wall remained continuous at that point (Fig. 52, 53).

Beyond the neckband two distinct layers were apparent in the stem wall and continued, without change in thickness, into

the wall surrounding the body of the haustorium (Fig. 63, 64).

(v) The Body

The two layers of the body wall were almost equal in thickness but differed sharply in electron opacity. The inner layer was electron dense, about 43nm thick, and the outer paler layer 42nm (Fig. 60). The pale layer was granular in texture and the electron dense layer faintly fibrillar, but both layers did not superficially resemble either the walls of the intercellular hyphae or of the host. In some haustoria the layers of the body wall appeared eroded and the pale layer was separated from the dark layer by thin gap (Fig. 61). In the more distal parts of the body the host plasmalemma was often loosely applied to the body wall and bridges of material from the pale layer extended across the gap giving the appearance of an 'encapsulation' (Fig. 61).

(f) Anomalies

A number of haustoria with very dense contents and undistinguishable cytoplasmic organelles were observed in apparently healthy host cells (Fig. 56, 57). These haustoria were probably necrotic, as their appearance differed from neighbouring fungal cells which appeared to have been living prior to fixation.

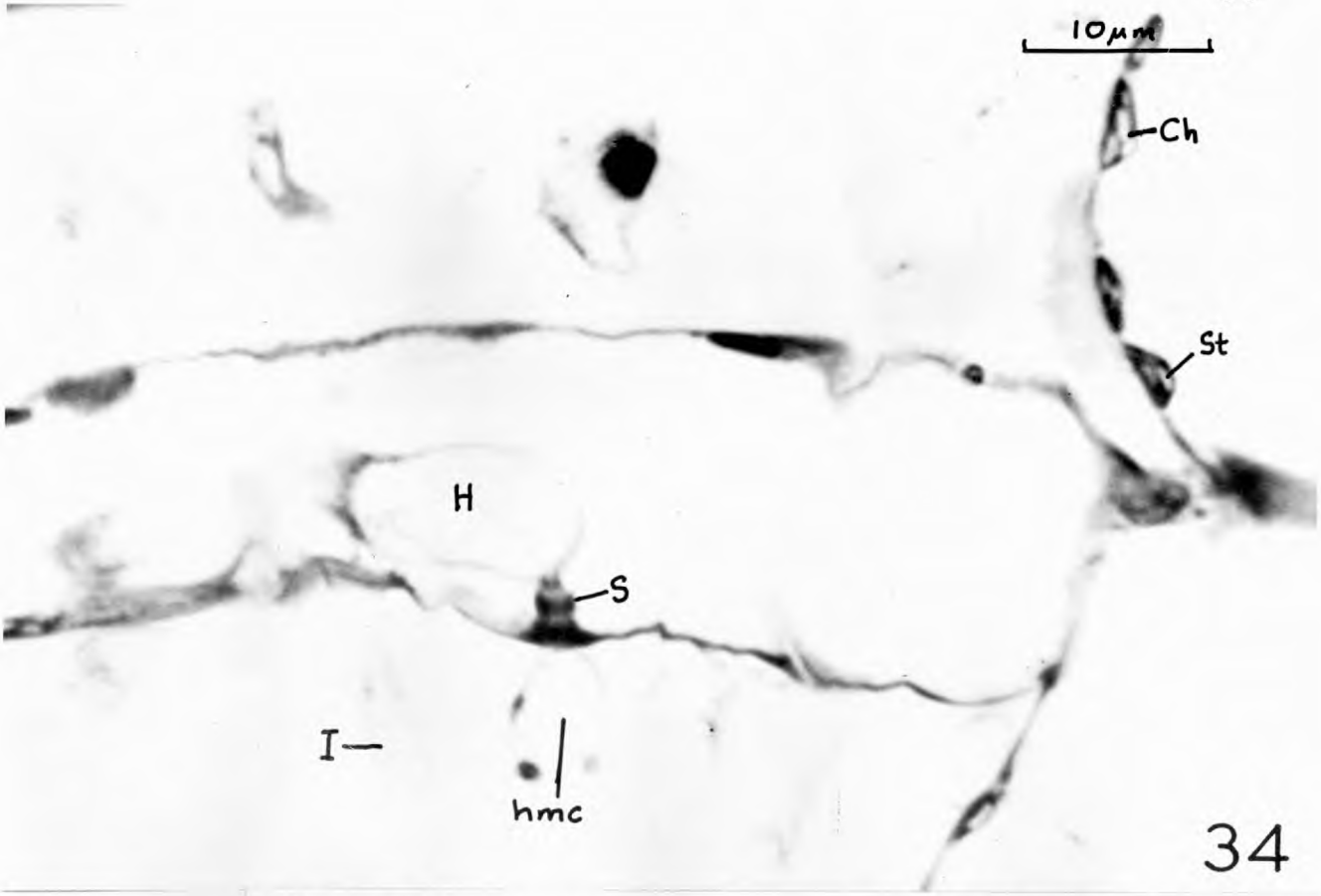
One haustorium was seen which lacked an h.m.c. (Fig. 58).

The outer layer of the host wall surrounding the penetration pore showed signs of having been disturbed as if the h.m.c. wall had been present; but possibly through lack of adhesion combined with the fact that the h.m.c. wall was not continuous with the wall of the haustorium, there was an inherent point of weakness and the h.m.c. was lost during fixation. The neck region, including the sheath, was everted but the host and fungal plasmalemmas over these regions remained in contact with the intercellular materials contained between them. The body wall was convoluted as if there had been a loss in tugor within the haustorium, brought about by the loss of the h.m.c., and the nucleus, seen in the neck and extending into the intercellular space, appeared as if it was in the process of being ejected (Fig. 59).

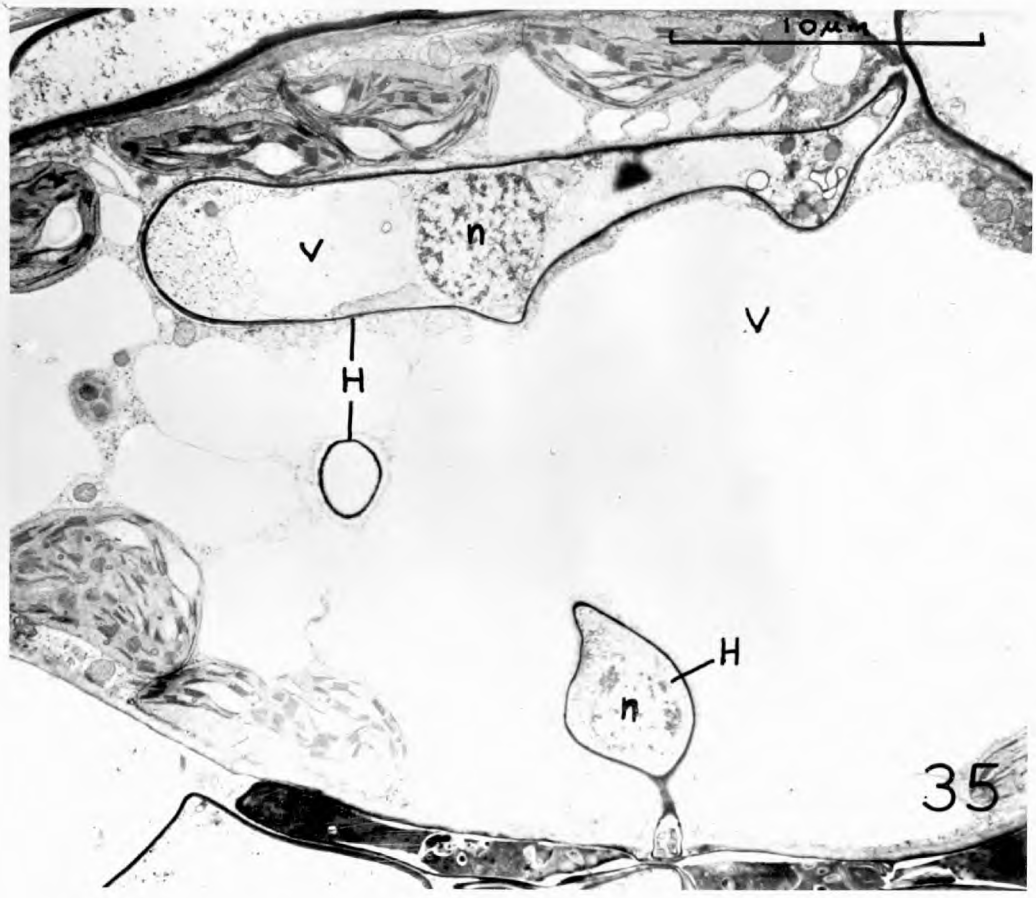
Some haustoria lacked a stem and were apparently completely surrounded by a sheath (Fig. 54). They were without any structural feature corresponding to the neckband and there were no conspicuous layers of host E.R. adjacent to the sheath. The cytoplasm of these haustoria contained a number of prominent vesicles with thick membranes.

34. Light micrograph of a palisade cell invaded by a haustorium (H). Note the large deposit of sheathing material (s) around the stem of the haustorium. A/G. Toluidine blue O. 1523. 535.

35. Spongy mesophyll cell invaded by at least two haustoria (H) sectioned in the region of penetration. Each contains a nucleus (n). 1382B/65. 6742.

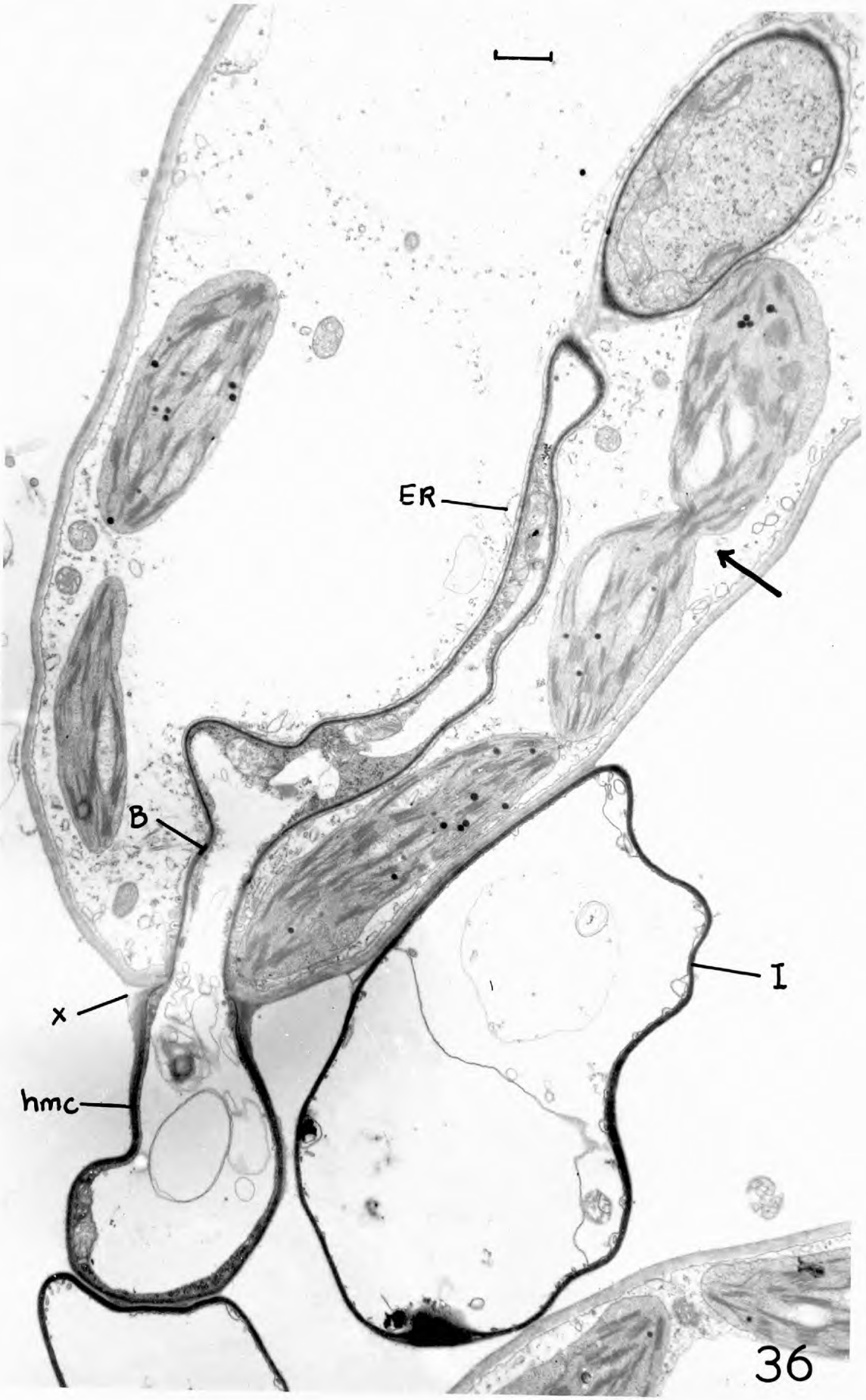


34



35

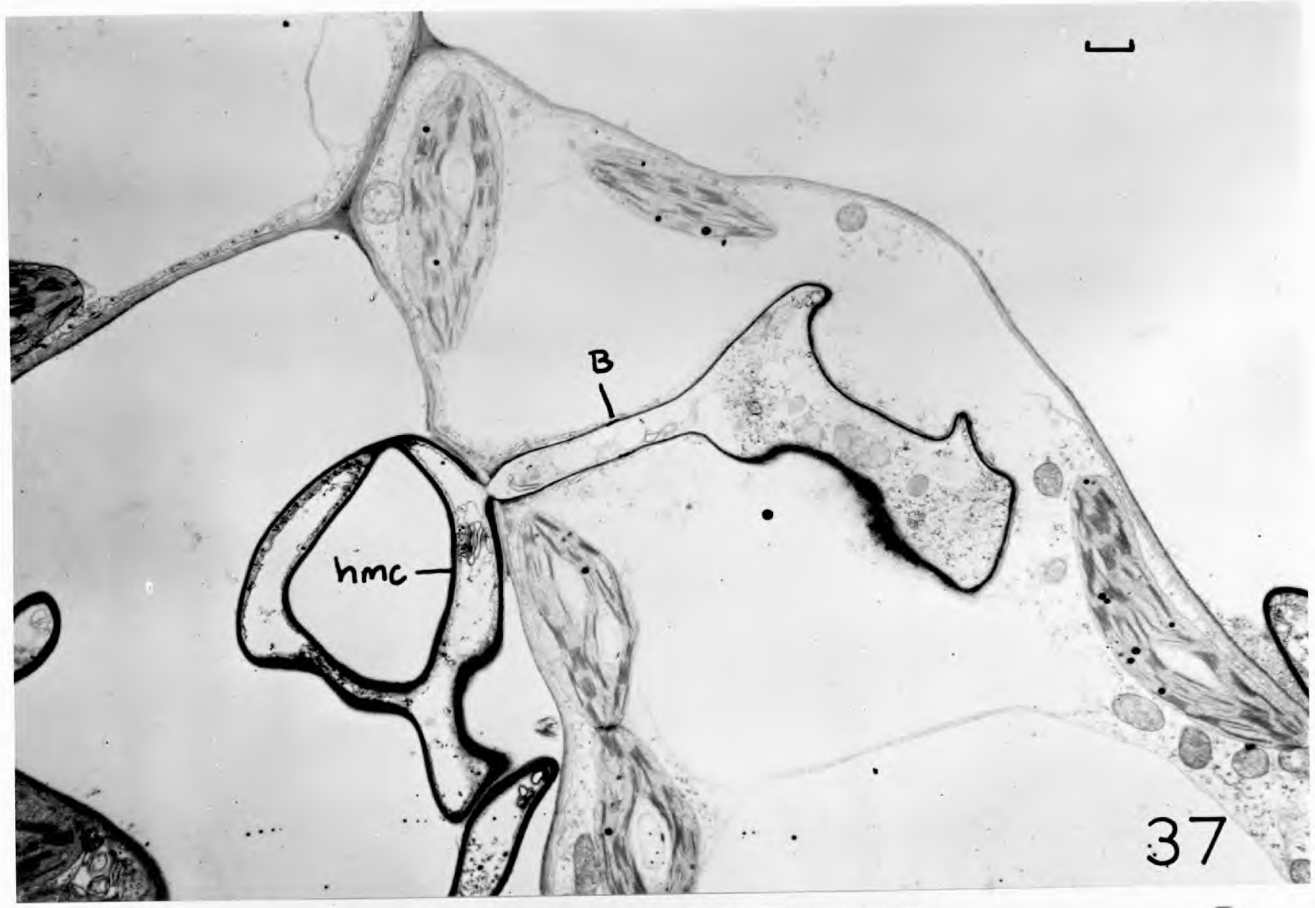
36. Section of a palisade cell showing a haustorium with base and stem in median L.S. and parts of the body, well filled distally with cytoplasm. One of the chloroplasts (arrow) has a median constriction. Note the dark staining band (B) in the stem of the haustorium. Intercellular hyphae (I) are characteristically devoid of contents and only a few scattered membranes remain. Some collapse of the body of the haustorium appears to have occurred causing disruption of the tonoplast. Compare the adhesive but unspecialised contact area between the host cell and an intercellular hypha with that of the h.m.c. Note material in the angle of contact between the h.m.c. and host cell wall (x). 1382B/6. 6868.

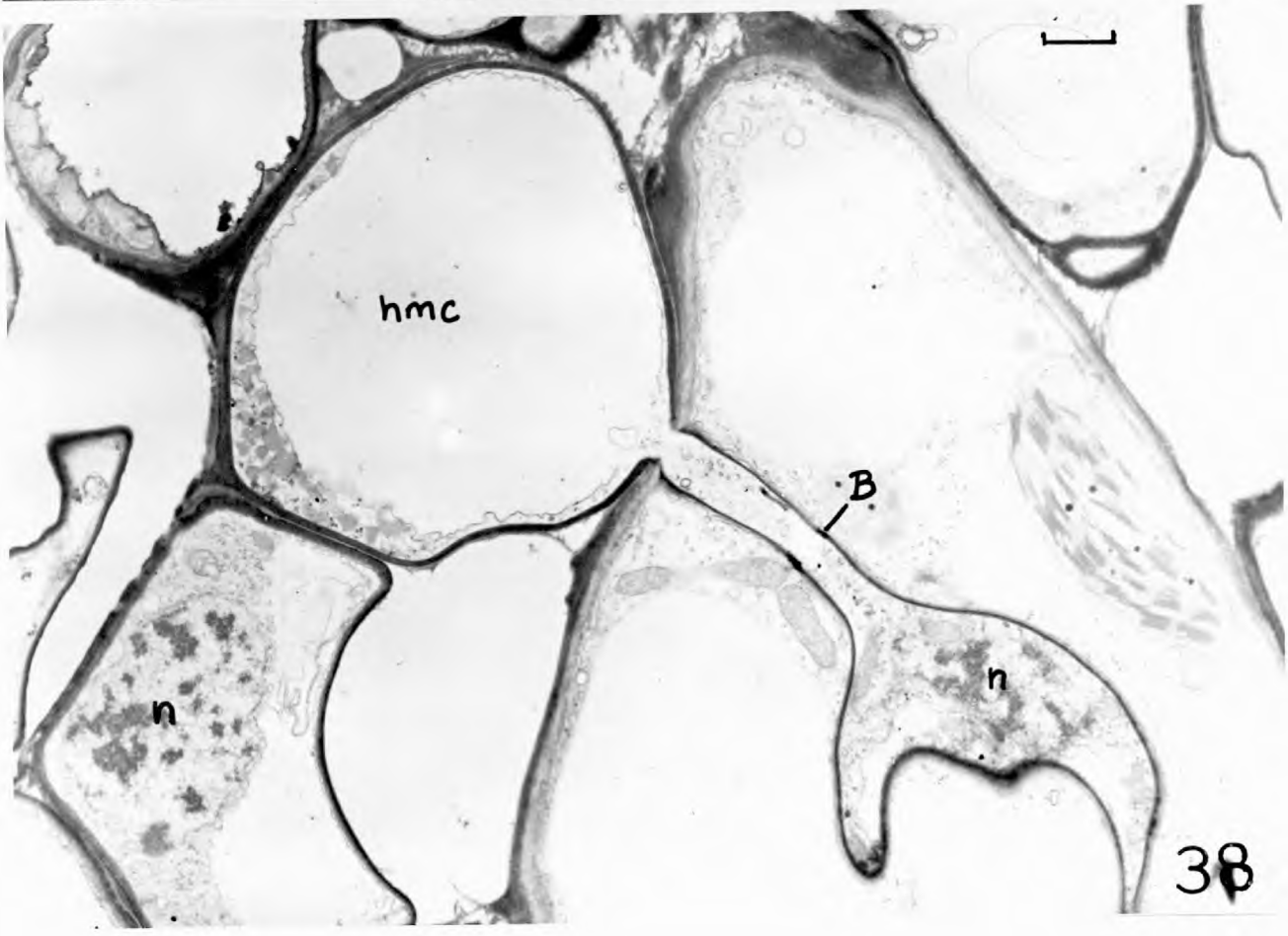


36

37. Haustorium within a palisade cell showing the neckband (B). The peculiar shape of the h.m.c. is caused by an invagination of one side of the cell to give the dimpled appearance. 1382B/30. 6710.

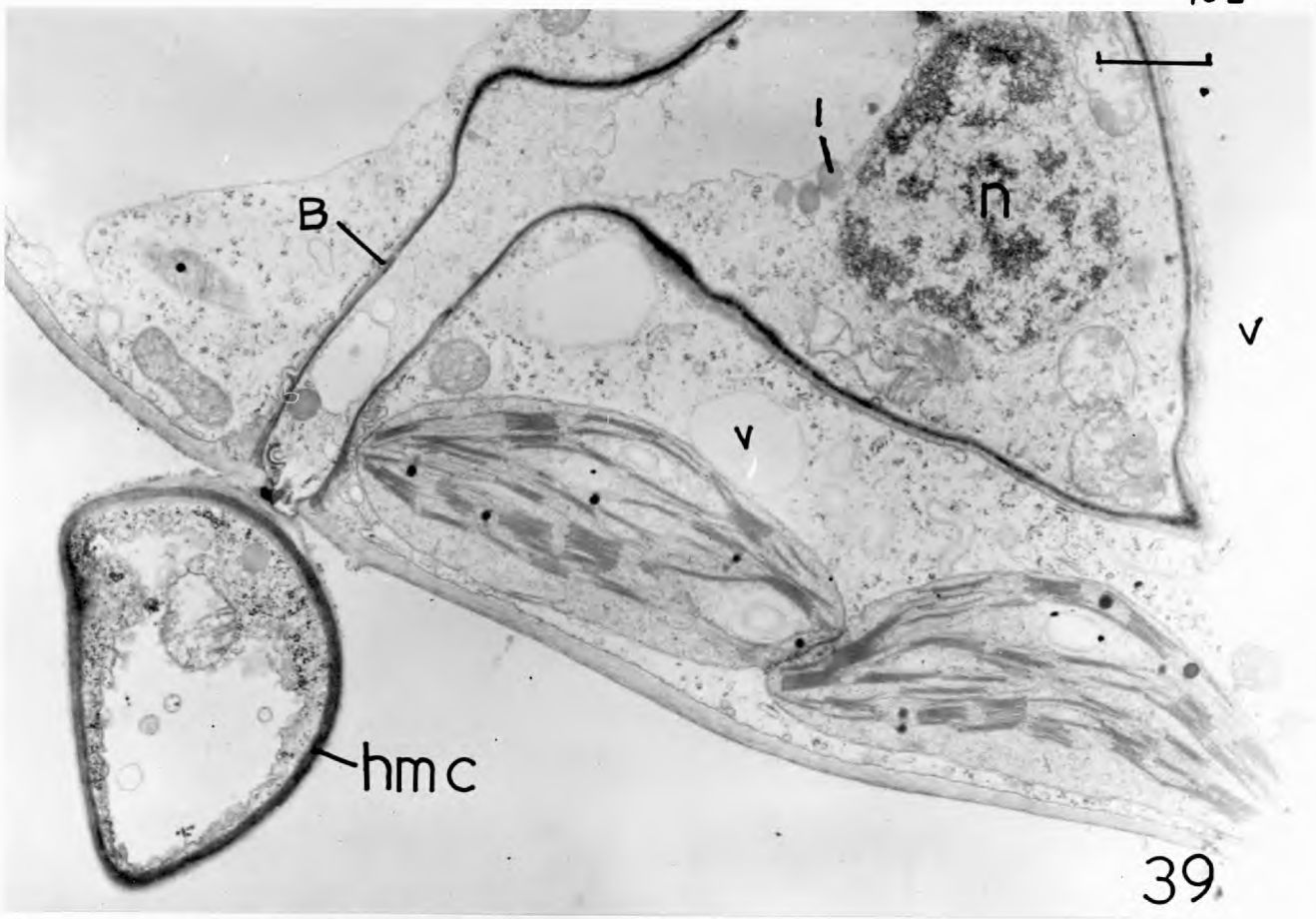
38. Haustorium showing neckband (B) and a nucleus (n). Nucleus also visible in the intercellular hypha. 1382B/21. 5843.


B
hmc
37

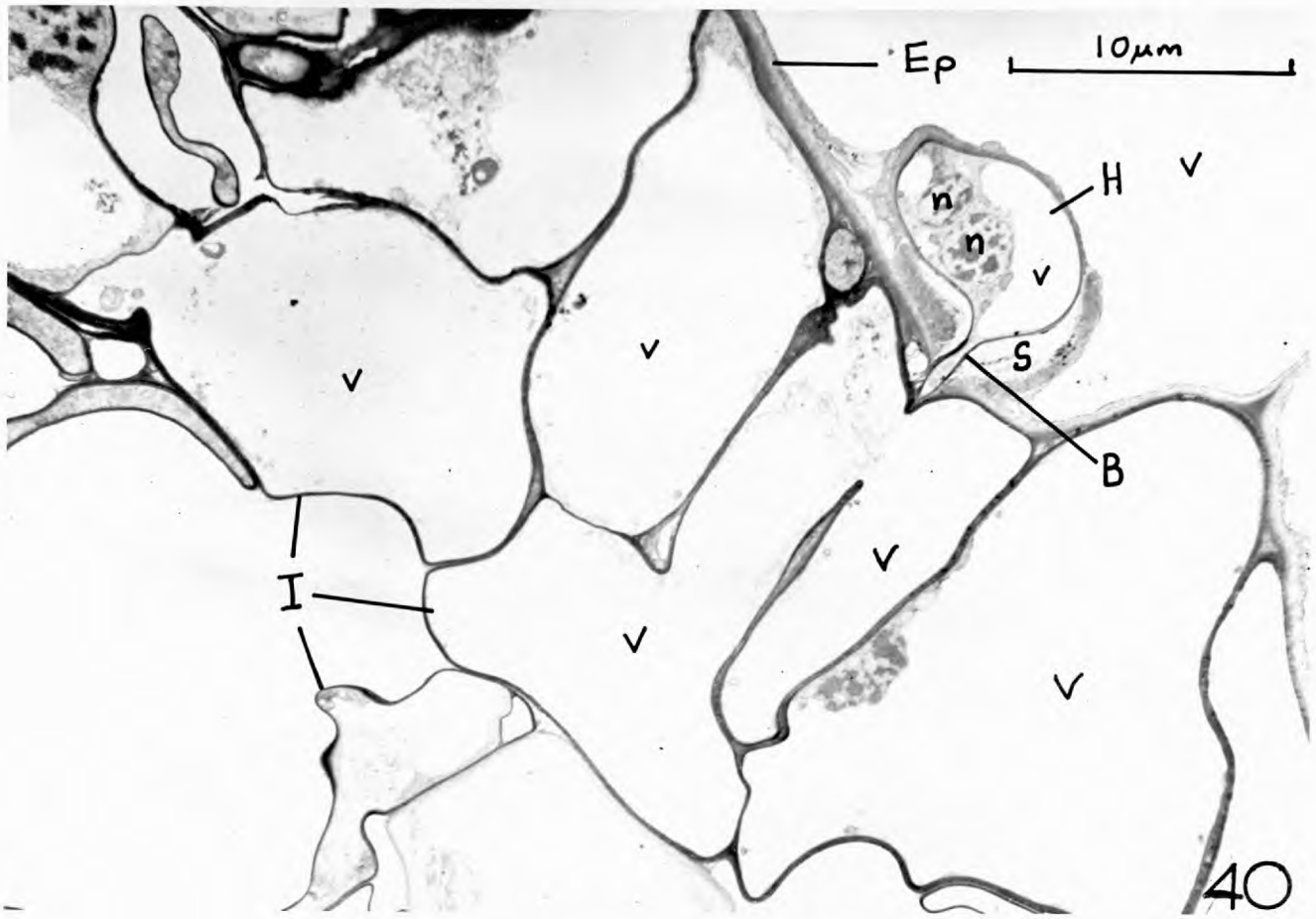

hmc
B
n
n
38

39. Section of a haustorium cut slightly off median to the penetration pore. Shows neckband (B), nucleus (n) and lipid droplets (l). 1382B/30. 5867.

40. Section of a haustorium within an epidermal cell (Ep), also showing a neckband (B). A large sheath (s) is present. The haustorium contains two nuclei (n). The intercellular hyphae are highly vacuolated (v). 1382B/12. 5832.



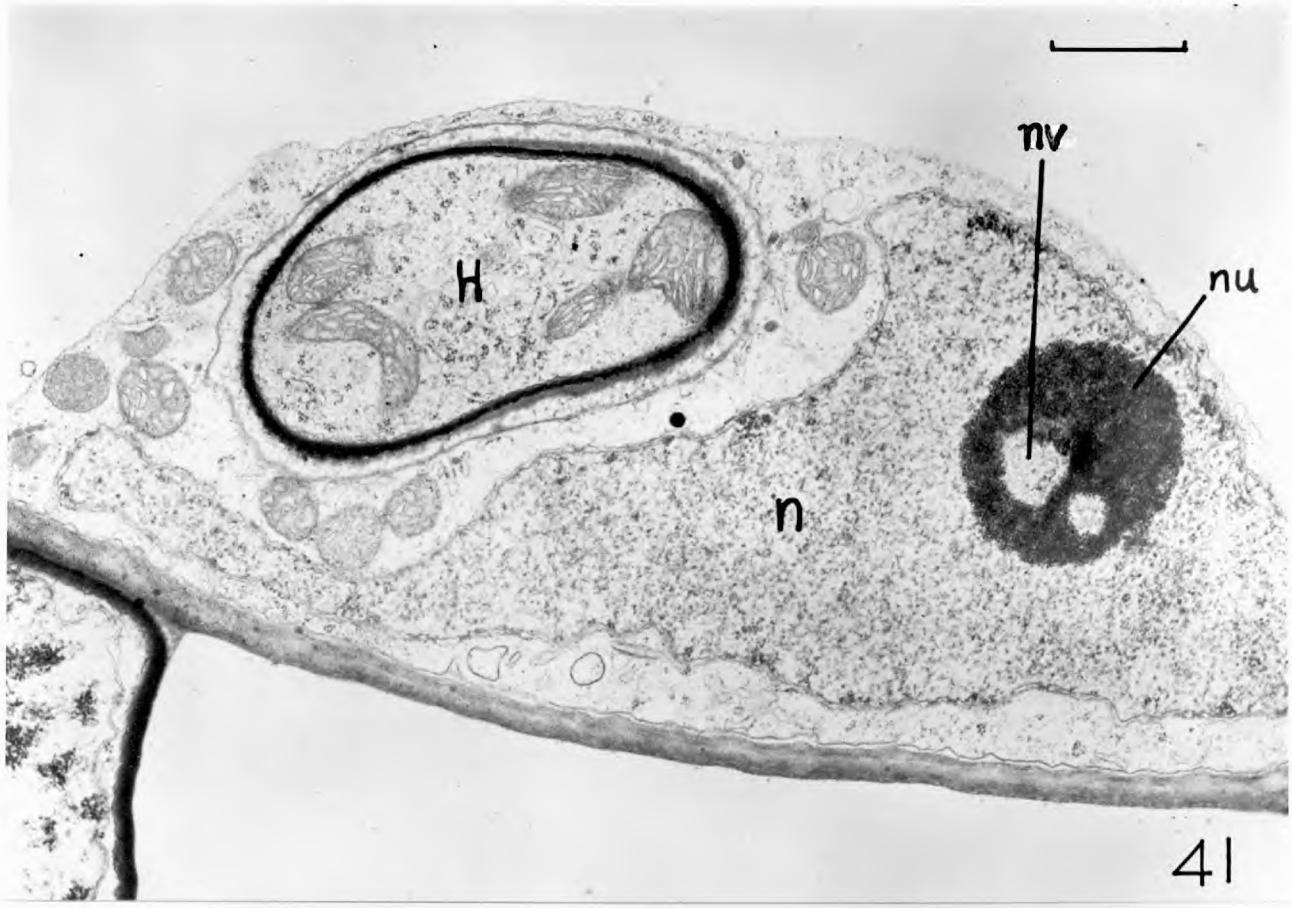
39



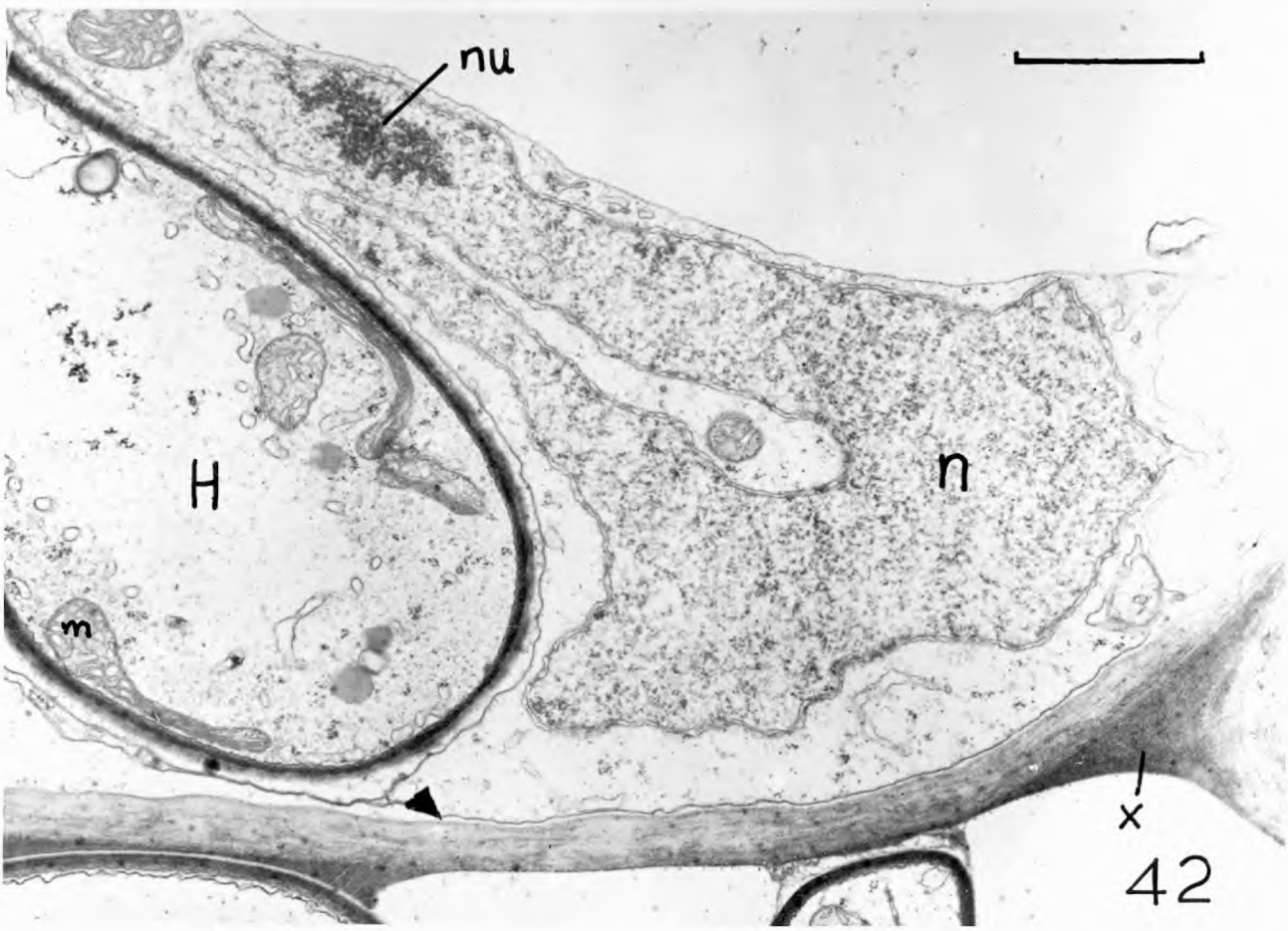
40

41. Host nucleus (n) distorted against a haustorium (H). Note the absence of aggregations of chromatin-like material. Nucleolus (nu) contains two nucleolar vacuoles (nv) of the same density as the matrix of the nucleus. 1381A/28. 5796.

42. An invaginated host nucleus (n) also distorted against a haustorium (H). Nucleolus is more dispersed than in Fig. 41. Disruption of the tonoplast has occurred in the haustorium with a consequent dispersion of the contents. Note the elongated mitochondria of the haustorium. 1382A/28. 6034.



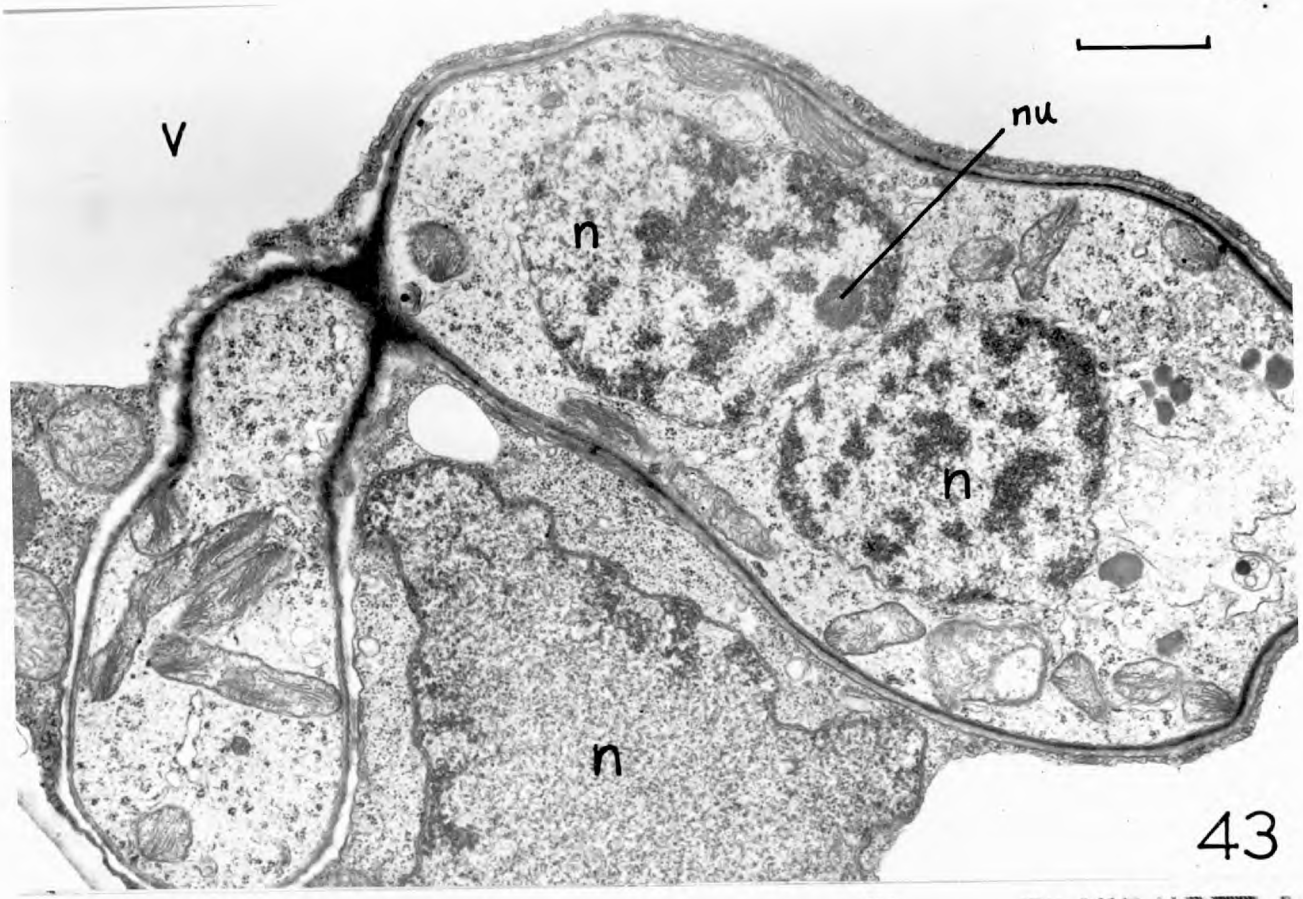
41



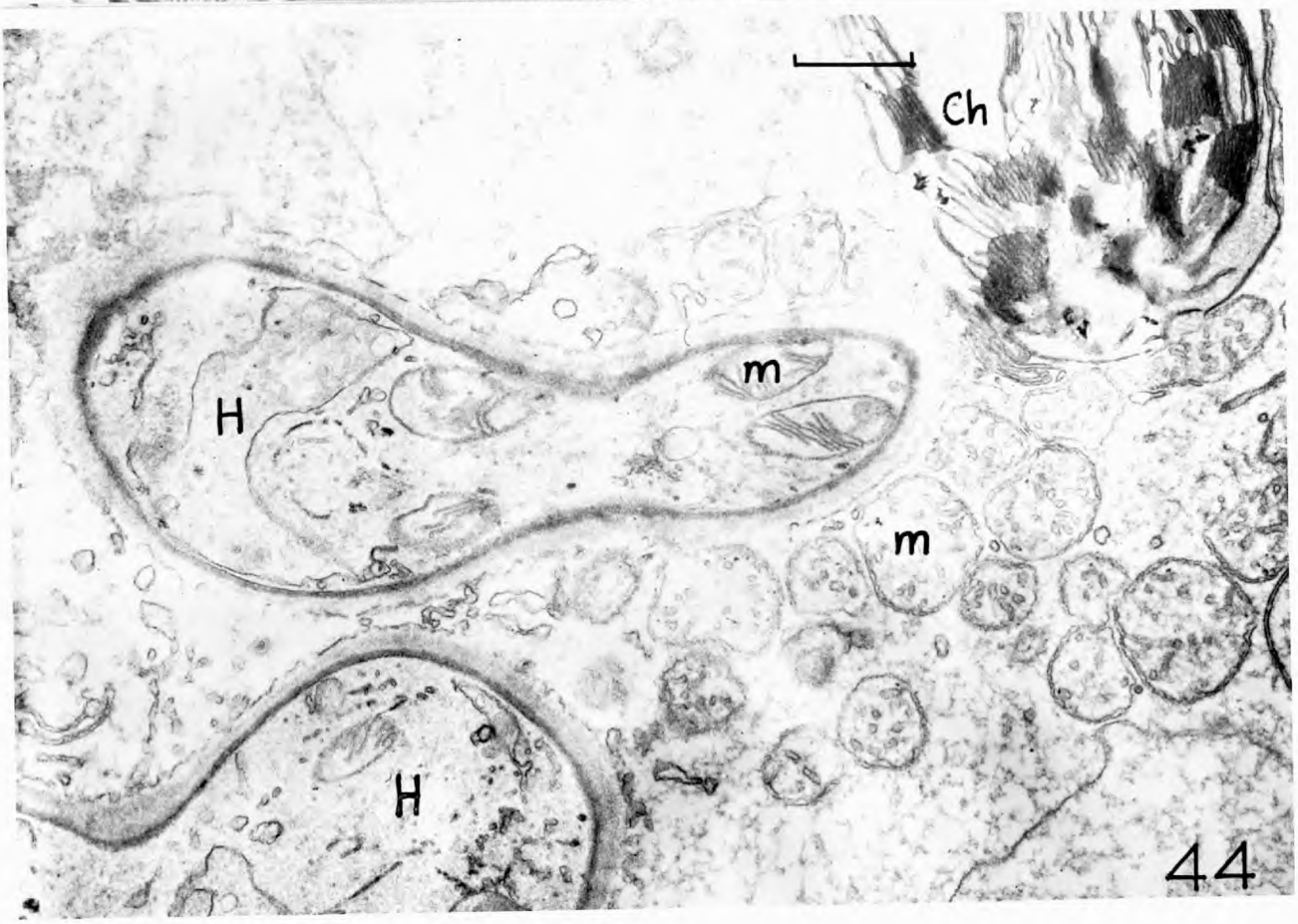
42

43. Section through the body region of a haustorium showing two nuclei (n) and nucleolus (nu). Compare the dense reticulum of chromatin in the haustorial nuclei with the absence of similar material in the nucleus of the host. 1382B/85. 8200.

44. Section through the body region of haustorium (H) surrounded by numerous host mitochondria (m). $KMnO_4$. 40KV. 1529/16. 6609.

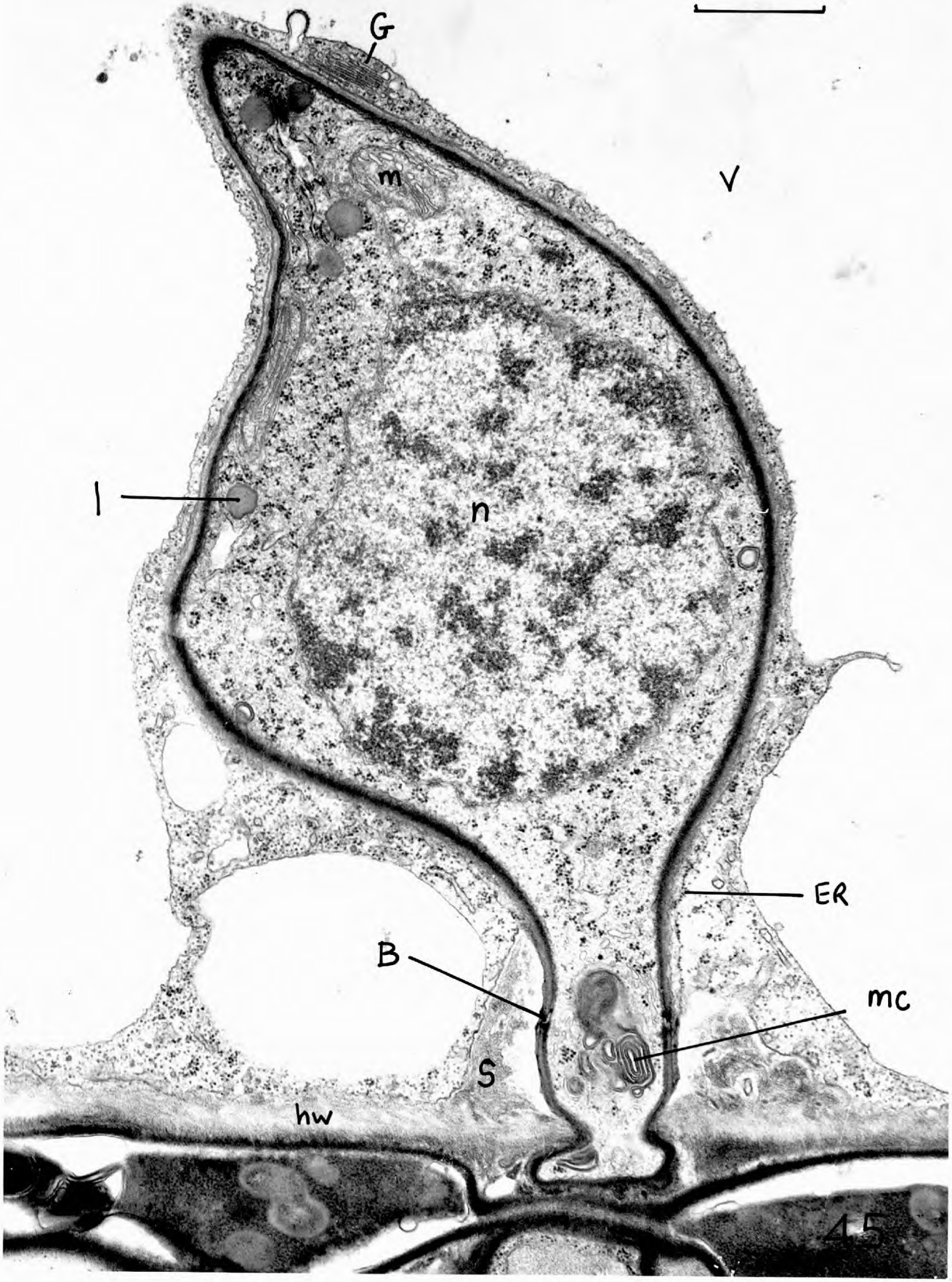


43



44

45. Section of a haustorium serial to Fig. 35, with a short stem containing conspicuous internal membranes (mc), a nucleus (n) several times the diameter of the penetration pore in the expanded part of the body. Note the heterogeneous materials of the sheath (s) and the coating of E.R. extending over the stem distal to the neckband (B).
1382B/65 S2. 6494.


G
m
V
l
n
ER
B
mc
S
hw
45

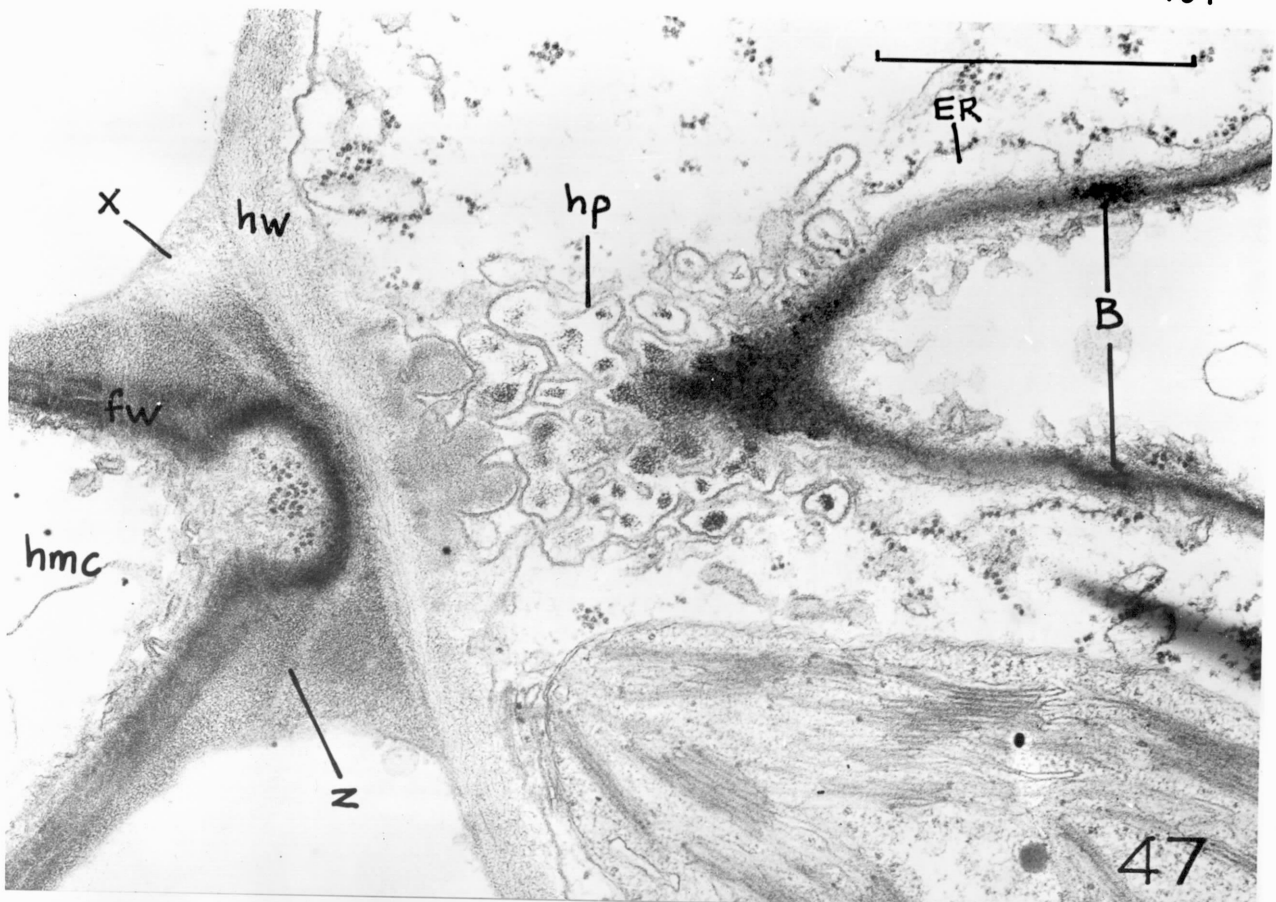
46. Section of a haustorium serial to Fig. 35 showing numerous lipid droplets (l), a nucleus (n) with nucleolus (nu). Note the sheathing material (s) extending over the length of the stem and including the neckband (B). 1382B/67 S1. 6514.

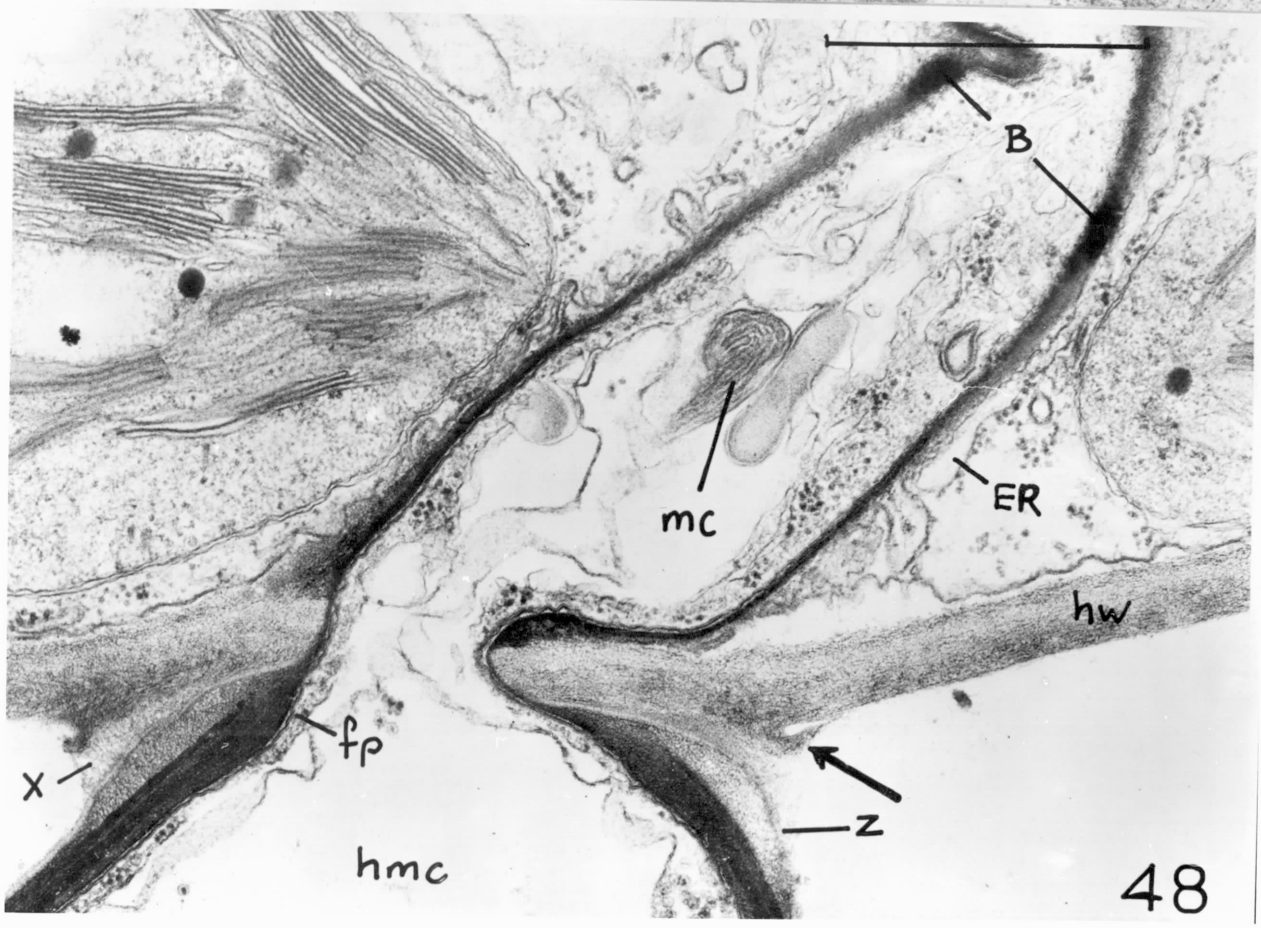


46

47. Glancing section over the neck region of the haustorium in Fig. 36, showing intercellular material (x) in the angle of contact between the host cell wall (hw) and the h.m.c. Note the pale zone (z) around the h.m.c. The host plasmalemma (hp) is thrown into folds loosely applied to the material of the neck wall which projects into the host cell. E.R., rough on the cytoplasmic side, covers the stem over the neckband (B). 1382B/6. 6360.

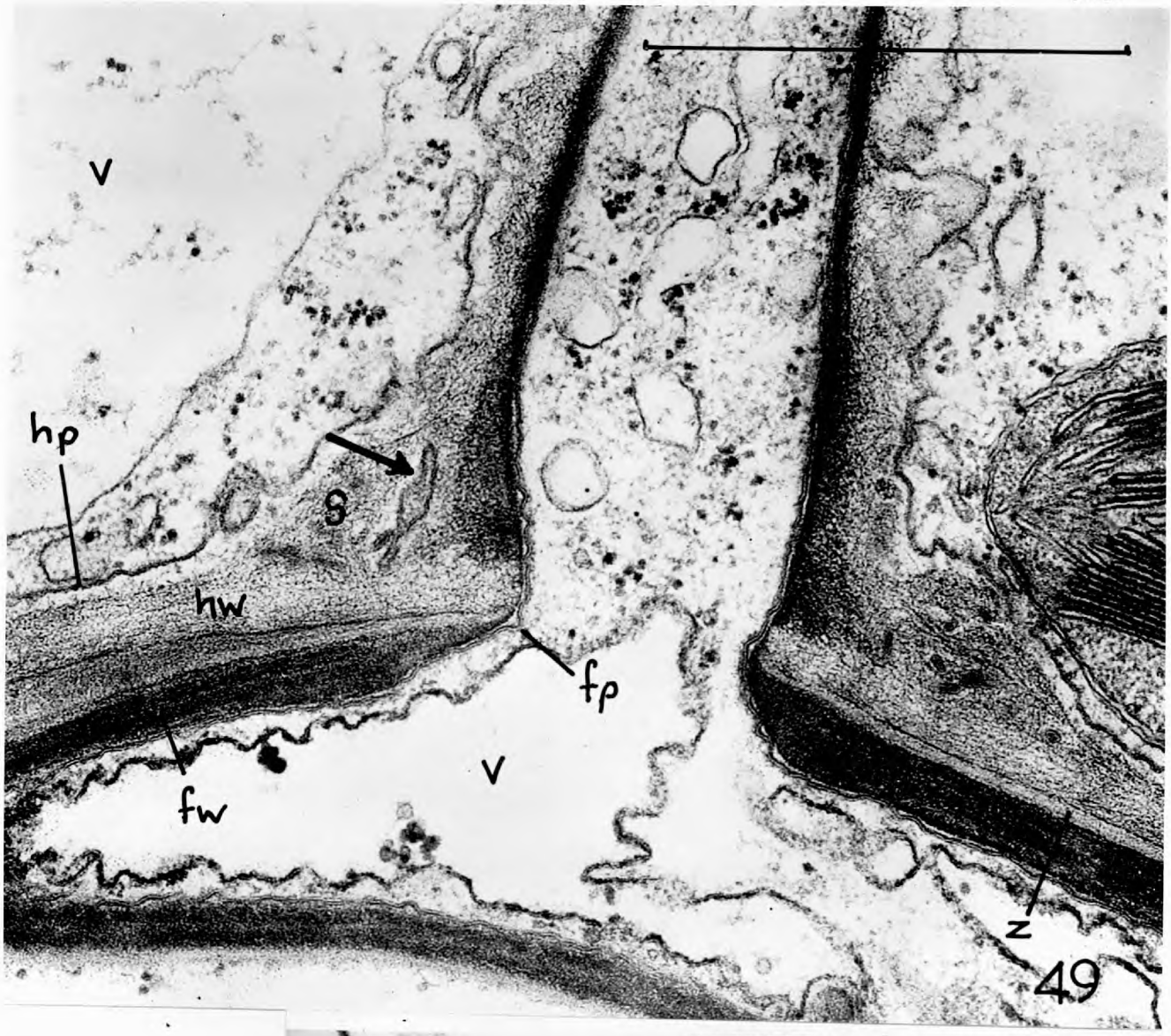
48. Section median to the base and neck of a haustorium. Note the small area of contact of the h.m.c. with the host wall, the swelling of the outer layer surrounded by the pale zone (z) revealing sub-layers within the remaining electron dense h.m.c. wall which terminate abruptly a short distance from the penetration pore. Note also the tapering of the h.m.c. wall to the penetration pore and 'scuffing' (arrow) of the host wall. 1382B/20. 6364.

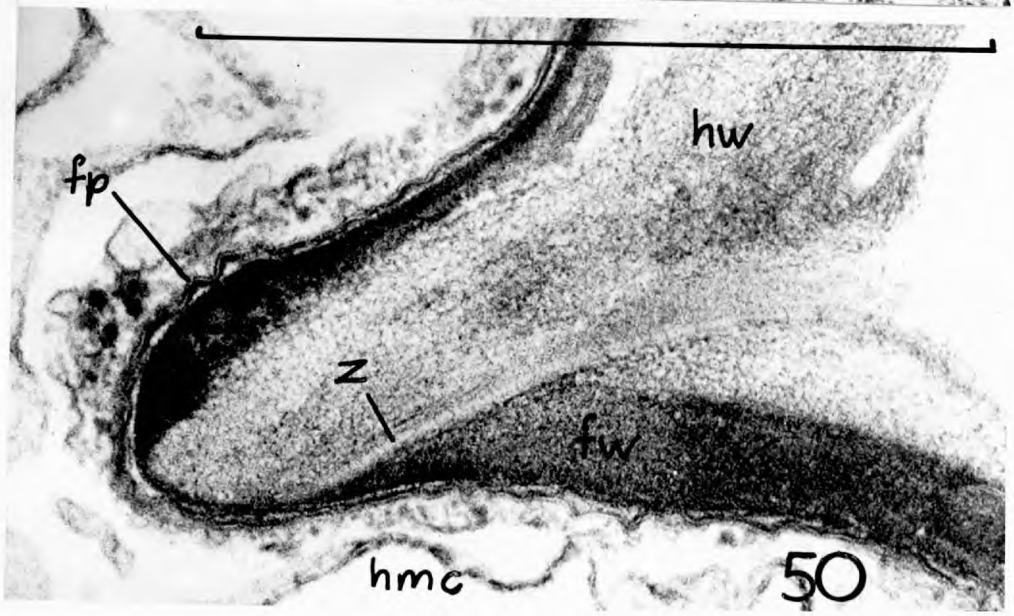

ER
X
hw
hp
B
fw
hmc
z
47


B
mc
ER
hw
fp
X
z
hmc
48

49. Basal part of a haustorium serial to Fig. 37 with little or no swelling of the h.m.c. wall. Shows naked fungal plasmalemma (fp) in the penetration pore, intermingled material in the base of the sheath and sequestered membranes (arrow), the tapering edge of the h.m.c. wall and the pale zone (z) of the latter separated from the host wall (hw) by a dark line. 1382B/30. 5869.

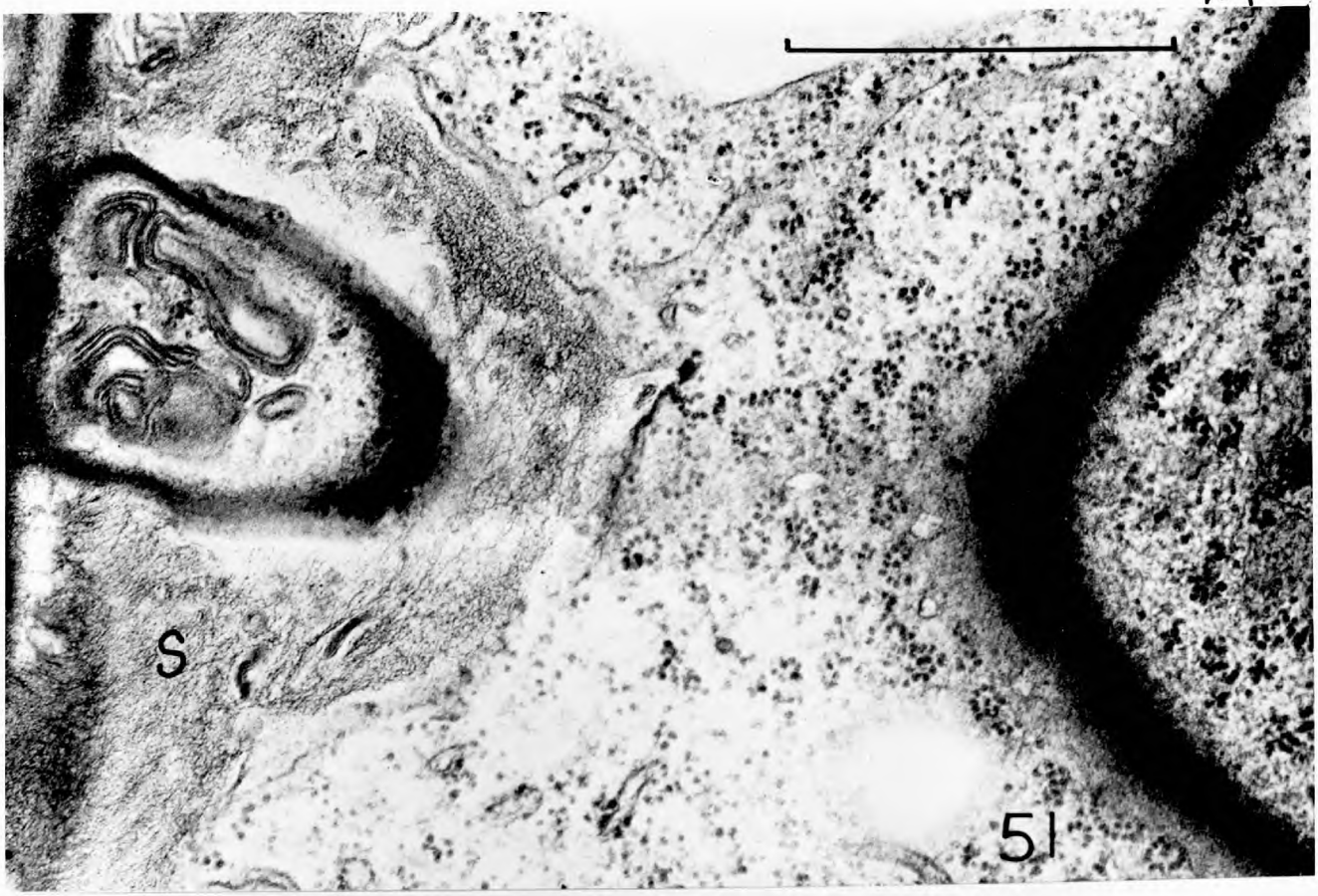
50. Detail of a corner of the penetration pore in Fig. 48 showing the continuity of the fungal plasmalemma (fp) from the h.mc. through the penetration pore into the stem of the haustorium, the superficial staining of the host wall in the penetration pore, mingling of material on the inside of the host wall (hw) and the termination of the pale zone (z) and h.m.c. (fw) at the penetration pore. 1382B/20. 6365.

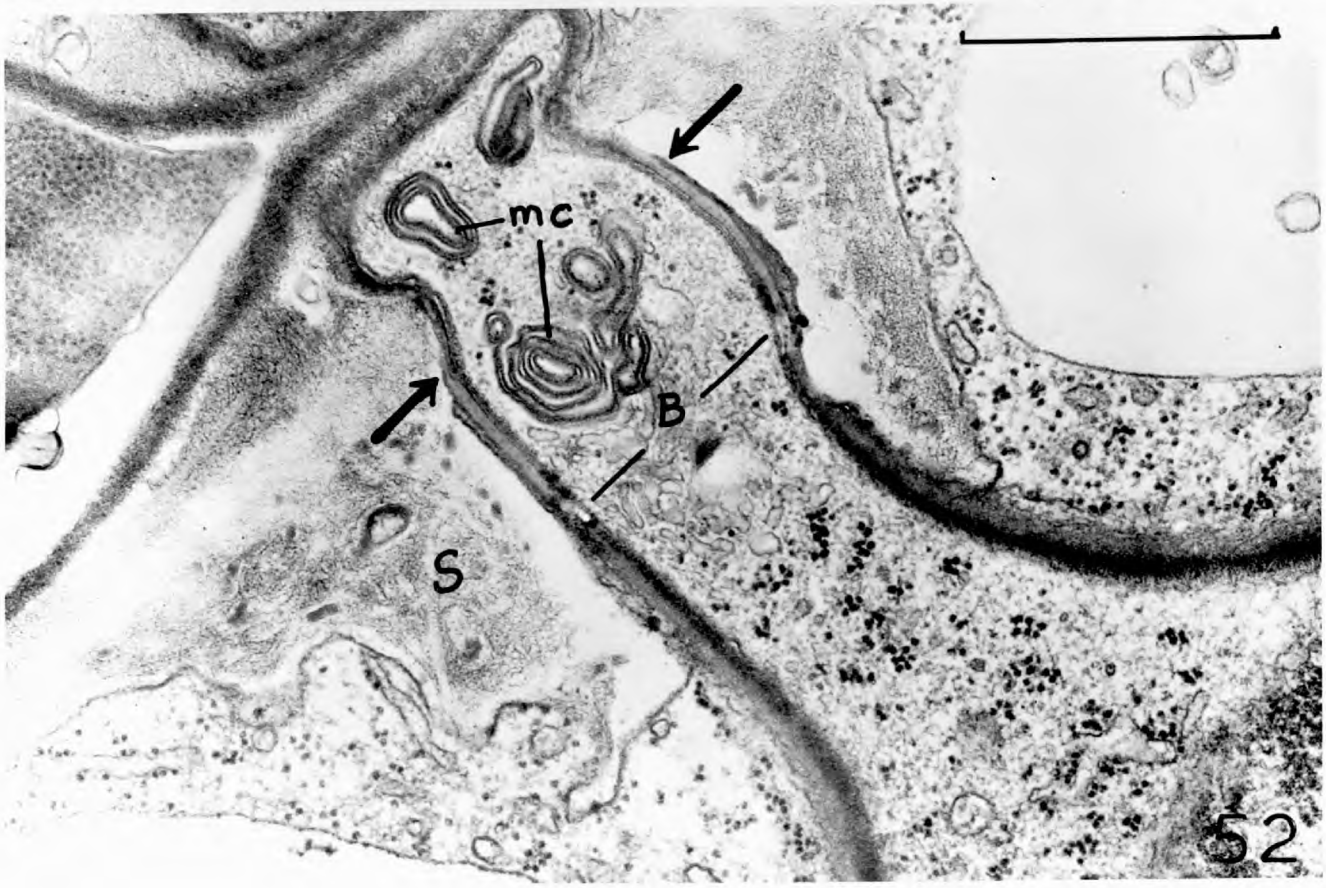

V
hp
S
hw
fp
V
fw
z
49


hw
fp
z
fw
hmc
50

51. Glancing section serial to Fig.45 over the stem region passing through the coating of E.R. showing the polysomal spirals distal to the sheath (s) and the dark underlying stem wall. 1382B/65 S6. 6503.

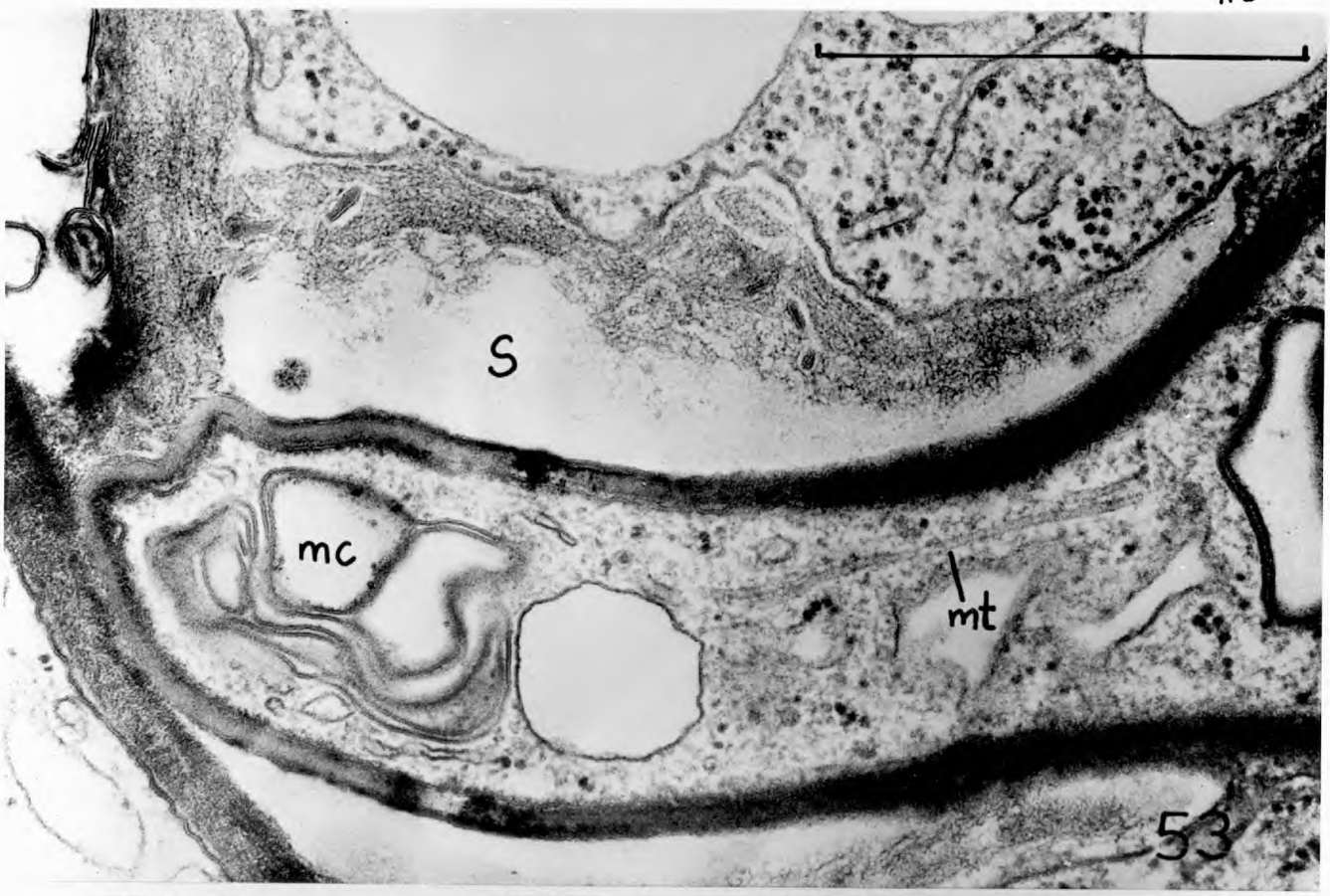
52. Section serial to Fig. 51 showing re-entrant plasmalemma (arrows) within the sheath to the beginning of the neckband (B). The distal end of the neckband is marked by a region of less electron density also containing clear patches. The sheath (s) contains an electron-lucent region devoid of a fibrillar element adjacent to the stem of the haustorium. 1382B/65 S4. 6499.

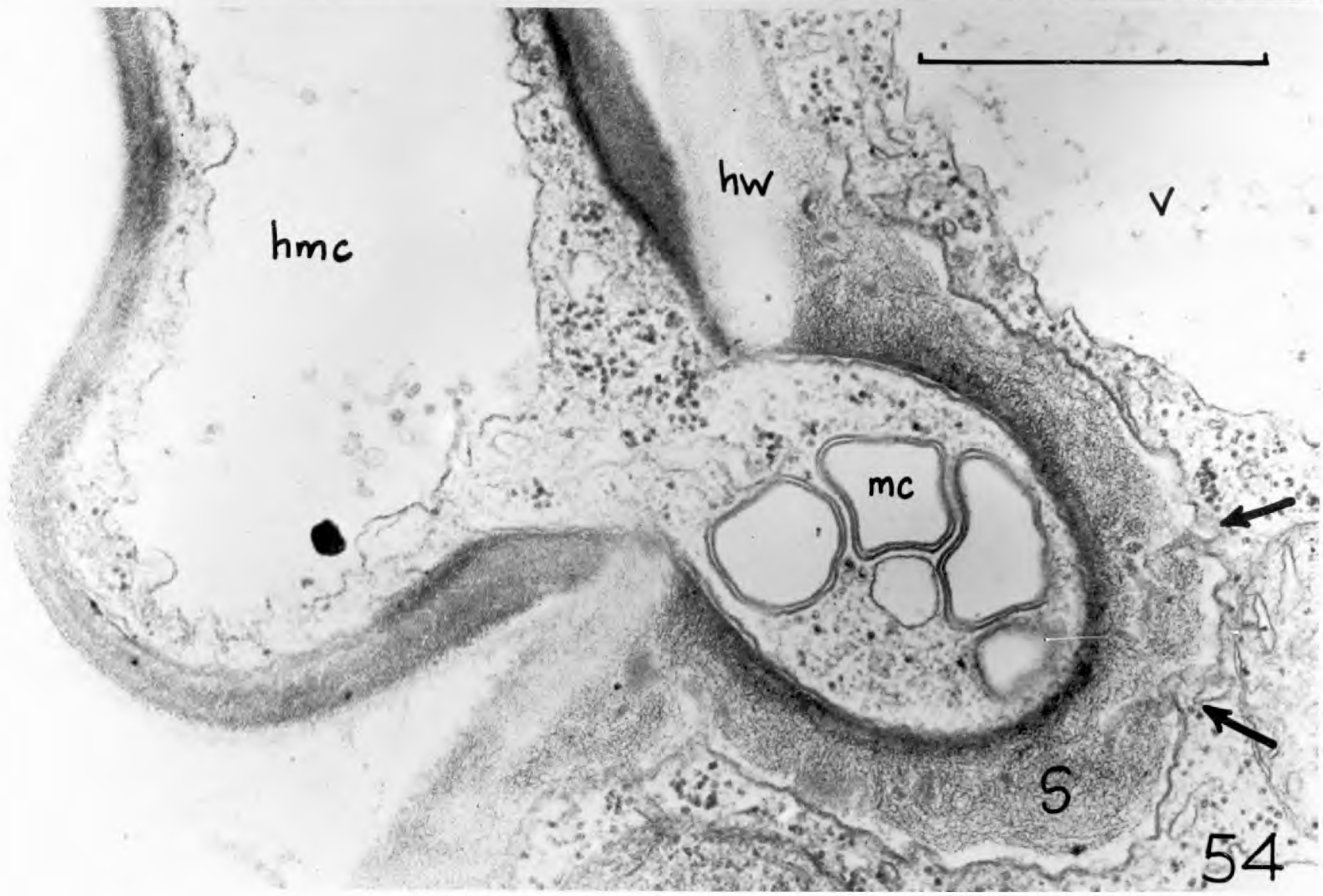

S
51


mc
B
S
52

53. The stem region of a haustorium serial to Fig. 46 showing a sheath (s) comprised of two layers, an inner electron-lucent region with no fibrillar element and an outer layer composed of fibrillar material with occluded membrane. Note material terminating the neckband (r) is more heavily stained than the adjacent areas of the stem wall. Microtubule (mt) present within the stem. 1382B/65. 6747.

54. Section of a short haustorium lacking a stem and neck-band and apparently enclosed by sheath (s). Note the evaginations of the plasmalemma (arrows) into the sheath. 1382B/65 S1. 6490.

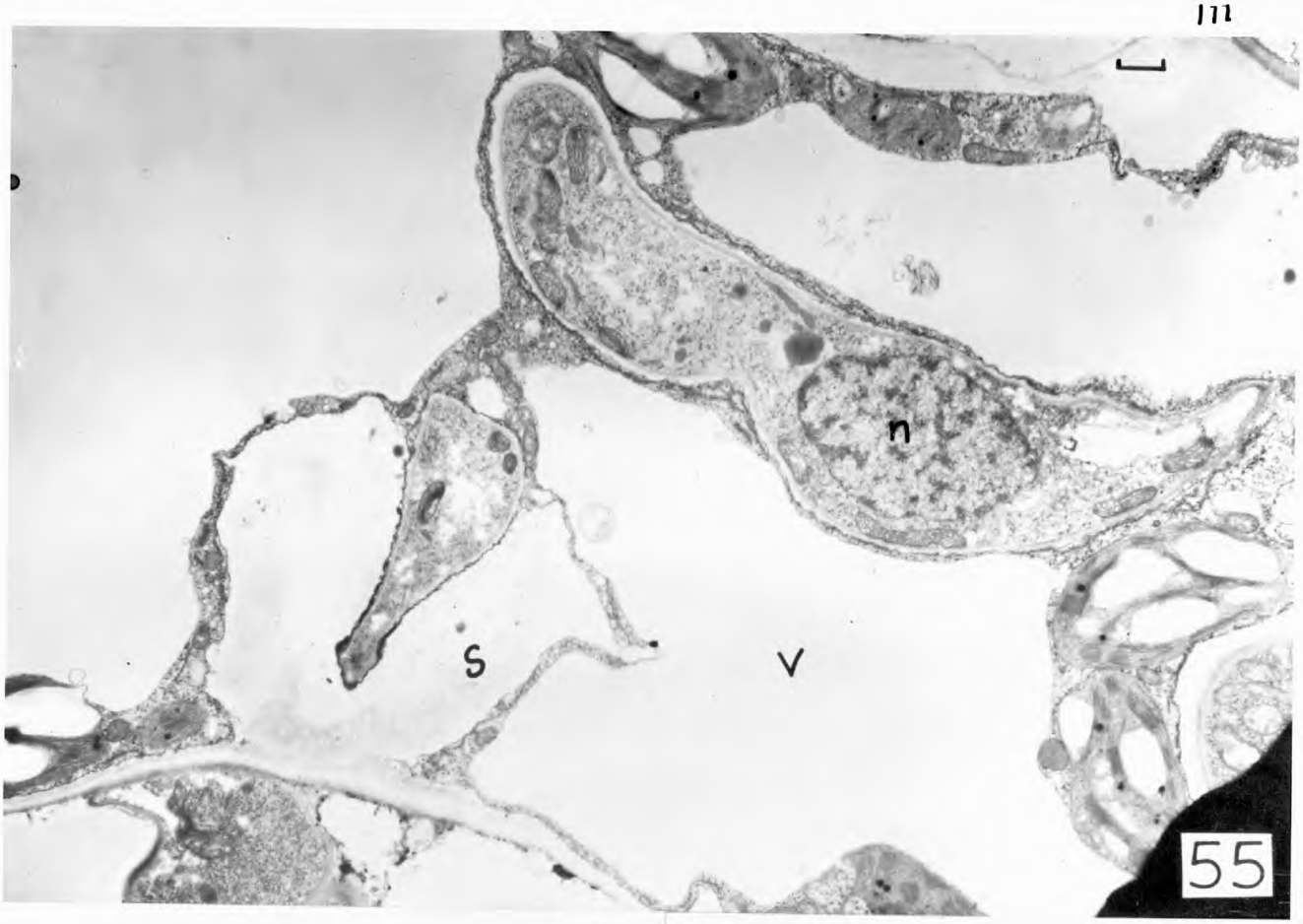

S
mc
mt
53


hmc
hw
V
mc
S
54

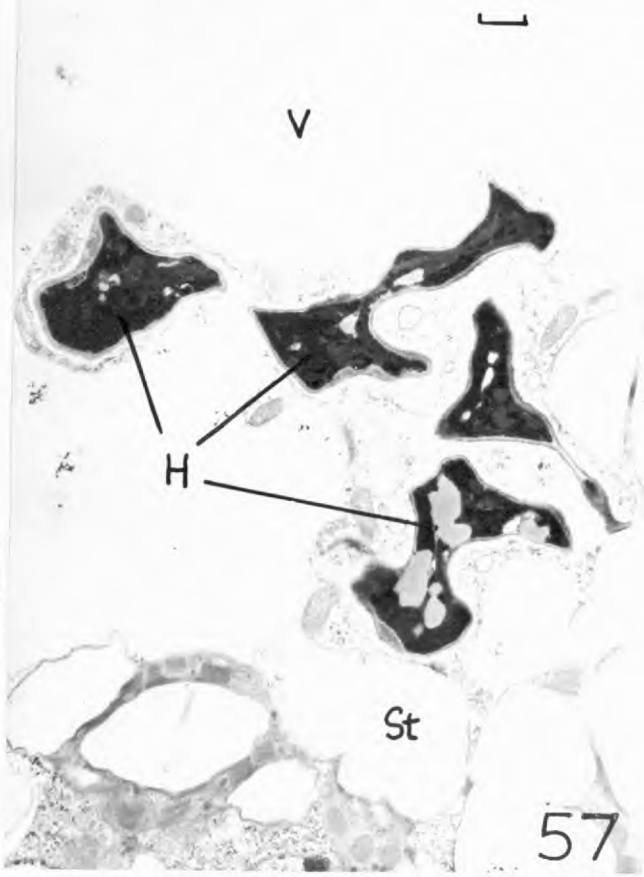
55. Section through part of the stem and body of a haustorium with a large deposit of sheathing material (s) over the stem region. Sheath consists of two regions, the inner elctron-lucent and the outer fibrillar. 1540B/13. 8317.

56. Section through a haustorium with dense contents appearing necrotic with a large sheath (s) around the stem. Some breakage of the h.m.c. wall has occurred. 1540B/12. 8313.

57. Section through the body region of necrotic looking haustoria in an apparently healthy cell. Chloroplasts contain large grains of starch. 1540B/2. 8295.


n
S
V
55


V
S
56

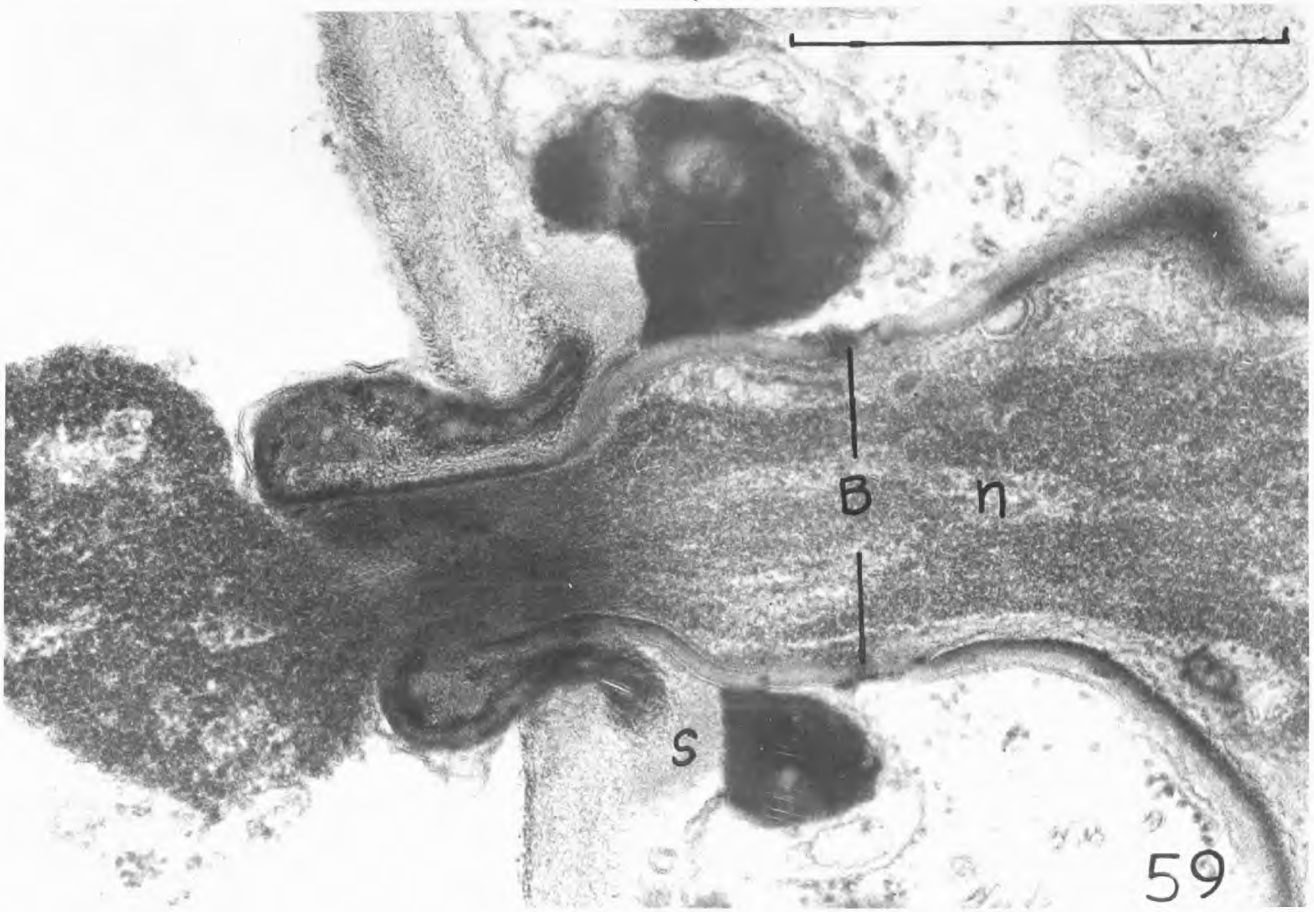

V
H
St
57

58. Section median to the penetration pore of a haustorium with its h.m.c. missing. The wall of the body is convoluted. 1382B/25. 14227.

59. Detail of Fig. 58 showing the eversion of the neck region, including the sheath (s) and the nucleus partly extending into the intercellular space. Electron dense material in contact with the sheath and neck wall appears to be lying outside the host plasmalemma. 1382B/25, 14229.

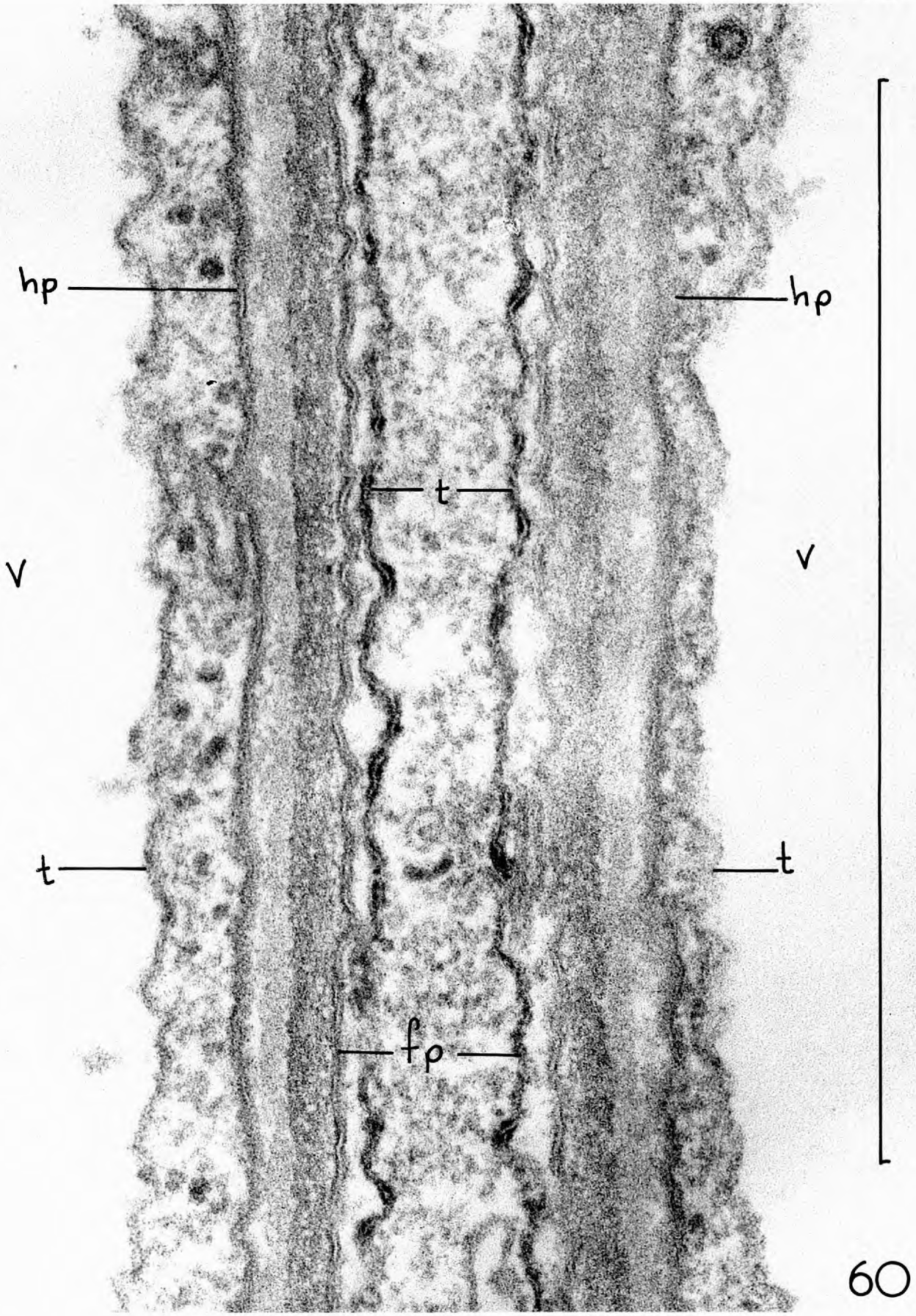


58



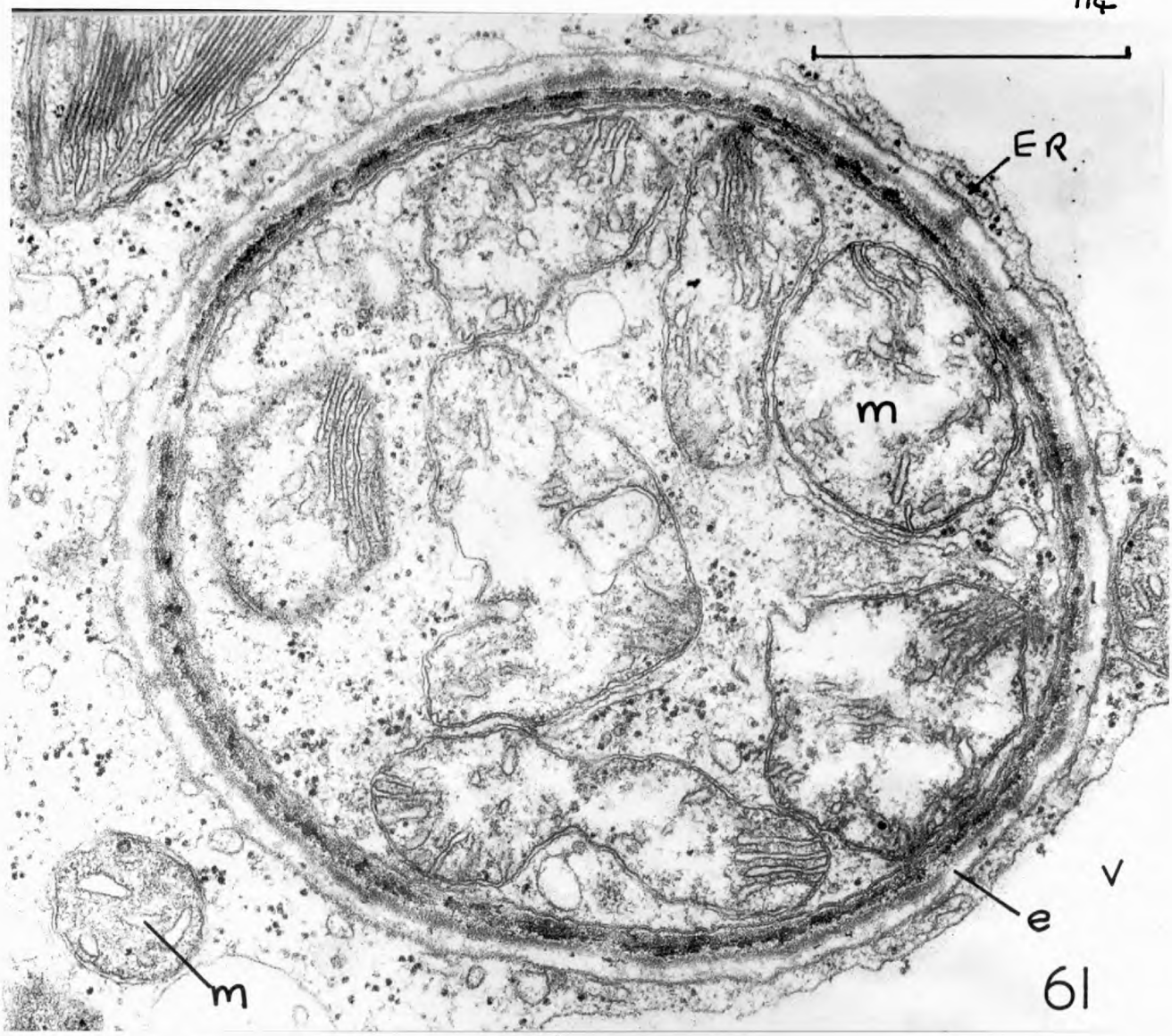
59

60. Detail of the body wall of the haustorium in Fig. 46 showing the two layers and their relationship to the host (hp) and fungal (fp) plasmalemmas. The fungal tonoplast (t) is heavily stained and the vacuole contains granular material. 1382B/67 S4. 6520.

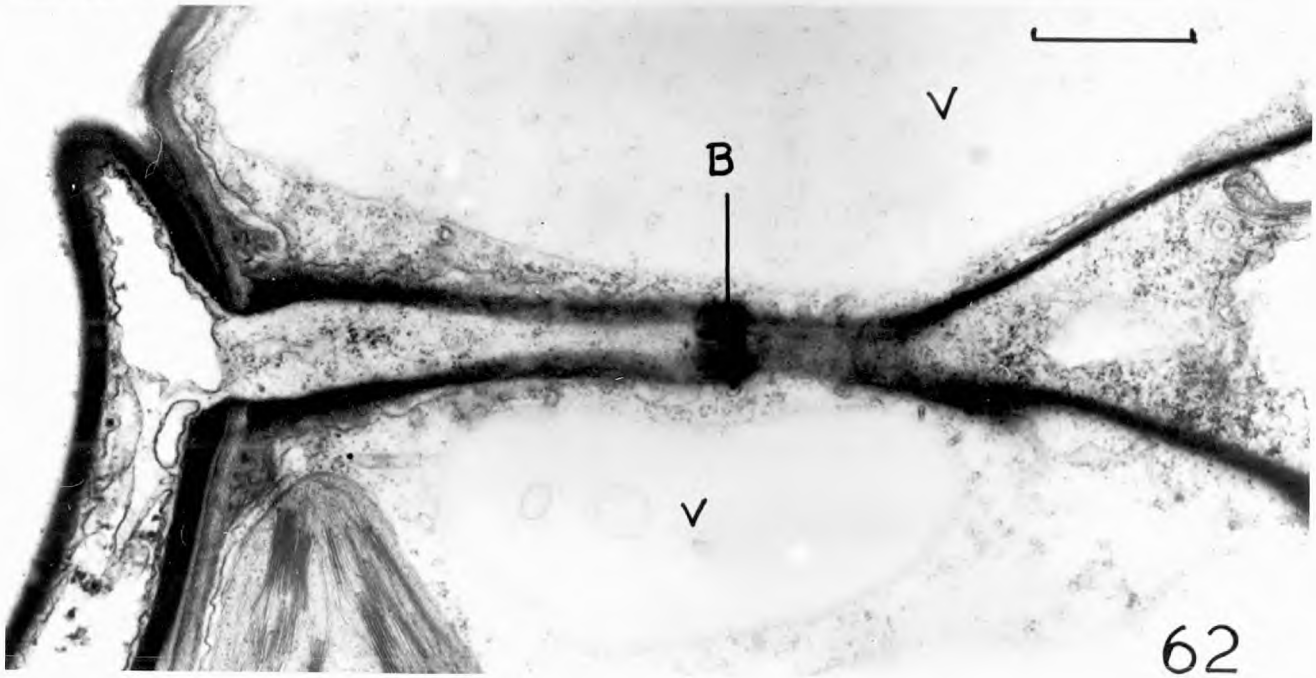

hp
hp
t
V
V
t
t
fp
60

61. Section across a haustorium showing the wall structure in a distal part. Its two layers appear somewhat eroded. The host plasmalemma is not closely applied to the body wall and bridges of material from the pale layer extend across the gap, giving the appearance of an 'encapsulation' (e). Note the numerous mitochondria and their structure in comparison with those of the host. 1382B/34. 6271.

62. Section serial to Fig. 37, illustrating the prominence of the neckband when cut at grazing incidence. 1382B/30. 6736.



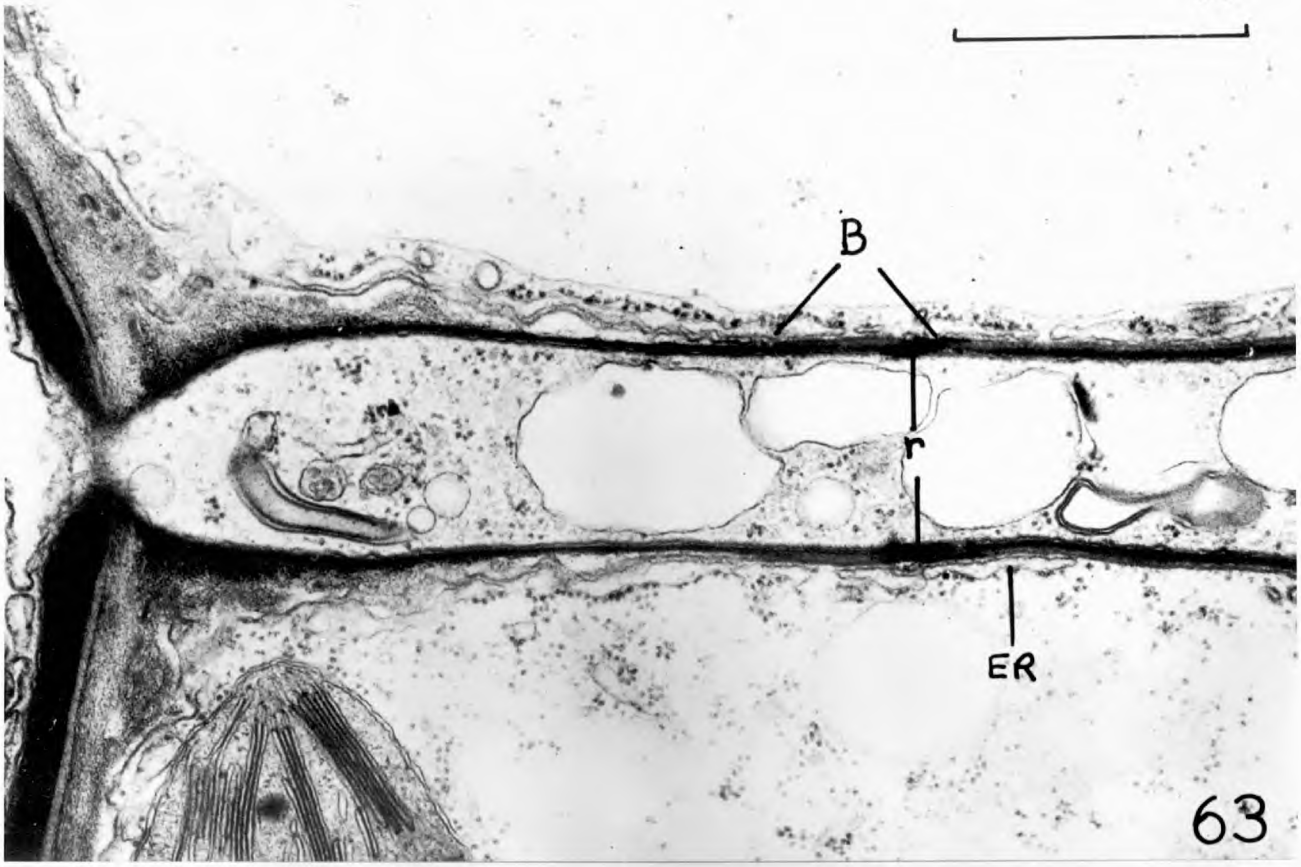
61

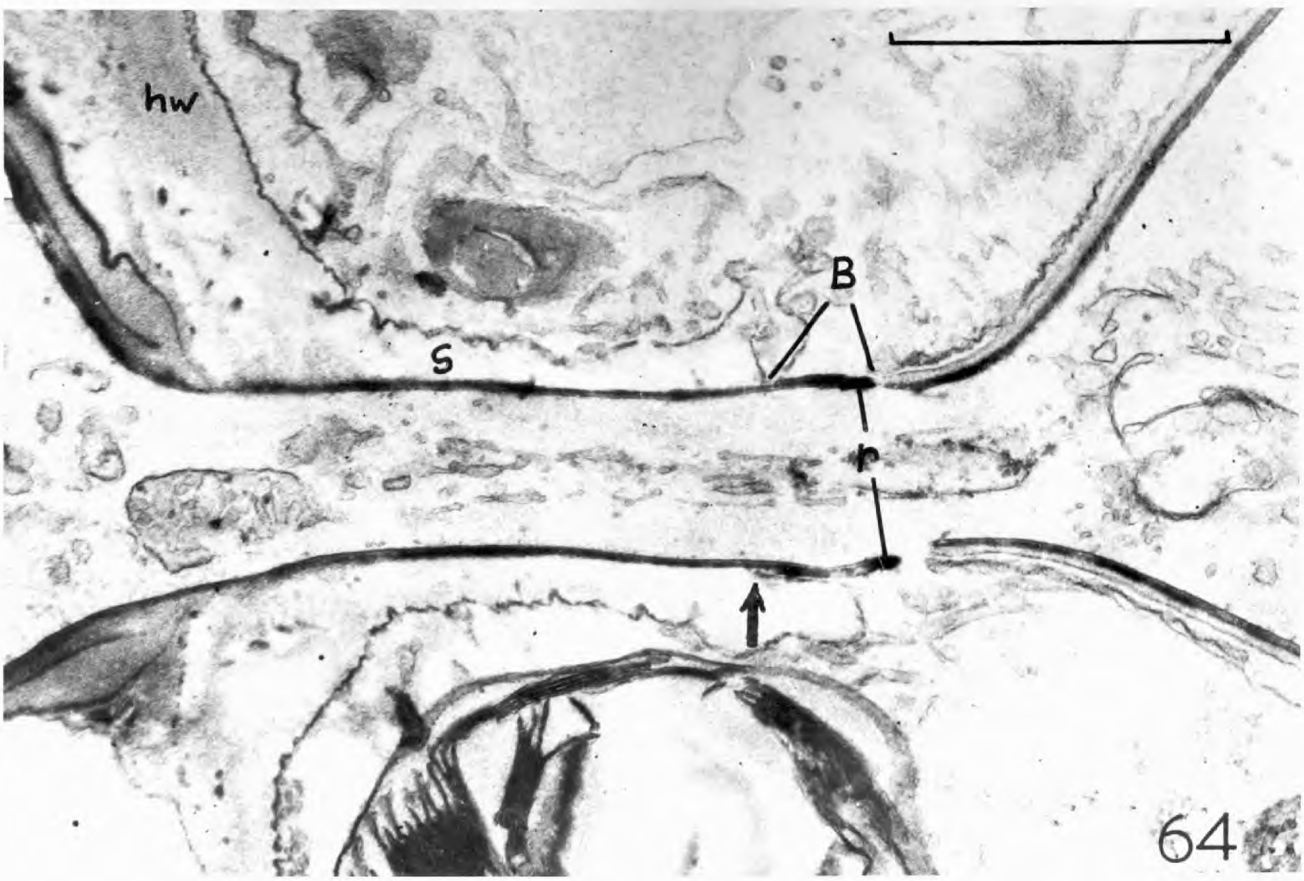


62

63. Section serial to Fig. 37 showing details of the neck-band. An expanded region of the neck wall terminated by an irregular deposit of staining material, the ring (r). Note cisternae of the E.R. applied over the sheath and stem. 1382B/30. 6711.

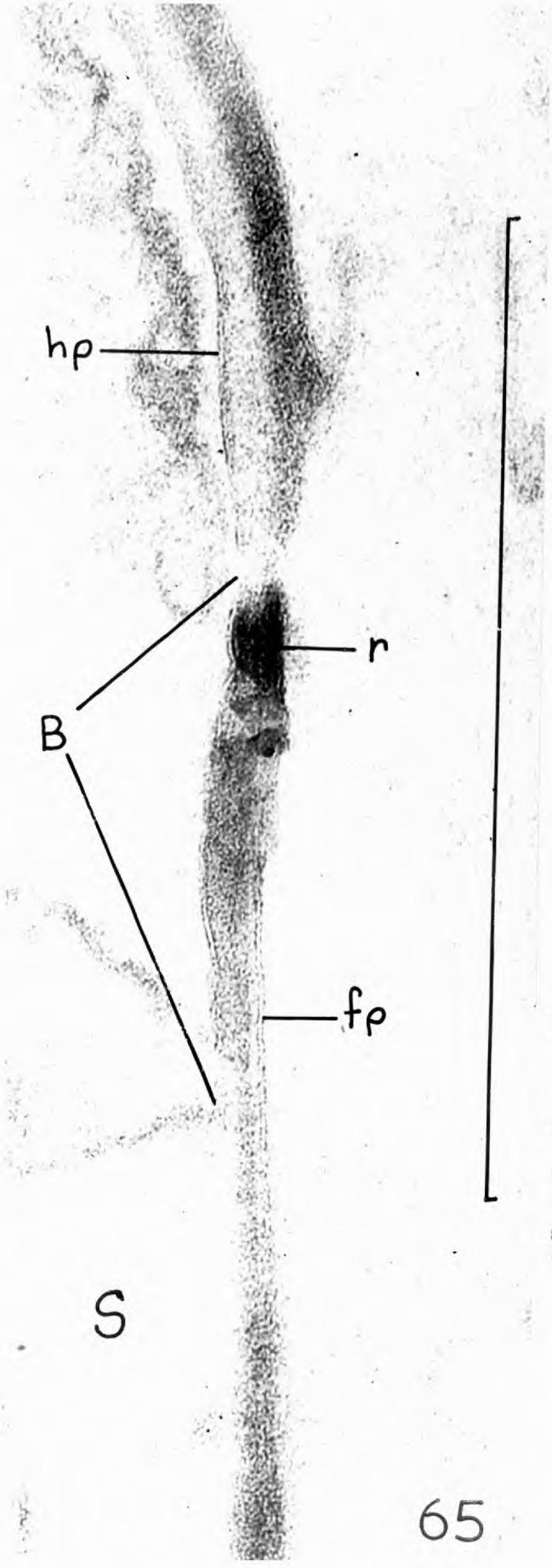
64. Haustorium with a cylindrical sheath & re-entrant plasmalemma to the neckband (arrow). Note the similarity between the material of the sheath (s) and the host wall (hw). The h.m.c. wall is swollen and the inner layer continues to the penetration pore where it becomes more diffuse before continuing in intimate association with the outer layer of the sheath. Beyond the ring (r) there is a break in the stem wall, after which the wall continues as a two layered structure and eventually expands to enclose the body. $KMnO_4$. 40KV. 1529/11. 6602.


B
r
ER
63

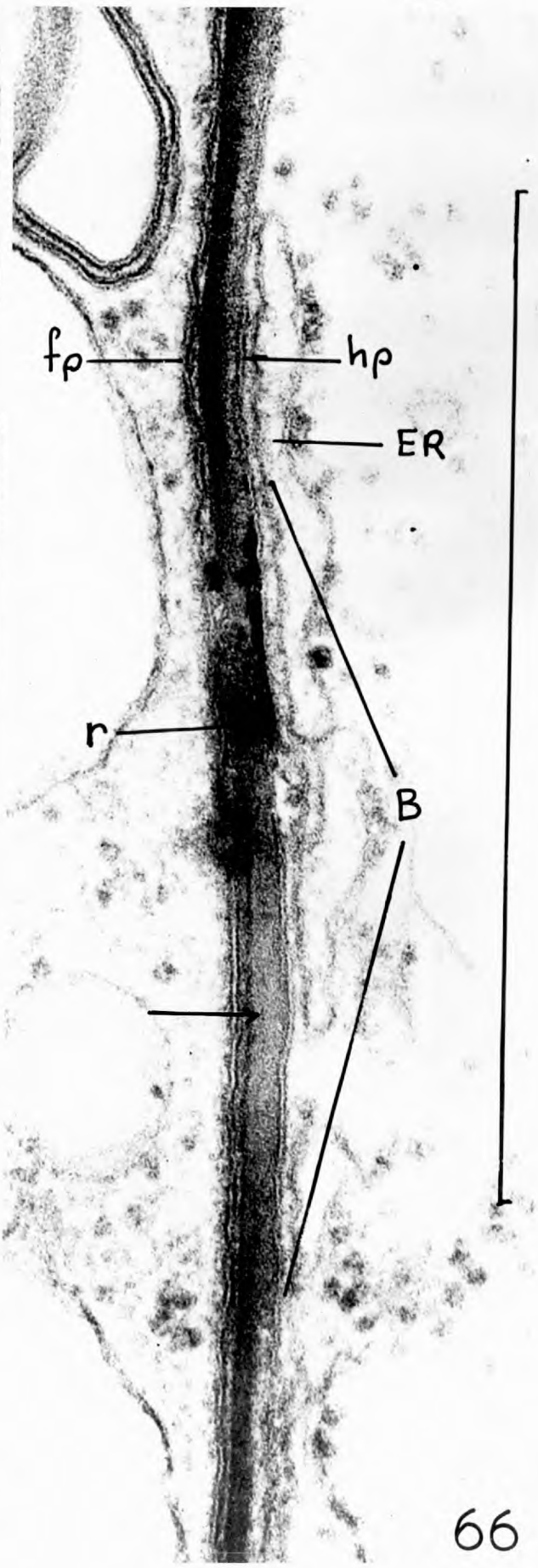

hw
B
S
r
64

65. Detail of Fig. 64 showing the beginning of the neckband, starting where the host plasmalemma (hp) contacts the electron dense region. The neck wall at this point is thicker than the electron dense lining to the sheath (s). The termination of the neckband is marked by a narrow region of increased density, the ring (r). $KMnO_4$. 1529/11. 6604.

66. Detail of Fig. 63 showing the continuity of the fungal (fp) and the host (hp) plasmalemmas over the neckband region of the stem. The electron dense layer of the sheath (bottom) can be traced to the ring (r) and is marked on the fungal side by an electron dense line and on the host side by a fainter line (arrow). Between this region and the adjacent host and fungal plasmalemmas material of a similar opacity and texture to the neckband (B) is present. The termination of the neckband at the ring is obscured by the presence of densely staining material of a diffuse nature. Beyond the ring the stem wall continues as two layers which differ markedly in electron density. 1382B/30. 6714.



65



66

4. THE STRUCTURE AND DEVELOPMENT OF UREDOSPORES

(a) The Mature Spore

Uredospores became mature whilst still supported individually on short stalks. Each spore was sub-spherical to ovoid in shape, within the range of 18-28μm in length by 18-22μm in diameter (Fig. 69, 72, 73).

The prominent spines of the uredospore were distributed more or less equidistantly apart but in no obvious pattern. They were conical in shape, usually with straight sides but sometimes bent over at the tip, and originated perpendicularly, though sometimes at an angle, from the surface of the spore (Fig. 77, 78). The larger spines were about 1,420nm long by 910nm in diameter at their bases which were usually eccentrically set within a slight depression of rather larger area defined by a small ridge (Fig. 77, 96). On two diametrically opposite sides of the spore the spines gradually diminished in size and number towards smooth central areas (about 5μm in diameter) free from spines which were the sites of potential germ pores (Fig. 74, 75, 77, 78). The wall of the spore was modified in these regions which were thickened towards the interior of the spore and appeared in section to be of low electron density and mottled texture (Fig. 106).

The spines were electron-lucent in thin section apart

from faint electron dense flecks (Fig. 95, 96, 98), which suggested a fibrous texture, orientated perpendicular to the bases of the spines. Each base had a deep central indentation filled with darker material, resembling the pulp of a tooth (Fig. 96).

The wall of the uredospore was thick (1.0-1.5μm) and electron dense and surrounded by a thin pellicle (about 7.0nm thick) which covered the spines and was continuous with the outermost layers of the wall of the stalk cell (Fig. 87). The stalk cell wall was slightly less opaque than that of the spore and between 100 and 150nm in total thickness.

The spore and stalk were separated by a septum with a central pore of diameter between 400 and 800nm through which cytoplasmic continuity was maintained (Fig. 72).

(b) Fine Structure of the Cytoplasm and Internal Organelles

(i) Nucleus

Uredospores and stalk cells were, like the vegetative hyphae, dikaryotic (Fig. 68, 72). Pores in the nuclear envelope appeared clustered as revealed by both glancing (Fig. 99) and median sections (Fig. 100).

Chromatin was distributed within the nucleus as a diffuse electron dense reticulum (Fig. 81). The nucleoli, which were found at all stages of spore development, were single, large bodies, comparable in electron density with

the chromatin inclusions of the nucleus, containing diffuse material, particles the same size as cytoplasmic ribosomes and clear areas, possibly analagous to the vacuoles of the host nucleoli (Fig. 101). The nucleolus was usually situated close to the nuclear membrane. The nuclei in mature spores contained two or more small round bodies which were more homogeneous and generally less dense than the chromatin and lacked the ribosome-like particles and clear areas characteristic of the large nucleoli (Fig. 102).

(ii) Mitochondria

Mitochondria were numerous and similar in general form to those of intercellular hyphae and haustoria (Fig. 94, 103).

(iii) Microtubules

Microtubules lacked contrast against the surrounding cytoplasm and were more clearly visible in cross section than in longitudinal section, but though present in the stages examined appeared to be few in number (Fig. 95, 103). These microtubules were about 22nm in diameter with less opaque centres, of inner diameter about 11nm, filled with amorphous material. Microtubules were surrounded by narrow zones of finely granular uniform matrix from which ribosomes and other cytoplasmic particles were excluded (Fig. 95, 98. 103).

(iv) Microbodies

Microbodies were present both in uredospores and stalk cells (Fig. 87, 88); they were vesicles bounded by single membranes, without the well defined triplet structure of the plasmalemma, and often contained crystalline inclusions in a matrix of amorphous appearance. Crystal containing vesicles, particularly in the stalk cell, tended to be grouped in the vicinity of the septal pore, where they were usually accompanied by vesicles without crystals. Microbodies could be distinguished from small elements of E.R. which were more empty in appearance and often flattened or tubular in form (Fig. 97, 98).

(v) Membranes

The plasmalemma of the spore possessed the usual triplet structure visible after Glut/Os fixation. However, in some spores the outer layer of the triplet stained more densely and appeared slightly thicker than the inner layer. The thickened plasmalemma was found in areas often closely associated with what may have been coiled invaginations of plasmalemma or detached multi-vesicular bodies whose membranes had similar staining properties (Fig. 103).

The membranes of some vacuole-like vesicles, but not the larger vacuoles, had a structure similar to that described above with the denser portion of the membrane on the vacuolar side (Fig. 95).

Numerous cisternae of E.R. were present. These could be traced more easily after permanganate fixation which removed the ribosomes and other cytoplasmic structures (Fig. 99, 100).

(vi) Other Cytoplasmic Inclusions

Numerous densely packed ribosomes about 21nm in diameter were present in the cytoplasm, often in small clusters, in the stalk and spore, but as the cytoplasm was so dense it was difficult to ascertain whether they were associated in any way with the E.R. (Fig. 98, 99). The cytoplasm adjacent to the spore wall was devoid of ribosomes up to about 80nm from the plasmalemma and appeared to contain microtubules and small spherical bodies about 40nm in diameter with amorphous contents (Fig. 98, 99).

Granular material with particles of slightly larger size (28nm) than ribosomes and less opaque was present as large aggregates in the cytoplasm of the mature spore, but was absent in early stages of development (Fig. 103, 104).

Droplets of lipid were more numerous in the spore initial than in the stalk cell (Fig. 81). In the mature spore lipid droplets tended to be uniform in size (about 30nm in diameter) and were often grouped around vacuoles (Fig. 72)

In some mature spores small, round electron dense bodies (about 50nm in diameter) were dispersed throughout the cytoplasm (Fig. 87), but were probably large aggregations of stain

or other extraneous matter introduced during the staining procedure.

(c) Spore Development

The intercellular mycelium from one infection centre developed a stroma below the epidermis of both the dorsal and the ventral sides of the leaf (Fig. 67). Special basal cells (Fig. 71) were cut off from the tips of the hyphae that formed the surface of the stroma. These cells unlike the majority of those of the intercellular mycelium were filled with dense cytoplasm (Fig. 68). The basal cells divided to produce two cells the upper one of which divided again to form a stalk cell surmounted by a uredospore initial (Fig. 79, 80). Both of these cells were filled with cytoplasm and lacked any large or central vacuoles, although a small number of very small vacuoles were present.

The uredospore initial was cylindrical below and expanded towards its apex in the form of a match-head. The wall of the spore initial was continuous with that of the stalk cell and was about 100 to 150nm thick (Fig. 80).

The structure of the septum separating the uredospore from the stalk was difficult to elucidate. The septum on the side of the stalk cell initial appeared to consist of a secondary deposit of wall material in the form of a perforated disk attached at its edge to the original lateral (primary)

wall of the cell (Fig. 97). There was no evidence that this half-septum was part of a more complete secondary wall developed over the lateral walls of the stalk cell. A corresponding deposit of new wall material appeared also to be laid down on the spore side. These two new deposits which formed the two halves of the septum were separated from each other by a narrow clear gap which appeared to continue to the edge of the septum around the pore (Fig. 85, 86). The septum thus appeared in some respects similar to a septum of a vegetative hypha (cf. Fig. 27-29), but differed in that the layer found on the spore side, unlike that on the stalk cell, was not bonded to the primary hyphal wall (Fig. 97). This difference between the two halves of the septum suggested that the deposition of new wall material on the stalk side occurred only over the septum and terminated when it attained a thickness not finally exceeding that of the original lateral wall of the stalk cell; whereas, on the spore side the septal layer was formed as part of a new wall deposited eventually against the whole of the original wall of the spore initial and attaining finally a thickness of almost ten times that of the primary wall (1.0-1.5μm).

At the earliest stage of development observed, spines were recognised in thin sections as small electron-lucent conical structures approximately 370nm long and 200nm in diameter at the base, lying outside the plasmalemma with their

apices in contact with the primary wall of the spore initial (Fig. 80). A single cisterna of the E.R. was associated with the base of each spine at this stage (Fig. 89).

Comparison of spines in a series of stages of development from 370 x 200nm (Fig. 89), 660 x 360nm (Fig. 90), 1,330 x 650nm (Fig. 91) to 1,420 x 910nm (Fig. 92) indicated that the increase in size took place by deposition of material at the base.

The first appearance of the electron dense secondary wall was at points immediately adjacent to the spines, but it did not become deposited over their apices (Fig. 90). the new wall spread mainly laterally at first between the spines, but in later stages of development it was deposited also on the sides of the spines (Fig. 91, 92) and finally beneath their bases after they were fully formed (Fig. 93). Cisternae of the E.R. remained in association with the bases of the spines throughout the development of the secondary wall (Fig. 94).

Shortly before the development of the spines was completed the primary wall began to disintegrate within sites localised at first at the points of contact with the tips of the spines (Fig. 91), but rapidly spreading to the whole of the primary wall (Fig. 82, 83, 92-95). The septum remained unaffected (cf. Fig. 97, 98).

When the spines were mature and dissolution of the primary wall had become extensive the persistence of a thin

electron dense superficial layer of the latter became clearly evident (Fig. 95). This finally remained as a pellicle enclosing the spore and in continuity with the outermost layer of the stalk cell primary wall which had remained entire (Fig. 98).

During these developmental processes the spore had increased slightly in size (from 12 x 8μm to 15 x 9μm: Fig. 79, 80) but with little change in the appearance of the cytoplasm in contrast to the stalk which had become highly vacuolated.

The spines when fully formed did not at first project beyond the surface of the spore and were completely enclosed by secondary wall on the sides and bases, but with their tips covered only by the pellicle (Fig. 93, 95). The spines were later elevated to the surface by a marked increase in the volume of the spore and the consequent elimination of the deep sockets in which the spines were situated. This process was associated with the sudden appearance of numerous small vacuoles in the cytoplasm of the spore (Fig. 72, 81, 82), which no doubt accounted for most if not all the increase in volume.

Successive stages of development and elevation of the spines are illustrated in the sequence in figures 89-94. The ridges on the surface of the spore within which the spines were set enclosed areas of the wall which originally formed the sides of the spine socket (Fig. 77, 78, 95, 96).

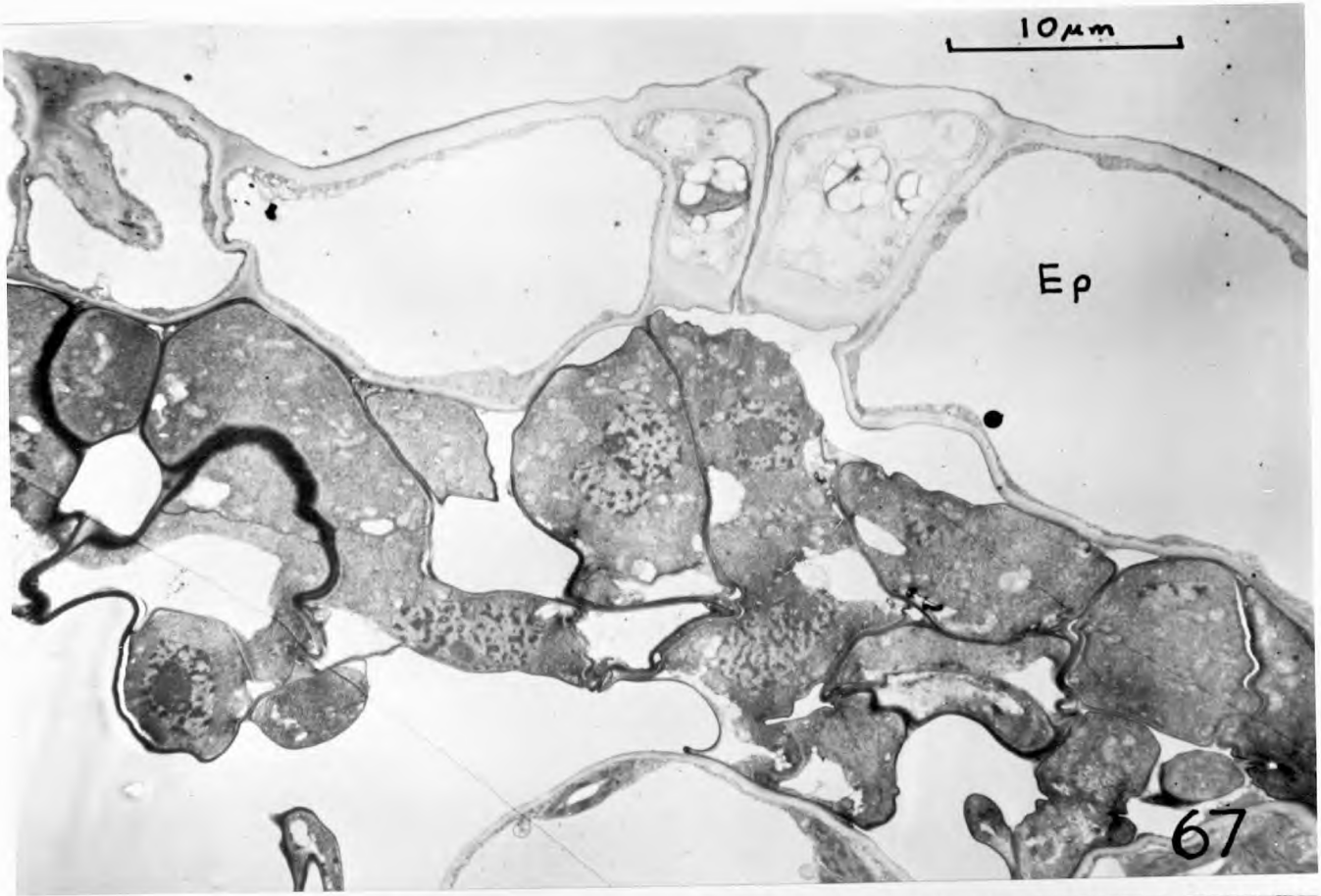
When the spore swelled the pellicle also became stretched and became closely adpressed to the surface of the secondary wall and spines of the mature spore (Fig. 84,96).

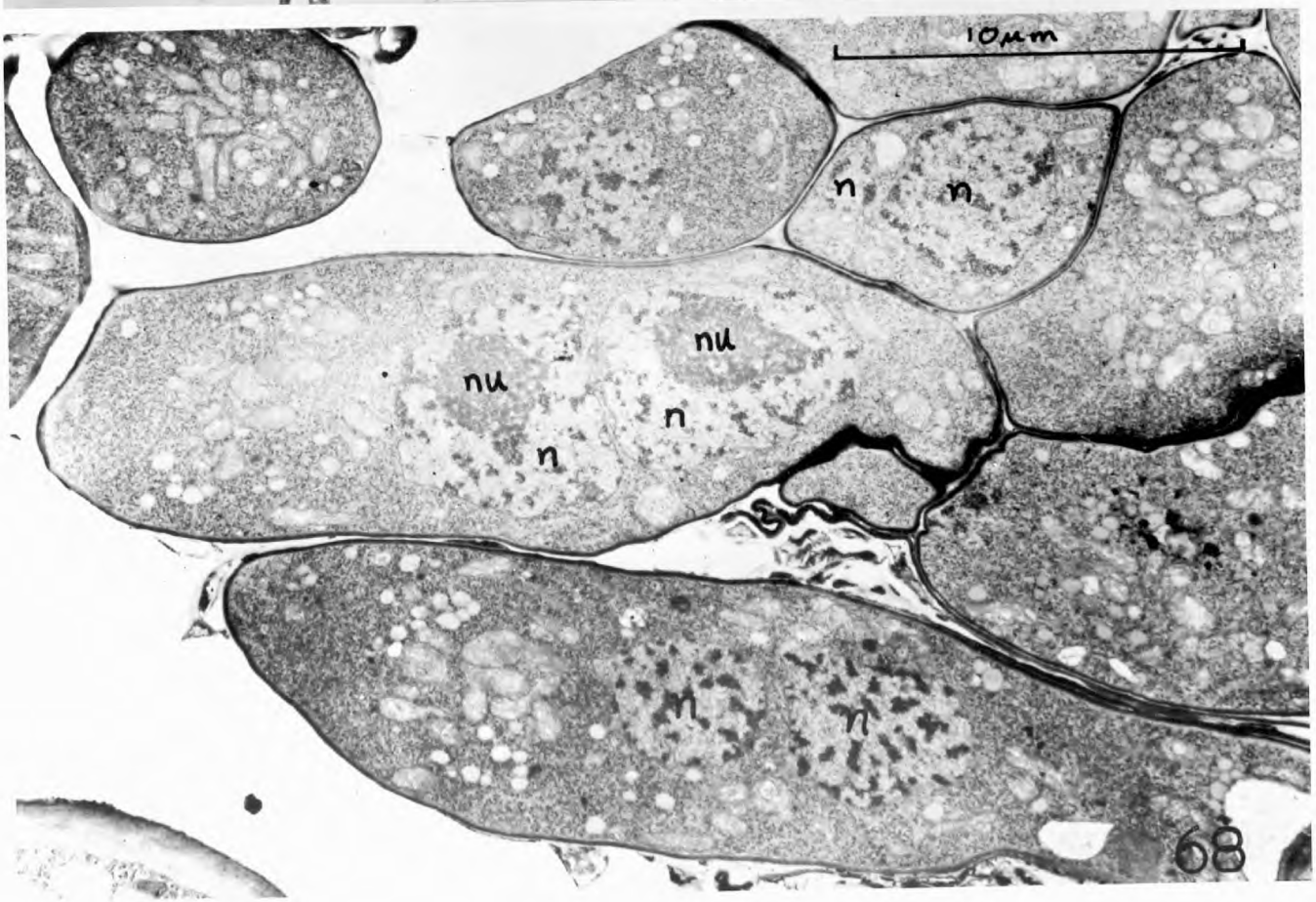
Released spores were contracted becoming depressed in the spine free areas of the germ pores and thus assuming the characteristic 'dough-nut' shape (Fig. 74, 75). Uredospores shaken from mature pustules and viewed unmounted under a light microscope also exhibited the dough-nut shape (Fig. 76) which was not, therefore, an artefact caused by the freeze-drying required for examination in the scanning reflection electron microscope. This change must inevitably have involved a loss of water but the spores had become difficult to fix and embed, the cytoplasm was dense and poorly preserved and its structure could not be examined (Fig. 70).

On detachment of the spore from its stalk it was difficult to determine the fate of the pellicle at the hilum region. In the germinated spore the pore in the hilum region appeared to have a thin covering layer (Fig. 84). However, the material observed may be extraneous material accumulated around the spore during its germination on the leaf surface because it was thicker, rather more diffuse and less closely applied to the spore than the pellicle (Fig. 96).

67. Low power showing part of the stroma beneath the epidermis (Ep) in the sub-stomatal cavity beneath the guard cells. 1540B/1. 8293.

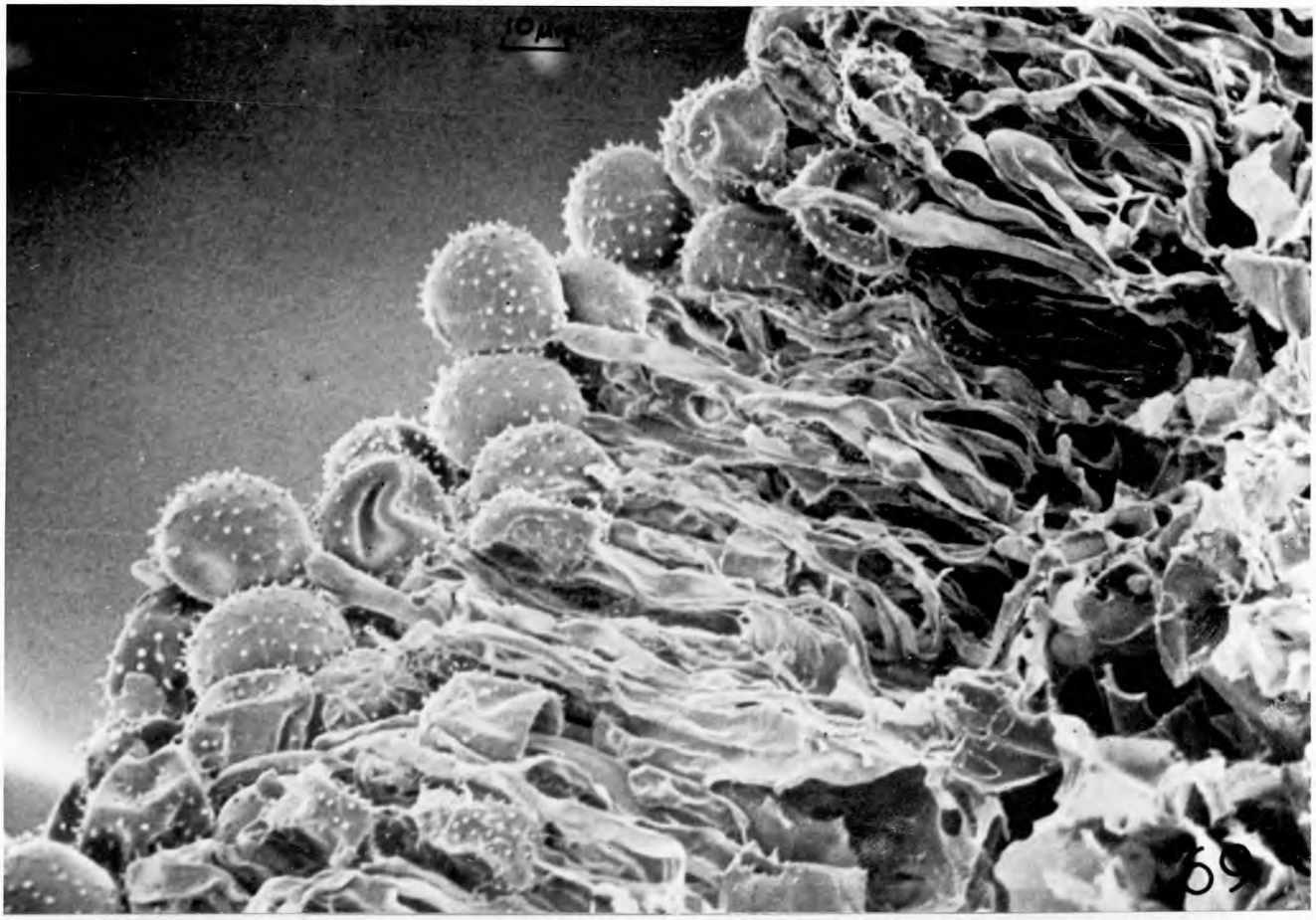
68. Part of the stroma bearing spore initials, each with two nuclei (n). 1540A/3. 8281.


10μm
Ep
67

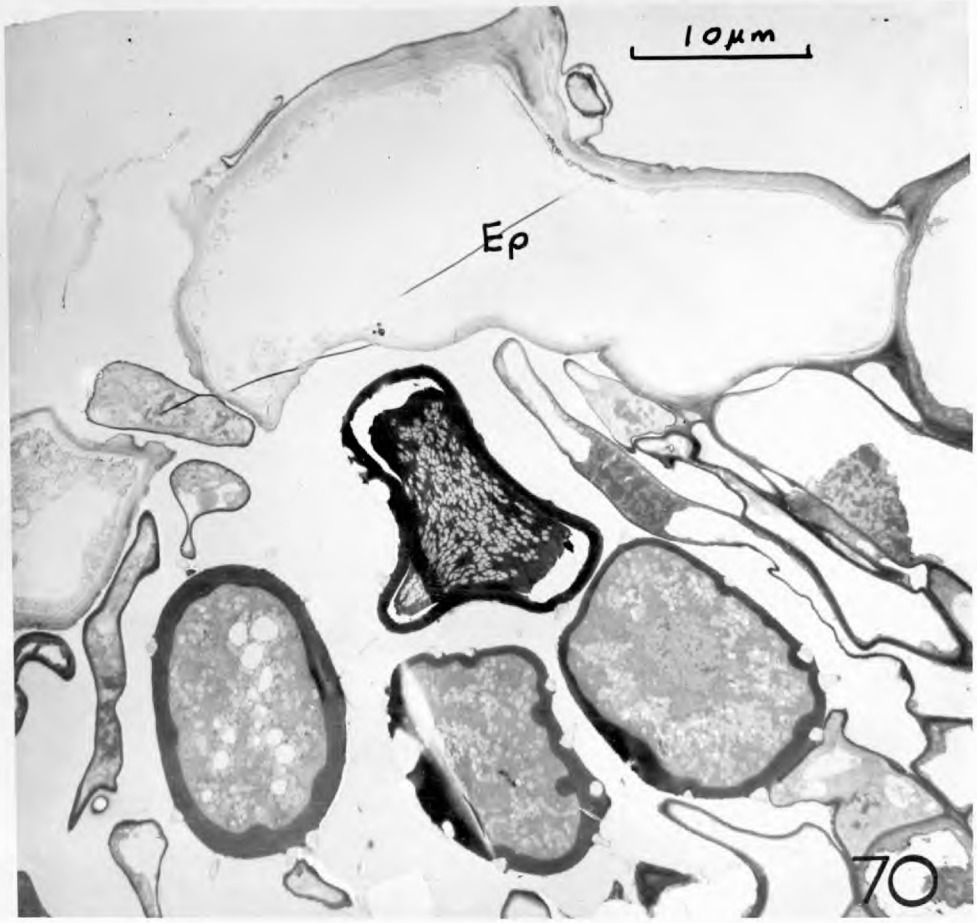

10μm
n
n
nu
nu
n
n
n
n
68

69. Scanning reflection electron micrograph of a uredosorus showing the uredospores attached to their stalk cells. 1790/5. G5A.

70. Uredospores beneath a ruptured epidermis (Ep). The electron dense, distorted uredospore (centre) appears to be poorly fixed and badly embedded. 1382B/17. 5838.



69



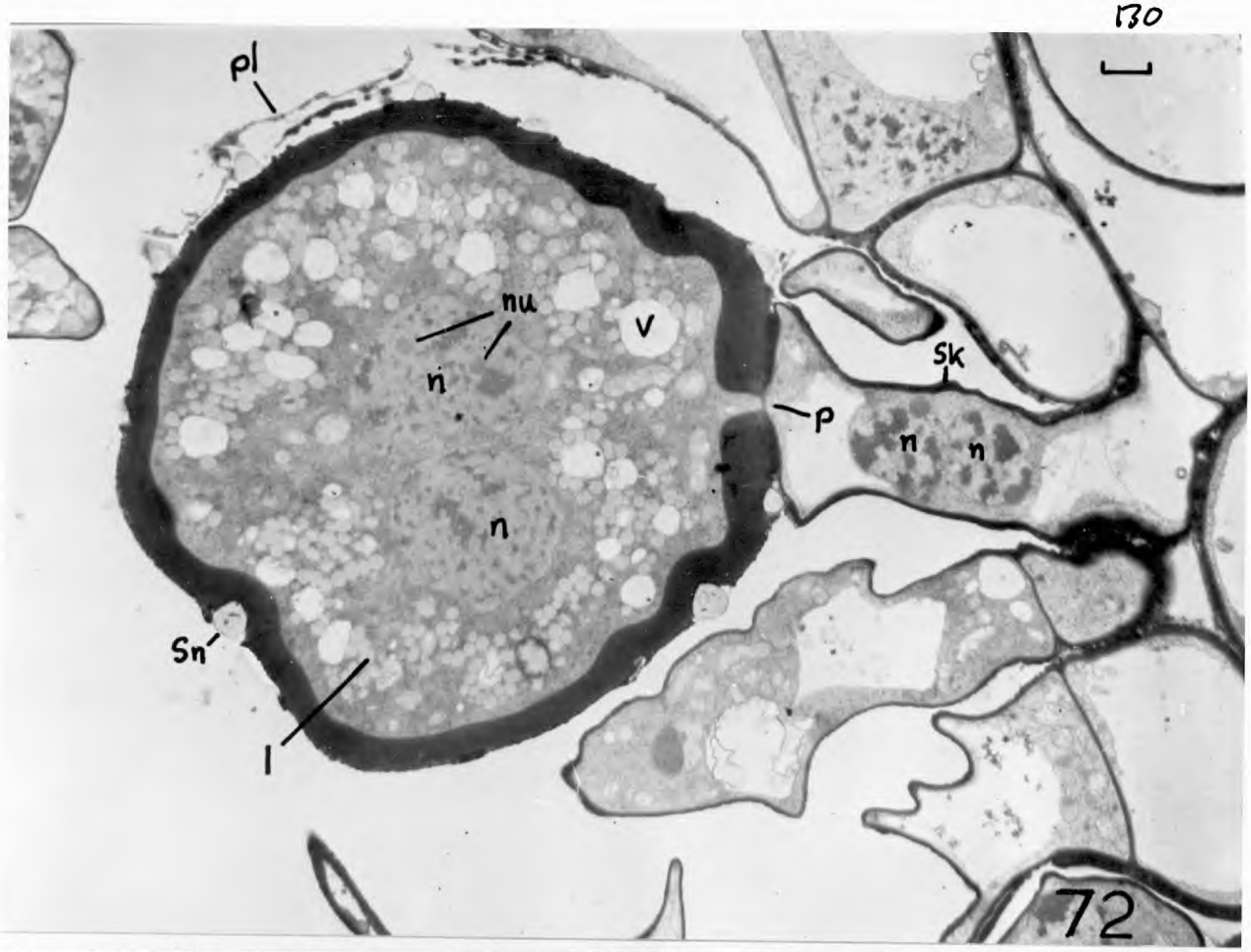
70

71. A developing uredospore with spines (Sn) still within the spore. The cell beneath the spore is its stalk cell, beneath the stalk the basal cell and finally part of the stroma. 1382B/68. 6522.

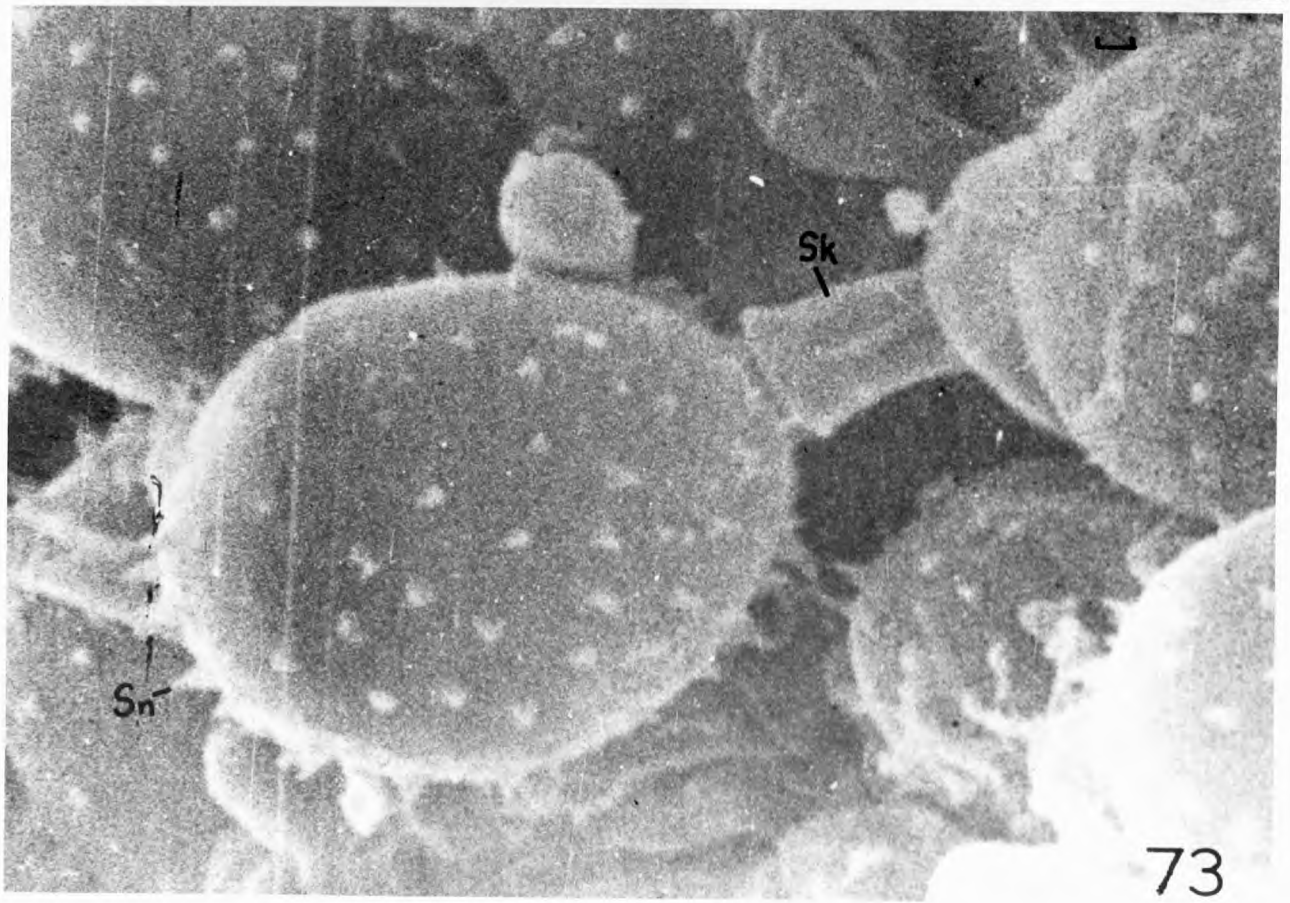

n
n
Sn
nu

72. Section of a uredospore on its stalk (St) near maturity. Note numerous spines (Sn), the covering pellicle (pl), lipid droplets (l) grouped near to the vacuoles (v). Also shows two nuclei (n) with nucleoli (nu). The pore at the base of the uredospore was not plugged by wall material. 1382B/11. 5829.

73. A scanning reflection electron micrograph of a uredospore at a similar stage to Fig. 72. Freeze-dried by method (i) (p. 56). 1295/5b. C42.



72

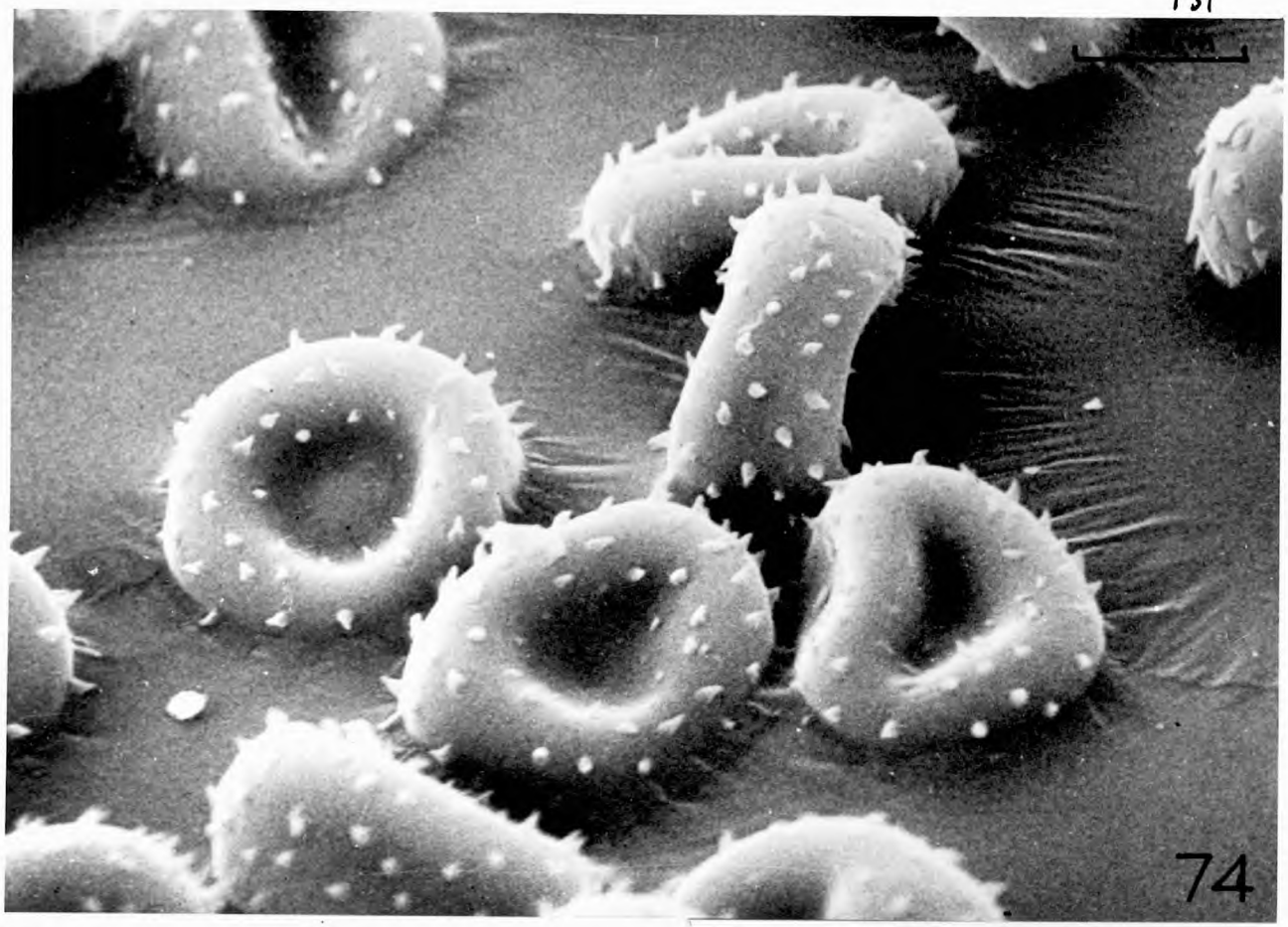


73

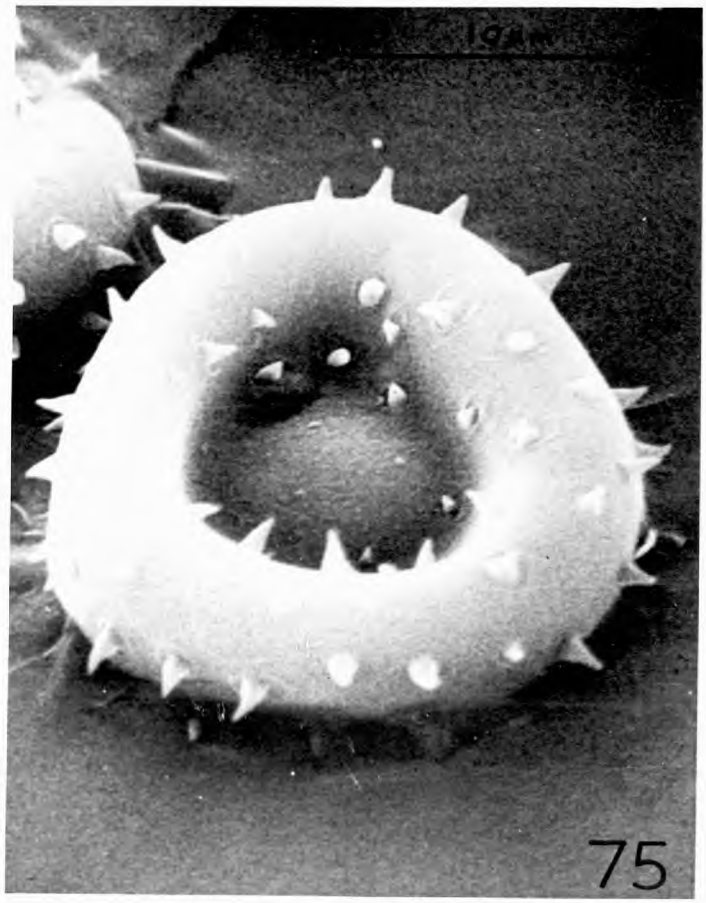
74. A scanning reflection electron micrograph of mature, detached uredospores exhibiting the characteristic 'doughnut' shape. 1790/5. A34.

75. A scanning reflection electron micrograph of a detached spore showing the conical spines set eccentrically within an eliptical ridge. 1790/5. B37.

76. A light micrograph of detached uredospores mounted, without prior treatment, in Canada balsam. -. 645.



74



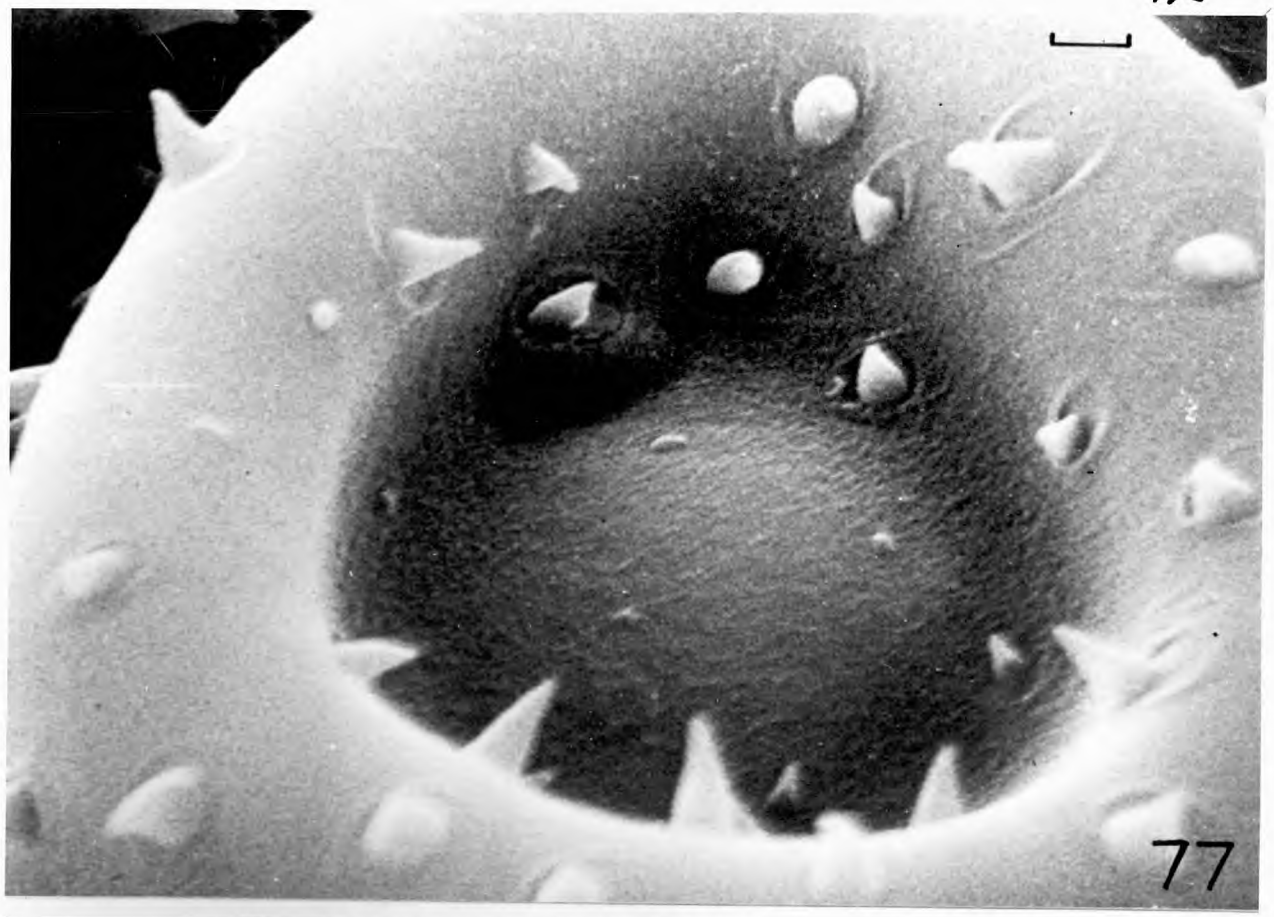
75



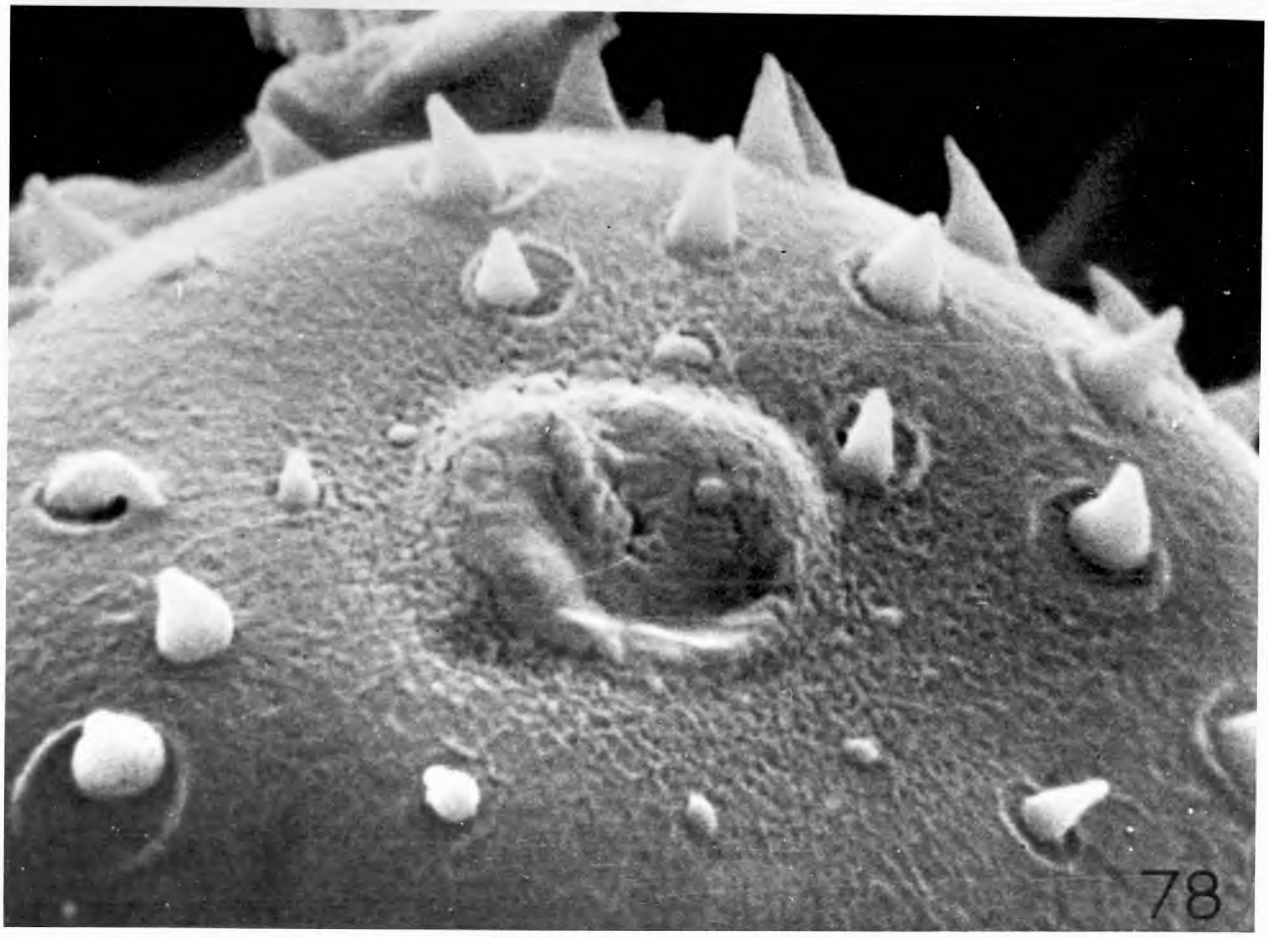
76

77. Detail of Fig. 75 showing the reduction in size of the spines towards the central depressed area. 1790/5. A36.

78. Detail of Fig. 142, a rehydrated uredospore, showing the area of the germ pore. 1802. D4.



77



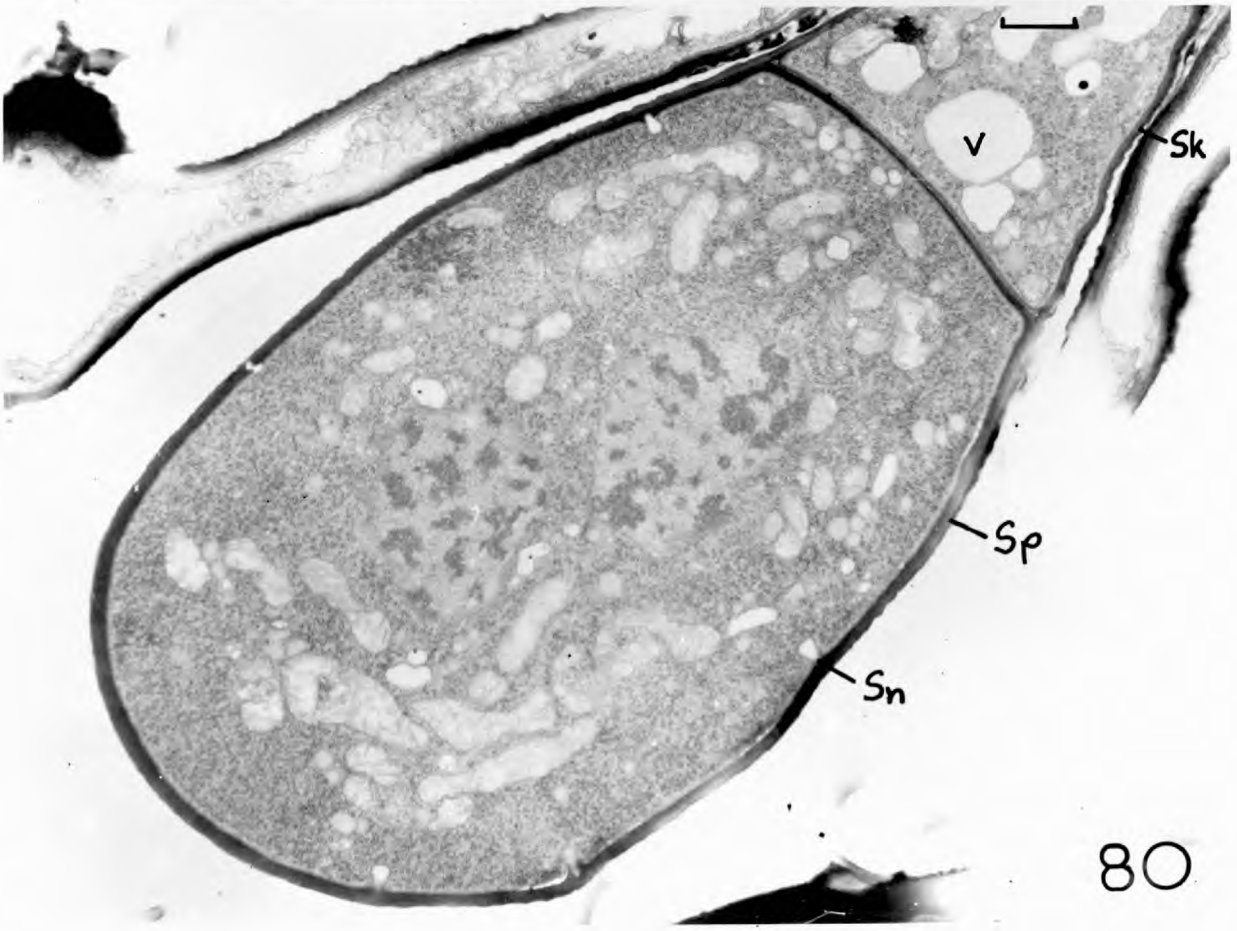
78

79. Uredospore initial before septum formation. Note the large nucleoli (nu) at the tips of the nuclei. 1382B/65. 14243.

80. Uredospore initial (Sp) after the septum has been formed. Note the increased vacuolation within the stalk cell (Sk) and also the spines (Sn) lying against the primary wall. 1382B/66. 14278.



79



80

81. Uredospore at a later stage in development to Fig. 80. Secondary wall material is apparent around the tips of the spines. 1382B/25. 14213.

82. Degeneration of the primary wall has occurred and in the upper spore some of the spines have been elevated to the surface. Lipid droplets (l) are present around the vacuoles (v). In the lower spore spines are still present within the spore, although some removal of the primary wall at the tips of the spines is apparent. 1382B/66. 14284.


n
n
nu


l
v
82

83. A developing spore showing dissolution of the primary wall and the retention of the pellicle (pl) which is continuous over the remains of the primary wall. 1382B/61, Pb only. 14238.

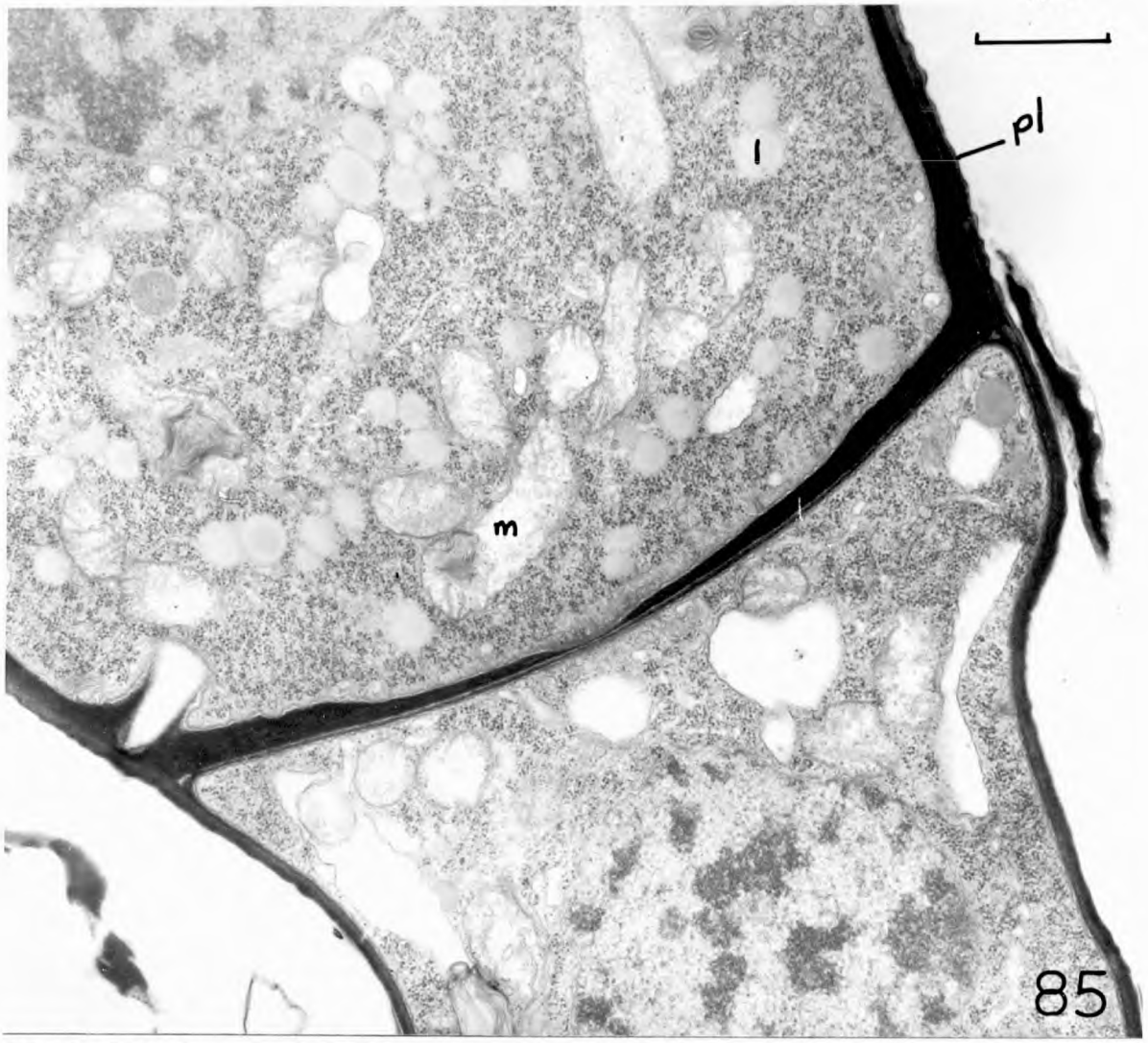
84. A mature, detached and germinated uredospore devoid of all cytoplasm except for a few membranes lining the wall. The hilum region (Hl) is thicker than the rest of the spore wall and possesses a less electron dense base. 1541A/27. 10079.

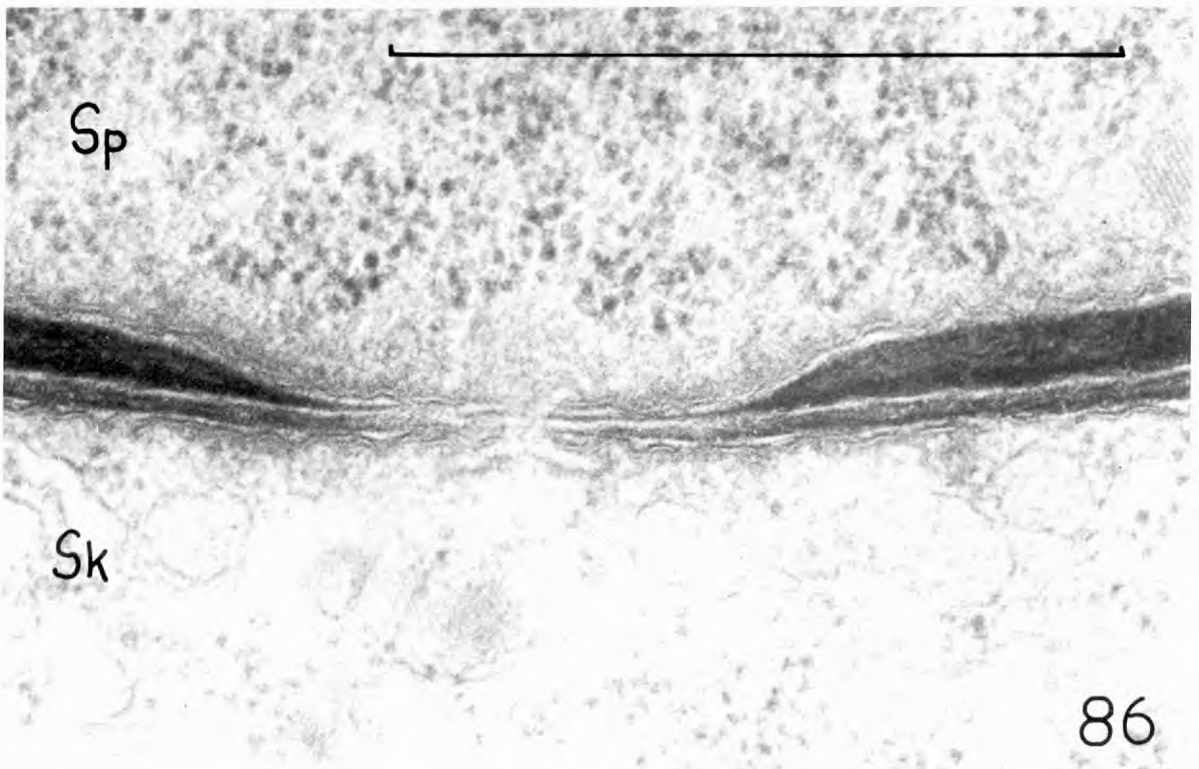

pl
83


Hl
pl
84

85. A stage during the deposition of secondary wall on the spore side (top) of the septum. 1382B/25. 141215.

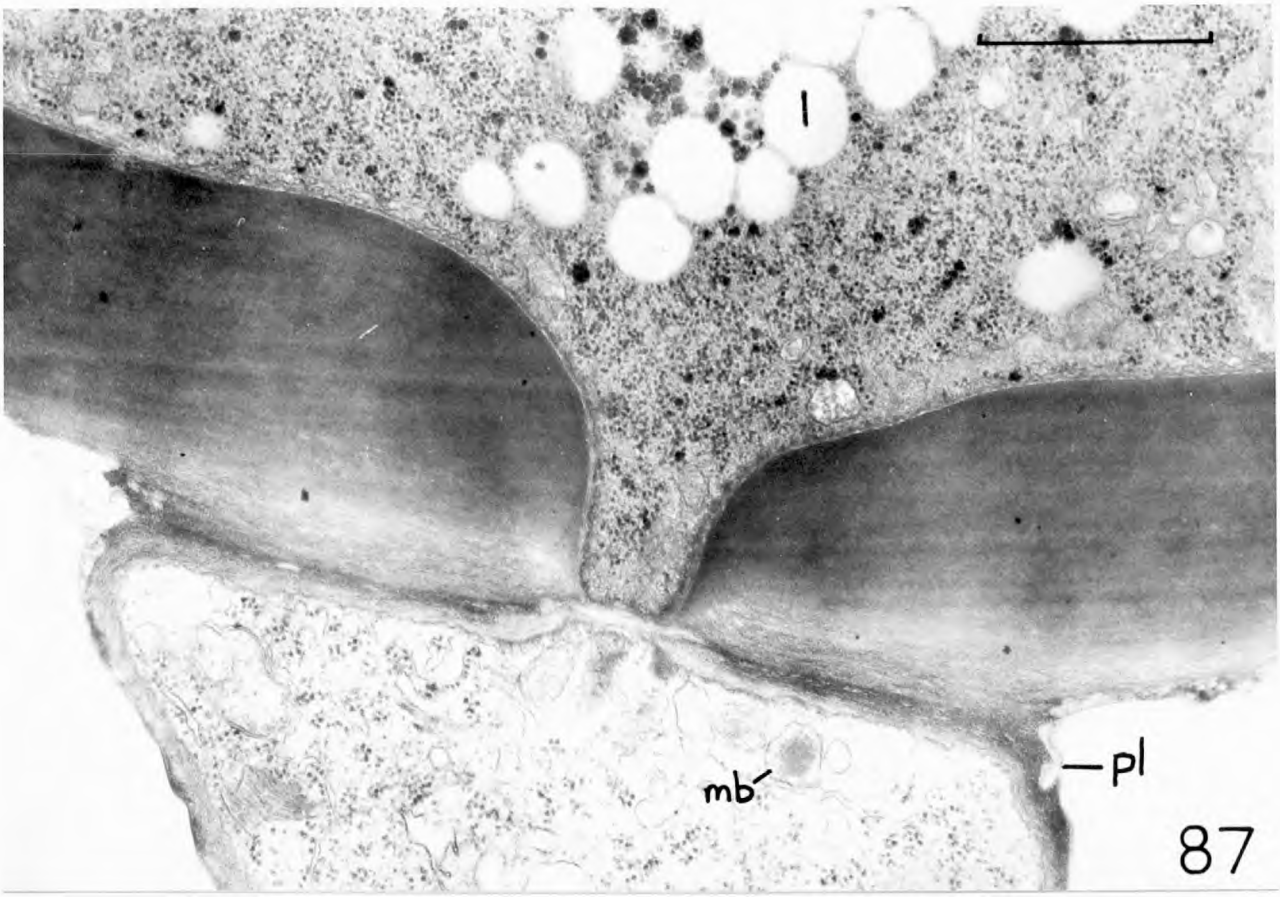
86. Detail of a serial section to Fig. 85. Note the electron-lucent zone between the two halves of the septum extending to the pore. 1382B/25. 141215.

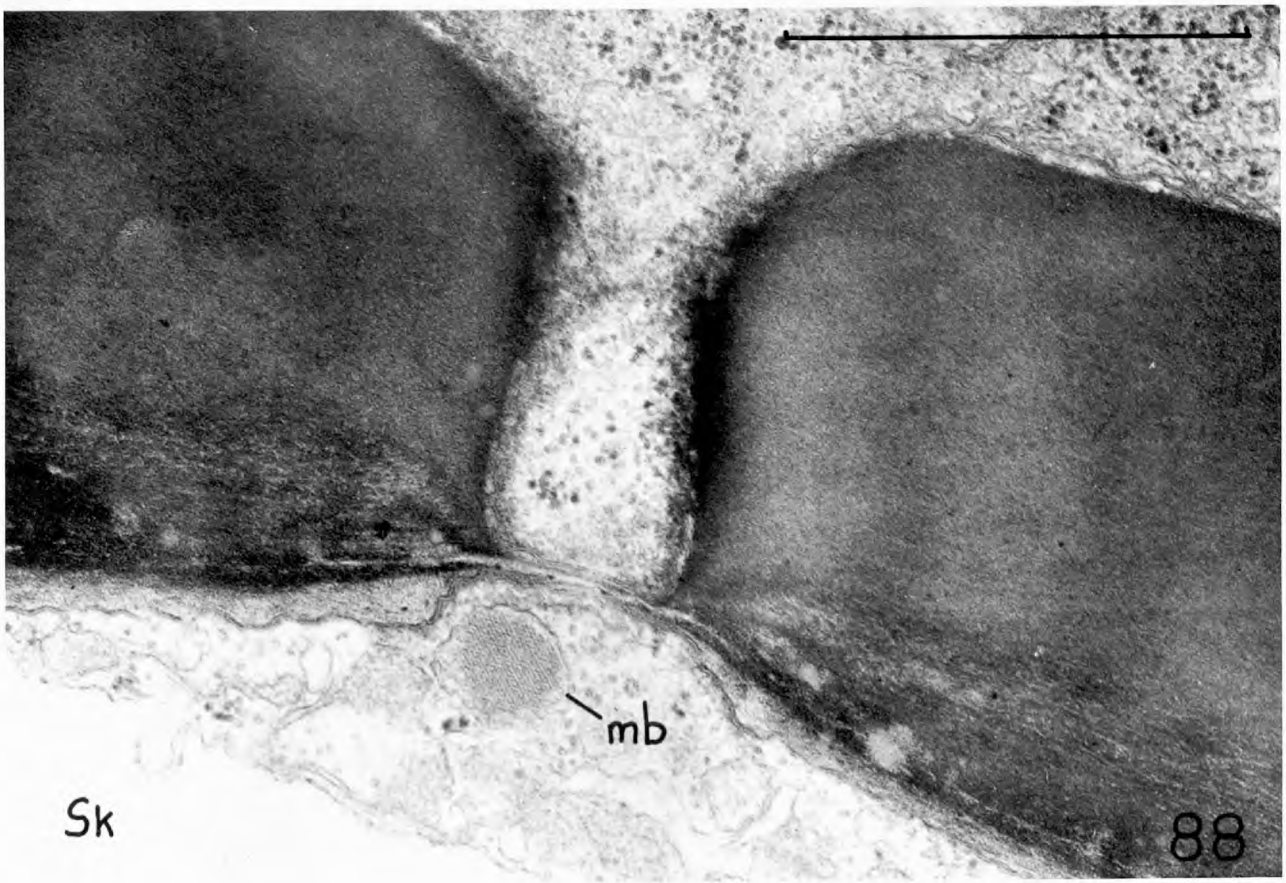

pl
l
m
85


Sp
Sk
86

87. Uredospore sectioned through the hilum region. Note the less opaque area of the outer part of the spore wall in this region and also the continuity of the pellicle (pl) from the spore across to the stalk cell. 1382B/67. 6513.

88. Detail of the pore region from Fig. 72. Note the microbody with crystalline inclusion. 1382B/11. 6361.


l
mb
pl
87


mb
Sk
88

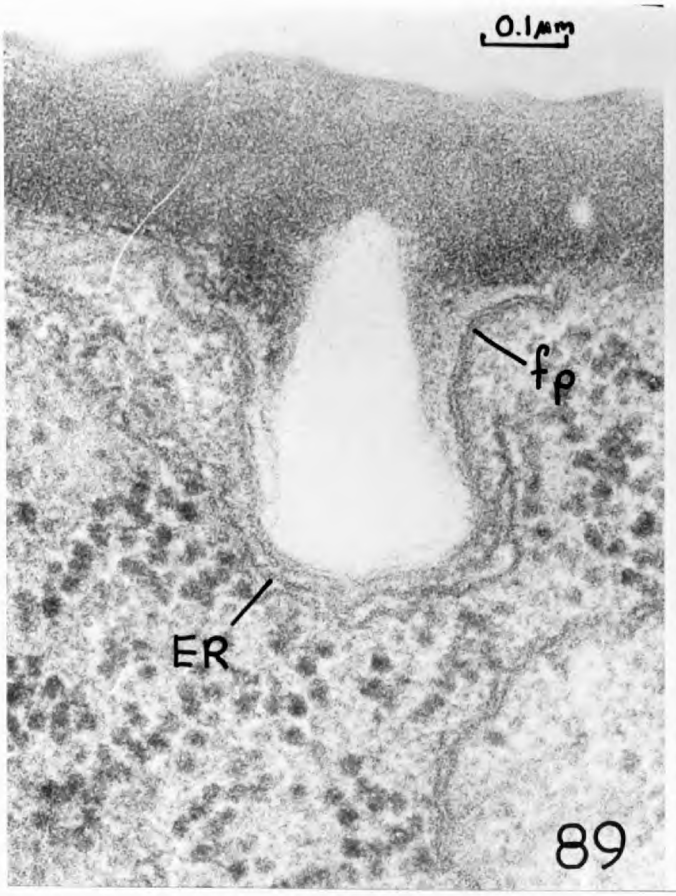
Figures 89-96 show a series of stages in the development of the spines.

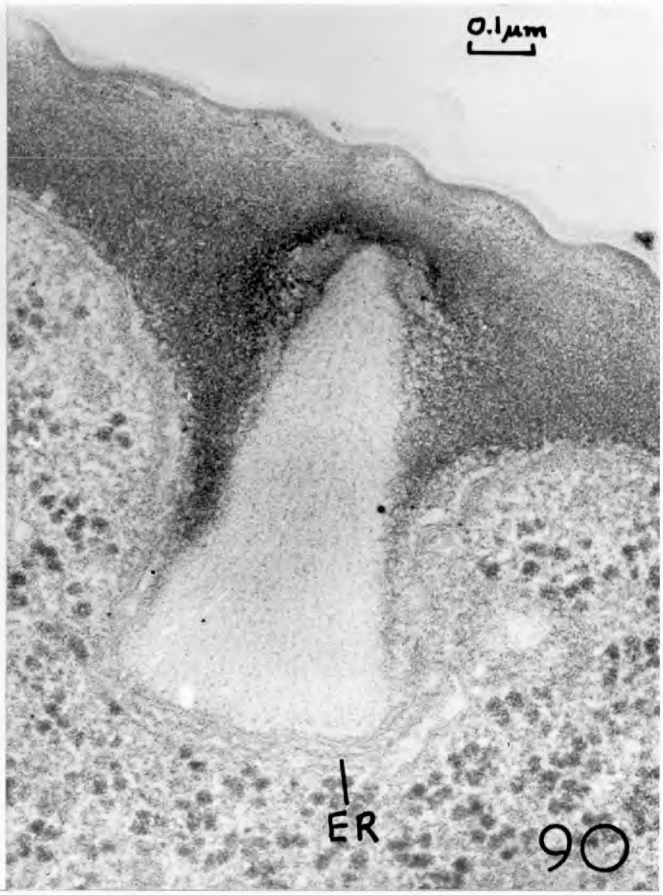
89. Section of a spine (the earliest stage obtainable) against the primary wall. Note the cisterna of E.R. at its base. 1382B/67. 14313

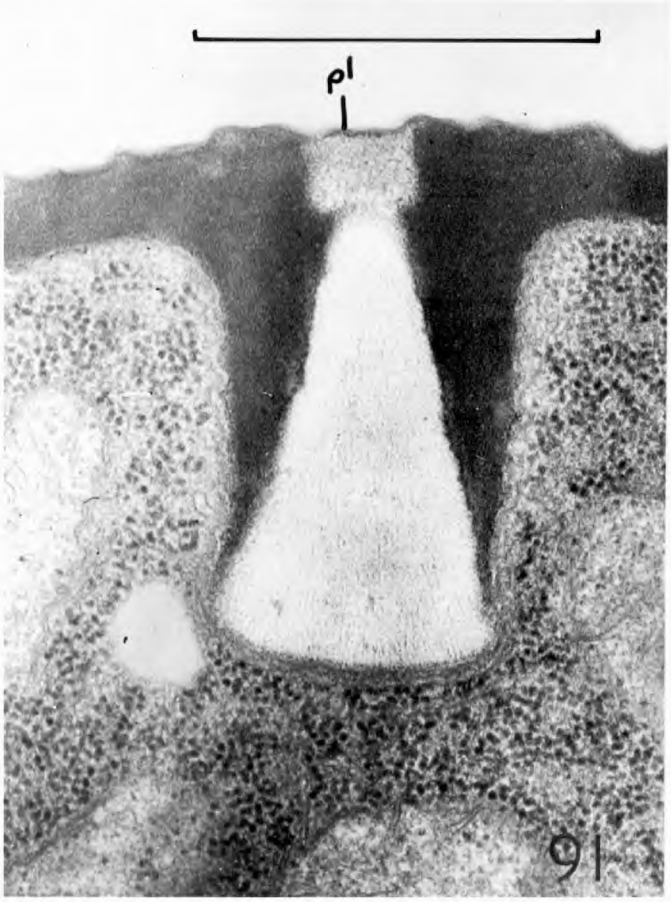
90. A spine at a later stage in development to Fig. 89. A deposit of secondary wall is apparent around the sides of the spine. Note the linear flecking within the spine. 1382B/25. 14222.

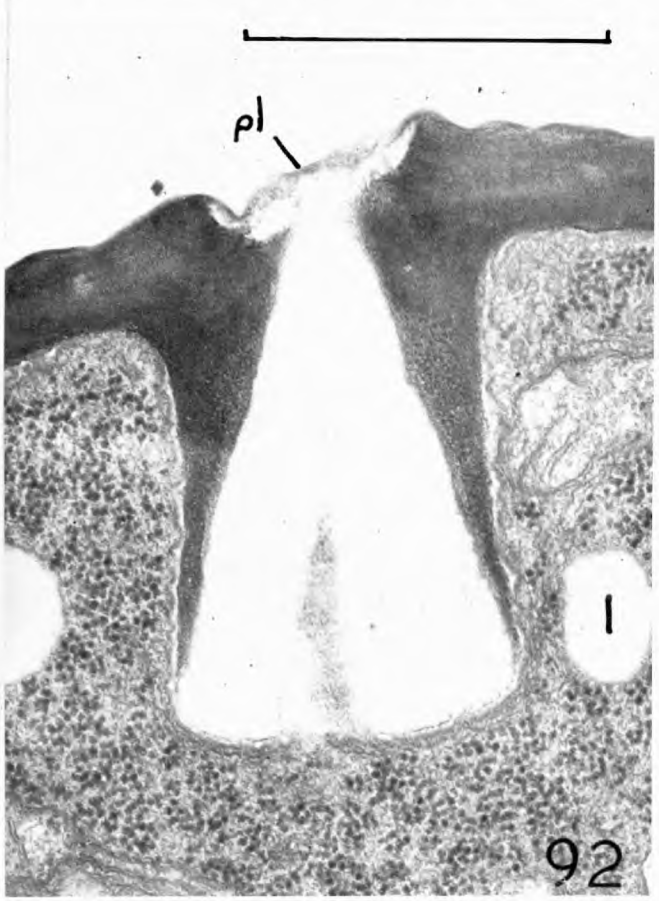
91. The secondary wall entirely covering the sides of the spine. Some dissolution of the primary wall at the tip of the spine is apparent. The pellicle is continuous over this region of dissolution. 1382B/22. 6415.

92. A fully mature spine, still retained within the spore, sectioned median to a central dense cavity. 1382B/65. 6745.


0.1μm
fp
ER
89

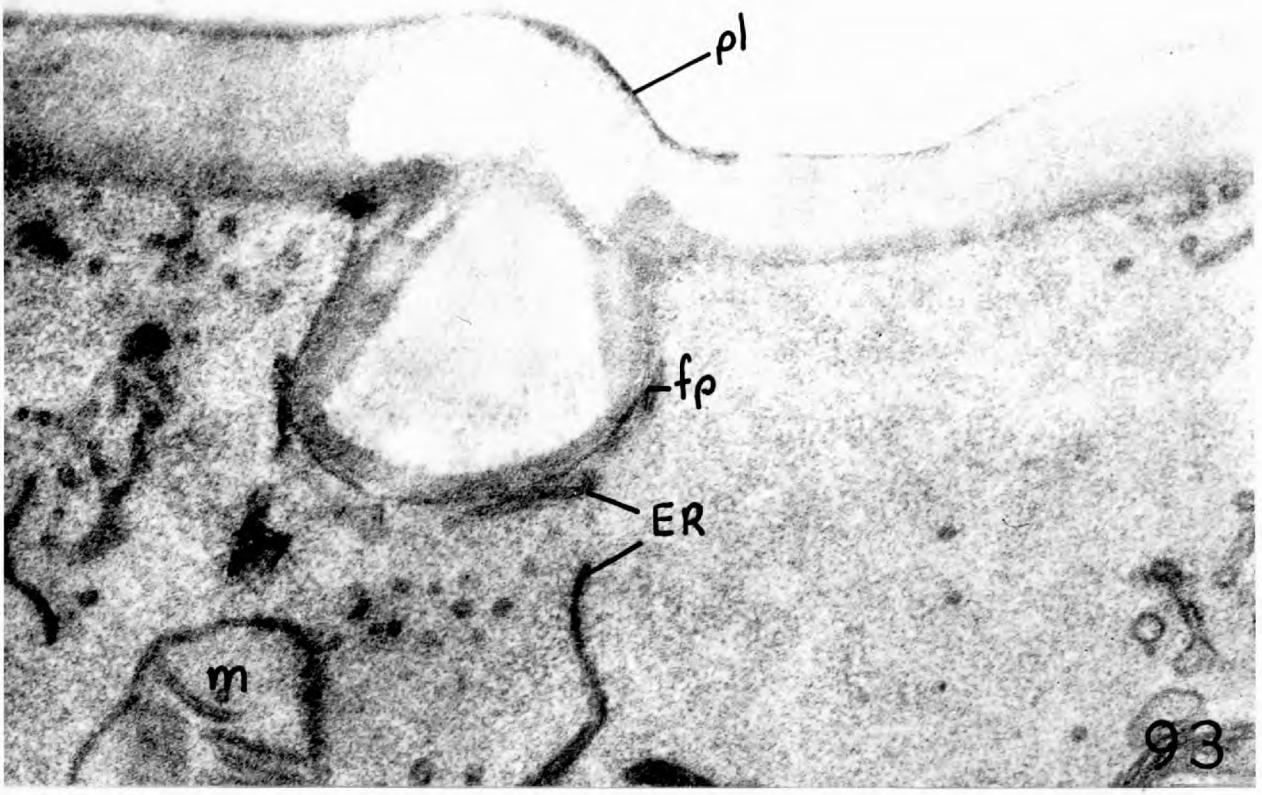

0.1μm
ER
90

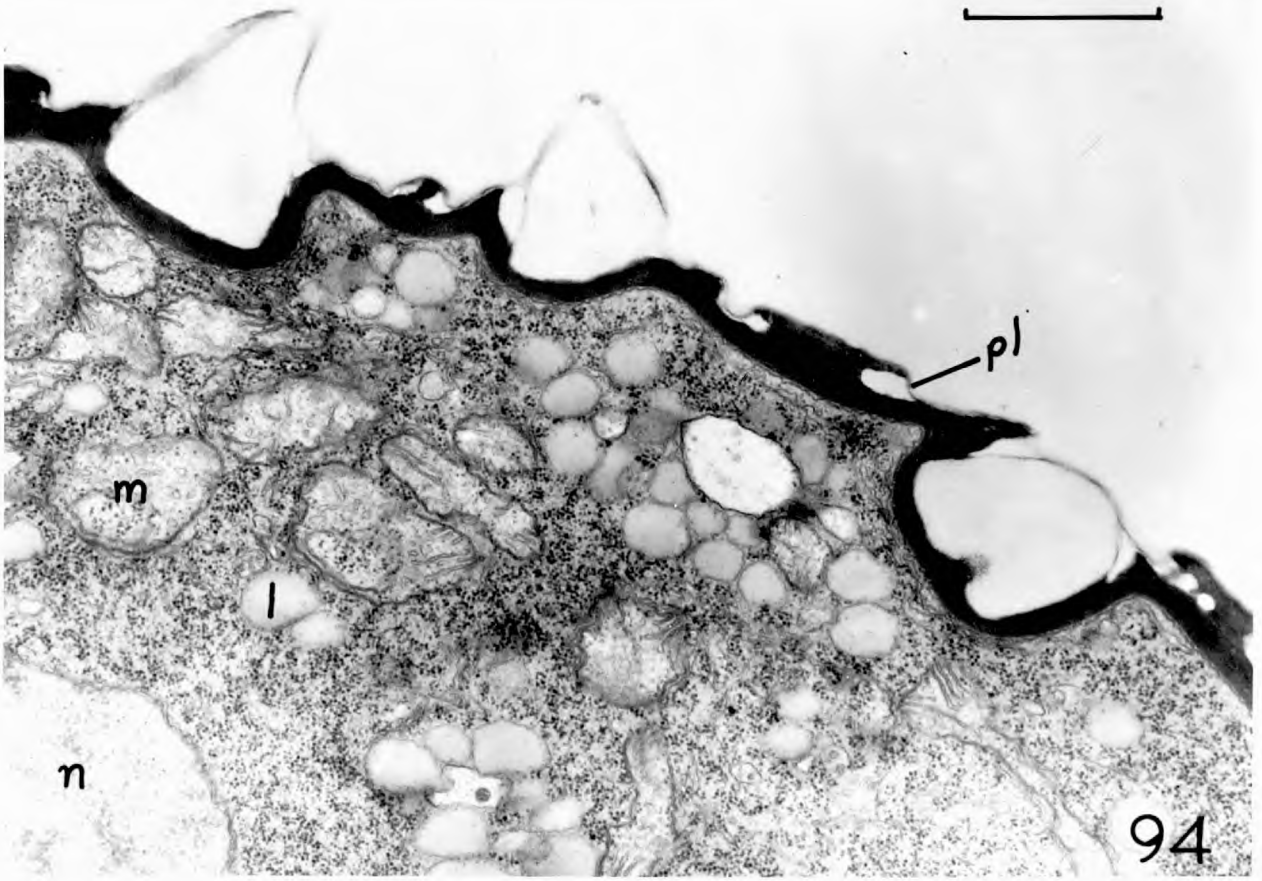

pl
91


pl
92

93. A fully mature spine with a deposit/of wall beneath its base. The E.R. remains associated with this region. $KMnO_4$. 1529/6. 14291.

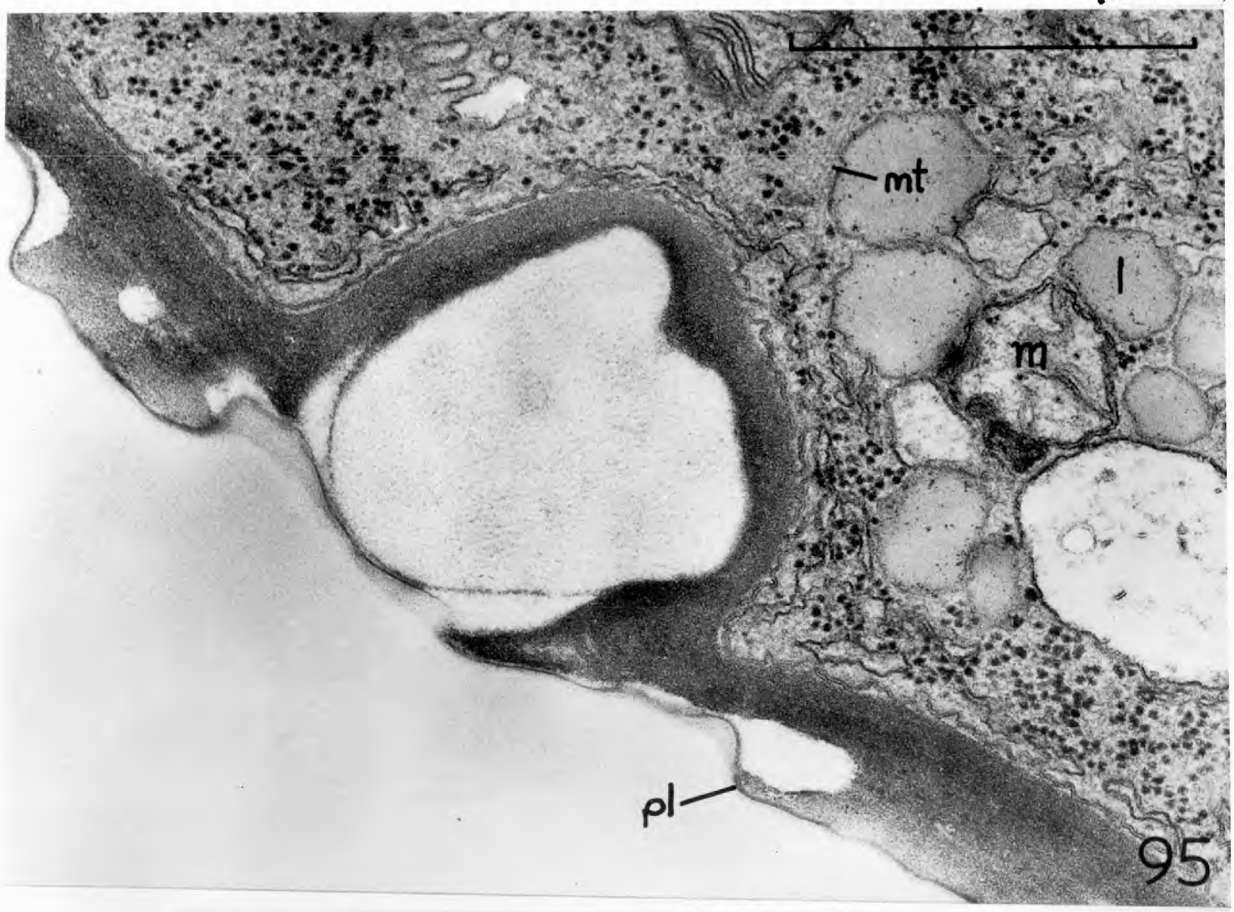
94. A section through three spines at various stages of elevation to the spore surface. Extensive removal of primary wall has occurred. 1382/B61, Pb only. 14214.


pl
fp
ER
m
93


pl
m
l
n
94

95. Section of a spine still within its socket formed by the enclosing deposit of secondary wall before elevation to the spore surface. Note the continuity of the pellicle (pl) over the degenerated primary wall and the tip of the spine. 1382B/61, Pb only. 14240.

96. Detail of Fig. 84 of a mature spine. The pellicle (pl) is closely applied to the spore wall and spine. A ridge is present on the outer surface of the spore wall and is probably equivalent to that seen in surface view (e.g. Fig. 77,78) and marks the boundary of the socket of secondary wall in which the spine was set. 1541A/27. 10080.



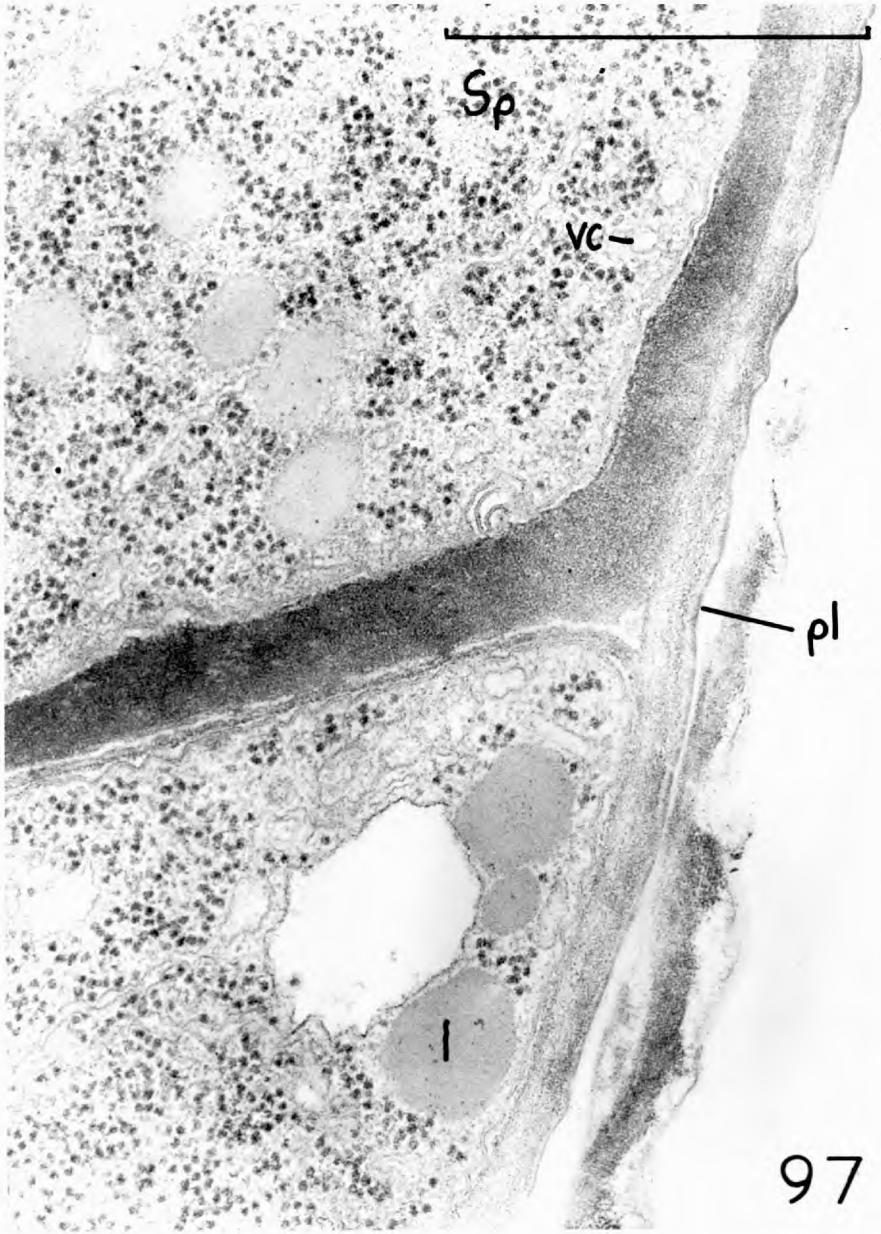
95



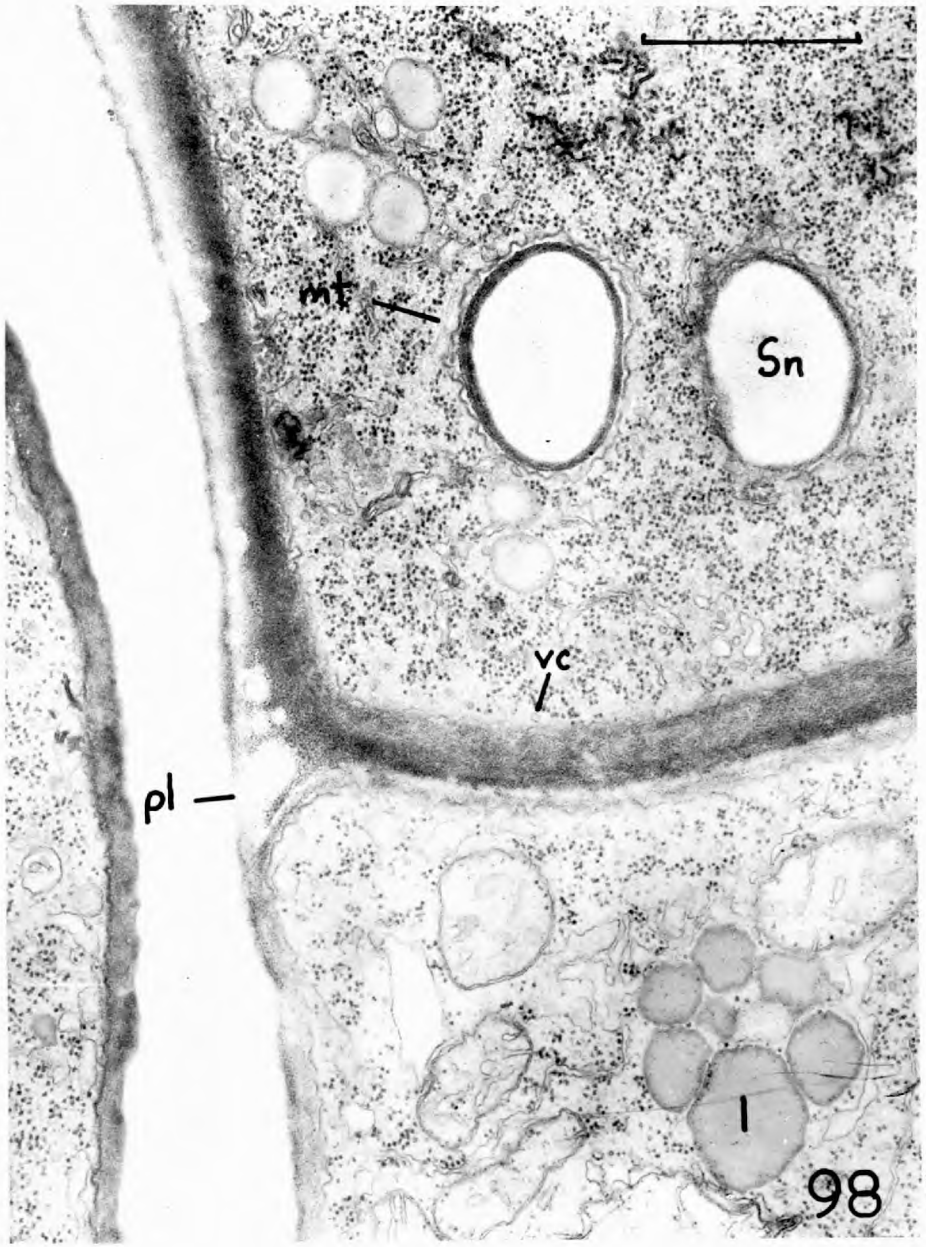
96

97. Detail of the edge of a septum between the spore and stalk cell. Note the deposit of secondary wall of the spore forming a complete covering to the primary wall and compare with the half septum on the stalk side where the septal wall terminates at its junction with the lateral wall. 1382B/25. 14218.

98. A similar section to Fig. 97, although at a later stage in the development of the spore, showing the beginning of the degeneration of the primary wall around the spore but not affecting the wall of the stalk cell or pellicle. 1382B/61, Pb only. 14239.



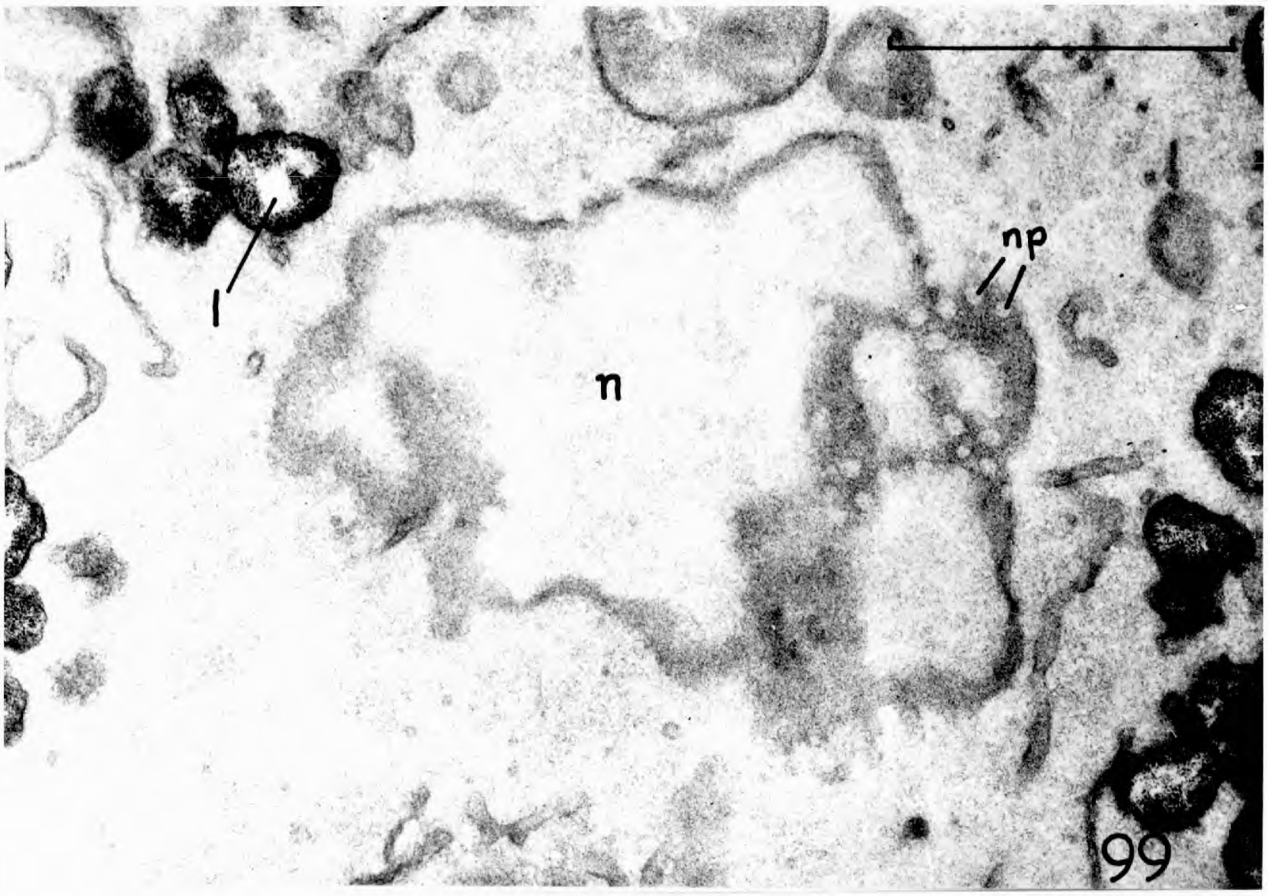
97

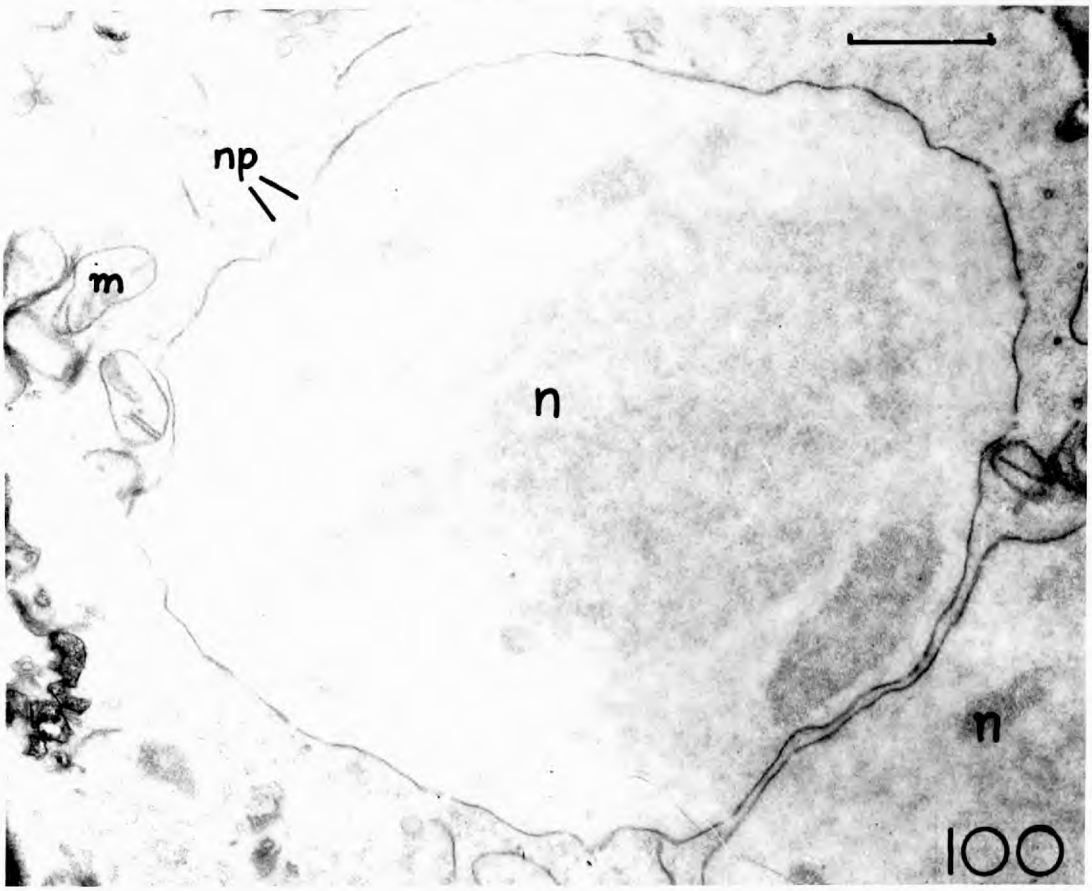


98

99. Section through a nucleus (n) of a uredospore showing the grouping of nucleolar pores (np) in surface view. Note form of the lipid droplets (l). $KMnO_4$. 1529/6. 14288.

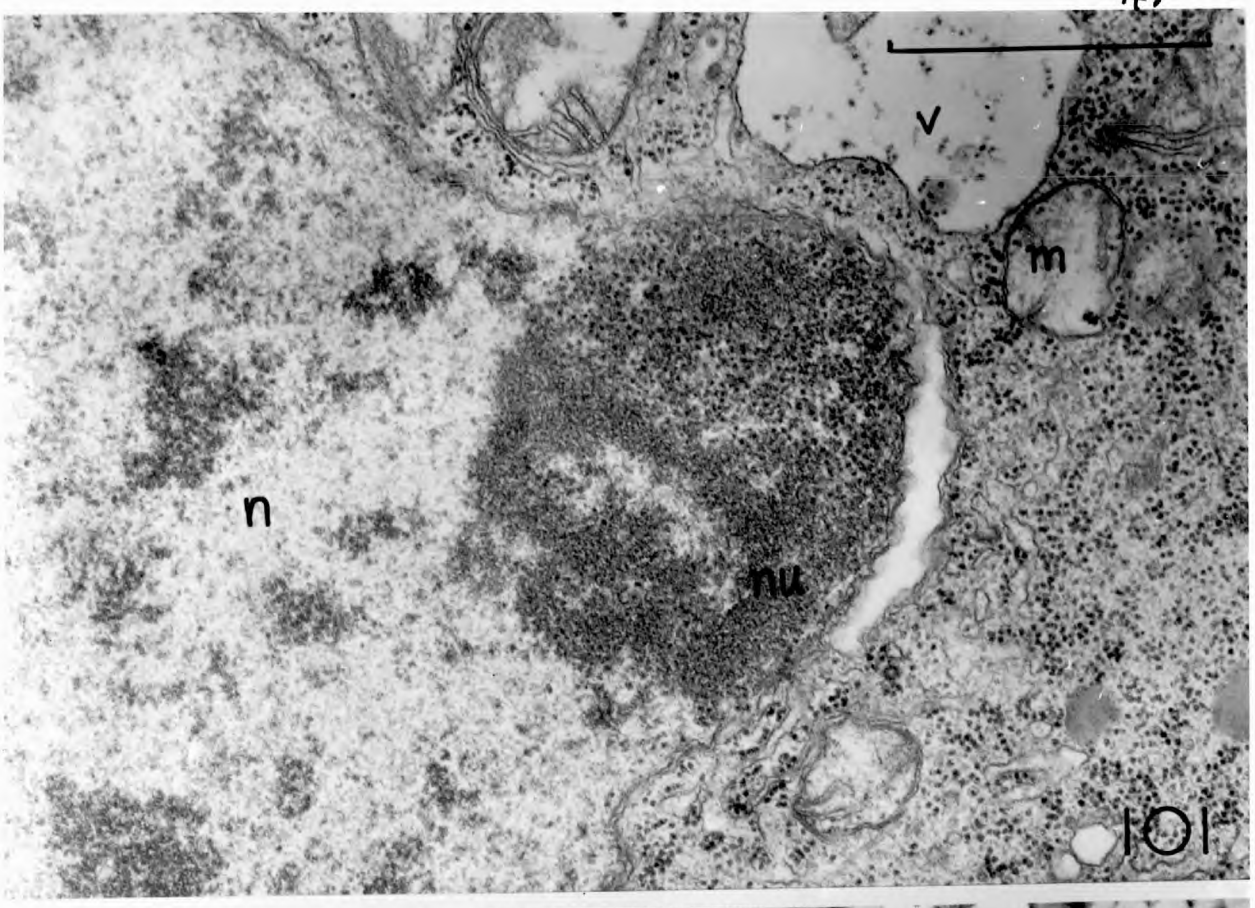
100. Section through the nucleus of a uredospore showing the grouping of the nuclear pores (np) in cross section. $KMnO_4$. 1529/7. 14298.


l
np
n
99

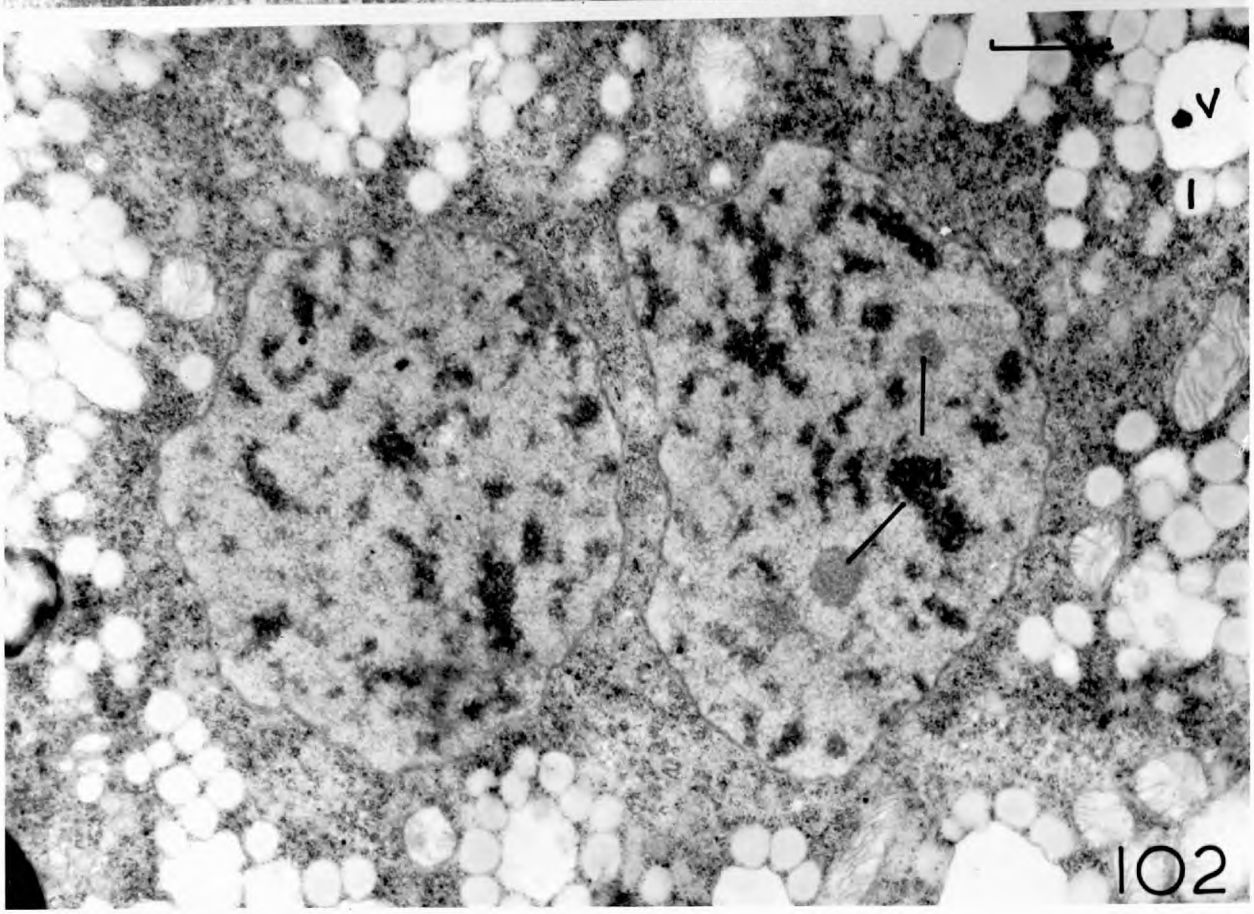

np
m
n
n
100

101. A nucleolus (nu) of a nucleus (n) in a developing uredospore showing three distinct areas, a dense finely granular area similar to the chromatin areas of the nucleus, an area of large granules, slightly smaller in size than the cytoplasmic ribosomes and clear areas. 1382B/65. 14248.

102. Section through the nuclei of a nearly mature spore showing two pale bodies (nu) within one nucleus. 1382B/11. 14189.



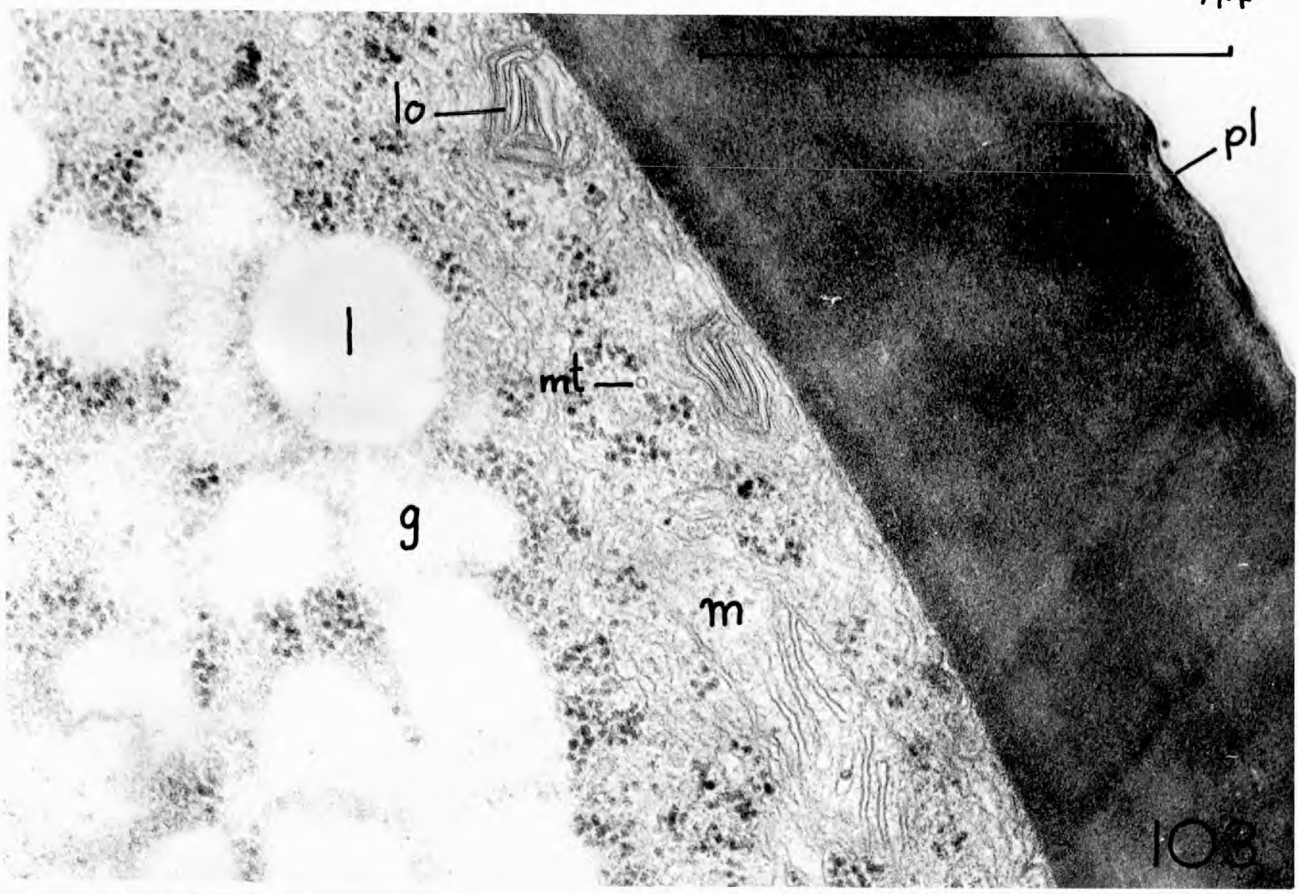
101

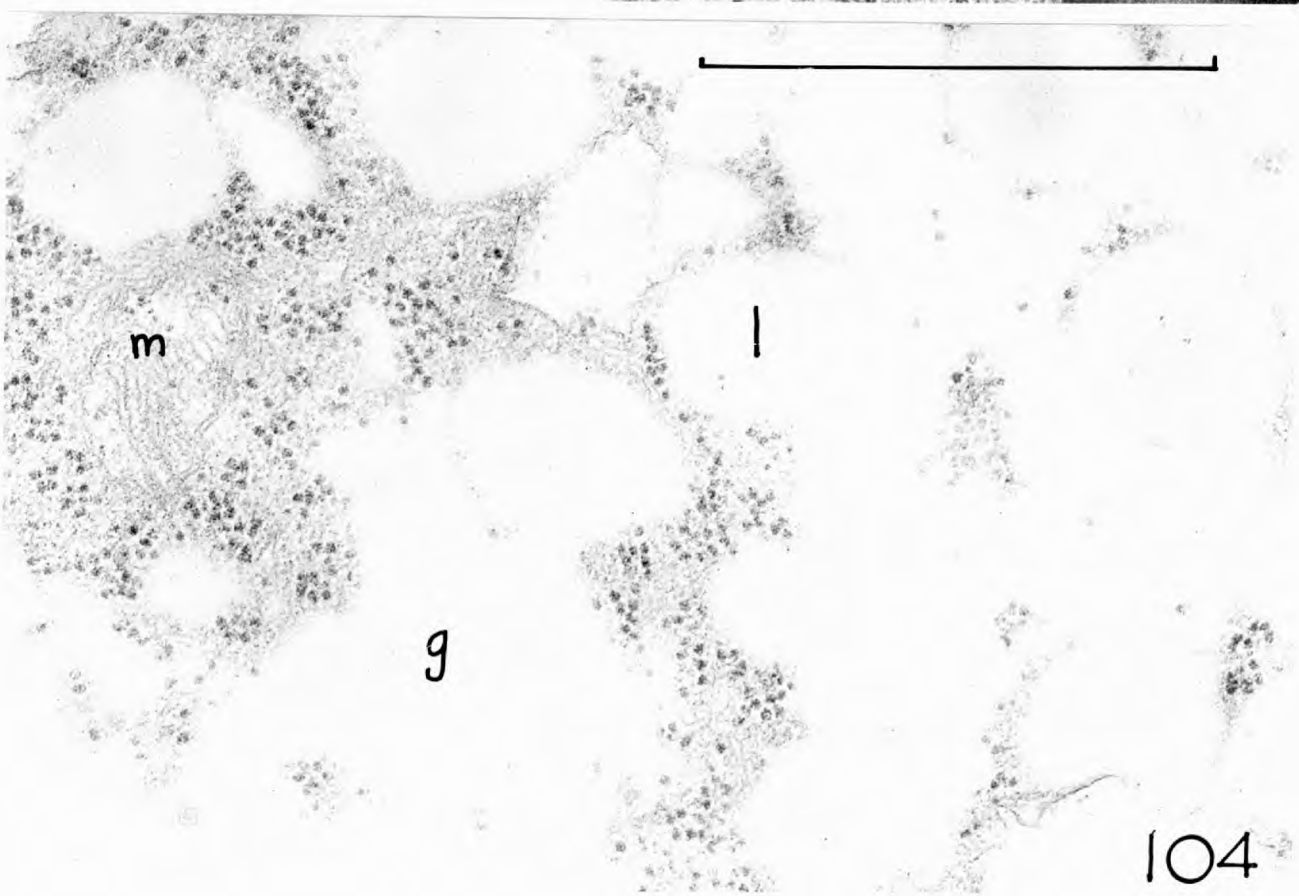


102

103. Section through a uredospore before detachment showing lomasome-like bodies (lo) against the spore wall. Note different staining characteristics of the outer part of the triplet membrane. 1382B/33. 6268.

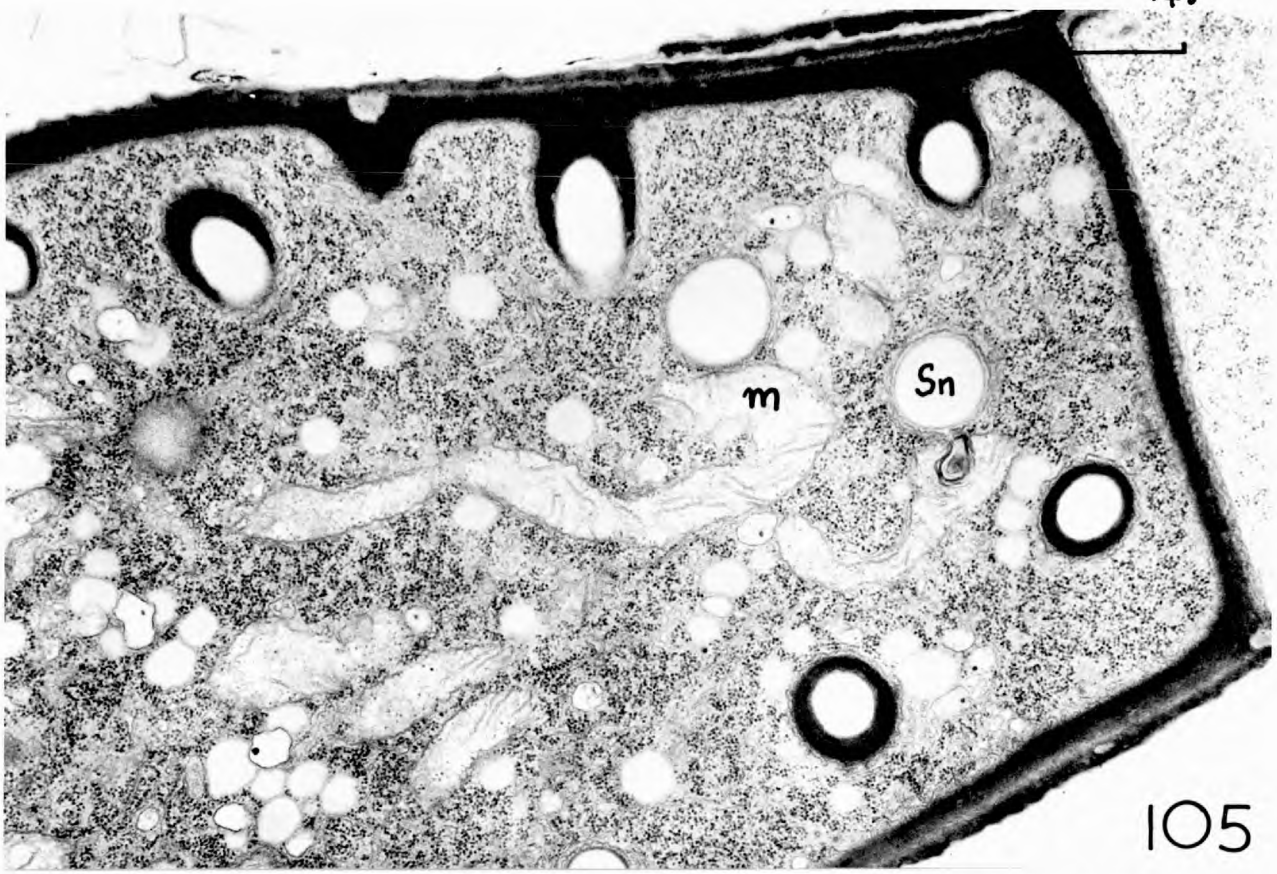
104. Detail of the same uredospore in Fig. 103 showing the large aggregates of granular material (g). 1382B/33. 6269.


lo
pl
l
mt
g
m
103

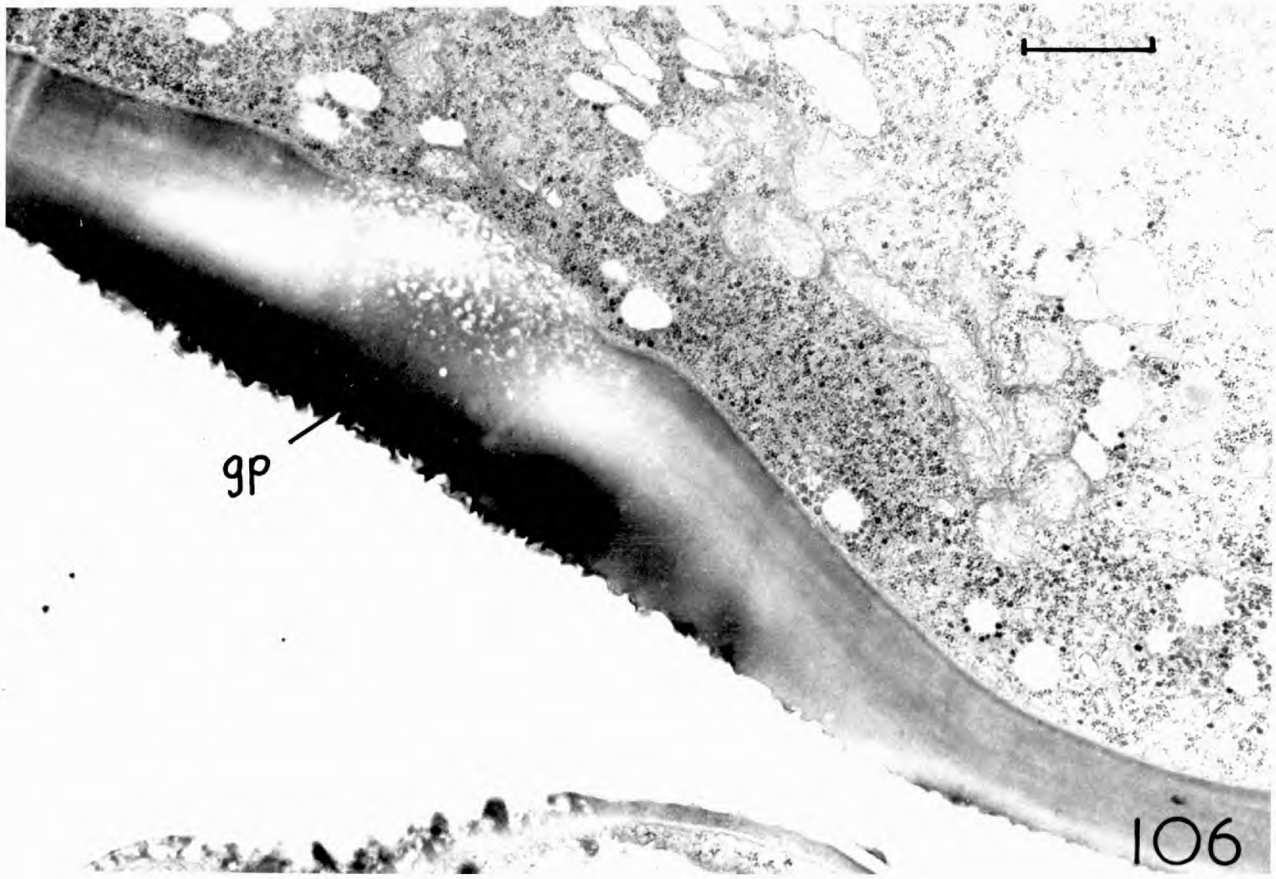

m
l
g
104

105. Section through developing spines (Sn) surrounded by secondary wall. Note the long mitochondrion (m). 1382B/23. 14194.

106. Section of a mature uredospore wall through the region of a potential germ pore (gp) (cf. Fig. 137). 1382B/76. 8193.



105



106

5. HISTOCHEMISTRY OF CELL WALLS

(a) Light Microscopy

(i) Staining with Toluidine Blue O

The metachromatic reactions of 2μm sections of A/G fixed material to toluidine blue O are given in the following table (from Fig. 8, 34).

STRUCTURE	COLOUR
Host cytoplasm	blue
Starch	none
Mesophyll cell walls	red-purple
Vessel walls	blue-green
Fungal cytoplasm	deep blue
Intercellular hyphal walls	blue
Haustorial body walls	blue
Haustorial sheath, inner layer	none
Haustorial sheath, outer layer	red
Uredospore walls	blue
Uredospore spines	none

(ii) Fluorescent staining with Aniline Blue

Sections of A/G material mounted in the stain and viewed with visible light showed no staining (Fig. 107).

When the same section was examined under U.V. light using the following combinations of filters: exciter filter UG 1/2.5mm + BG 12/3mm, placed between the source and the specimens and absorption filter GG 9/1mm, between the

objective and the ocular lenses a yellow fluorescence could be observed against the dark background in haustorial sheaths (Fig. 108). This reaction was not apparent in all haustoria examined.

In fresh hand sections of Vitis vinifera treated in a similar way a yellow fluorescence was observed in areas of the sieve plate regarded as containing callose.

(iii) Staining with Resorcinol Blue

The sieve plates of phloem cells of V. vinifera stained blue as did also some sheaths of haustoria in 2μm sections of A/G infected leaf tissue.

(iv) Reactions to IKI/H_2SO_4

Fresh uredospores mounted in IKI did not become stained blue in any part. Immediately on being irrigated with 63% sulphuric acid the spores contracted suddenly, taking the form of a 'dough-nut' and then re-expanded more slowly to their original shape, but with hemispherical protrusions at each germ pore. No features of the spores stained on irrigation with the acid. Spores irrigated with concentrated H_2SO_4 underwent the same changes but cytoplasmic material was then seen to move into the protrusions at the germ pores, followed by the sudden rupture of the covering membrane of the pores and release of the contents of the spore into the mountant. The spore did not become completely dissolved but

was rapidly reduced to an empty, round translucent shell, devoid of spines and with two pores in the position of the original germ pores.

(v) Staining with Ruthenium Red

Ruthenium red gave a general staining in A/G sections and no differentiation of specific structures could be obtained.

When fresh uredospores were mounted in this stain the spines were not stained but the wall appeared pink, with a greater concentration of the colour in the hilum region. It could not be determined whether the wall or the cytoplasm in this region was reacting more intensely to the stain.

(vi) Examination in Polarised Light

Fresh and F.A.A. fixed but unembedded sections of infected tissue were examined. No parts of the fungal walls in intercellular hyphae or in uredospores exhibited birefringence.

(vii) Reactions after extraction with KOH/Acetic Acid

Unembedded material autoclaved in KOH and treated subsequently with either 1% H_2SO_4 in IKI or 2% acetic acid gave the reactions summarised below

REAGENT	MYCELIUM	UREDOSPORES	CHITIN (BDH)
1% H_2SO_4 in IKI	violet colour	negative	violet colour
2% Acetic Acid	dissolved	dissolved	dissolved

(b) Electron Microscopy of Epon Embedded Specimens

Untreated material comparable with that described in Sections 2, 3 and 4 was used in these experiments.

(i) Material extracted with Schweitzer's Reagent

The same results, except where otherwise noted, were obtained using fresh material, or material fixed in F.A.A. or Glut/Os prior to extraction.

Cytoplasmic contents were severely disrupted and unrecognisable except in uredospores (Fig. 121) where, although all cytoplasmic membranes were lost, remnants of the nuclei and nucleoli could still be identified after F.A.A. fixation.

The cell walls of the intercellular hyphae and the host were altered and appeared looser in general texture to those of the controls (cf. Fig. 23, 109). There was also a decrease in the overall electron density of fungal walls and an increase in that of the host wall so that at low power they appeared similar in general contrast (Fig. 114). However, the decrease in electron density did not apply to the electron dense layer of the body wall of the haustorium (Fig. 111) or to the wall of the uredospore (Fig. 122) which

appeared unaltered in their staining reactions and structure.

Material in the angle of contact between adjacent cells persisted after treatment (Fig. 109) as did also some rows of superficial fibrils projecting into the intercellular spaces attached apparently to the pellicle (Fig. 110).

The fine structure of the more important features is given in greater detail below.

1. Host Walls

Host walls appeared to be composed of a residue of loosely arranged electron dense fibres (Fig. 115) in which some degree of orientation in directions parallel to the surface of the wall was evident (Fig. 117). The walls appeared generally to be swollen, although to varied and not precisely measurable extents, and they were convoluted, as if the host cells had partially collapsed with a consequent folding of the wall (Fig. 114).

A loss of fibrillar material was apparent within a zone extending for a distance of about 0.8μm from the margin of the penetration pore (Fig. 117, 118). The fibrils remaining in this region although in more random orientations were in other respects similar to those of the fibrillar material elsewhere in the wall.

2. Fungal Walls

Walls of the fungal hyphae were more open and granular in texture than untreated walls, but since the latter varied

considerably in thickness it was difficult to determine if any swelling had taken place on treatment (Fig. 110). A dark layer, corresponding to the pellicle, was present and also a thin dark layer lining the inside of the hyphal wall (Fig. 110).

3. Haustorial Mother Cell

The wall of the h.m.c. appeared similar to those of the intercellular hyphae (Fig. 114). There was no conspicuous swelling of the fungal wall in the region of contact with the invaded host cell, even when this area was small (Fig. 114, 115). The material remaining in the angle of contact between the h.m.c. and the host cell was often distorted and did not possess the smoothly curved surface found in untreated material (Fig. 117, 119). The h.m.c. wall tapered to the penetration pore and appeared closely bonded to the outer margins of the host wall (Fig. 117-119).

The inner, thin, electron dense layer in the h.m.c. appeared to pass through the penetration pore (Fig. 117, 119), although within the pore it was slightly altered being less electron dense, more uneven and frequently discontinuous (Fig. 118).

4. The Sheath

The bulk of the sheath consisted of bundles of fibrillar material, randomly orientated, but similar in appearance to that remaining in the host cell wall. These bundles often appeared isolated from each other by clear gaps (Fig. 117) and there were also patches of amorphous material (Fig. 117,

118), presumably the degraded remnants of the pockets of sequestered membrane typically present in sheaths (e.g. Fig. 49). An electron-lucent region, bordered on its external face by fibrillar material, was often present adjacent to the stem wall (Fig. 120). The area of contact between the sheath and the inner face of the host wall surrounding the penetration pore usually extended considerably beyond the zone of host wall which had lost fibrillar material (Fig. 115).

5. The Stem Wall

The thin electron opaque layer that could be traced through the penetration pore continued along the stem where it appeared to be bonded by bridges of material to the enclosing sheath (Fig. 116).

After treatment the neckband could no longer be recognised with certainty, but in one example the dense layer of the stem wall terminated a short distance from the end of the sheath and a body wall was not present beyond, although remnants of host and fungal cytoplasm formed a cup over the end of the stem (Fig. 114, 115).

6. The Body of the Haustorium

The residue of the body wall was composed of a granular, electron dense layer of uniform thickness (approximately 44nm) (Fig. 112). The outer pale layer of the body wall (e.g. Fig. 60) was absent, instead fibrillar material attached to the dark layer of the body wall, similar to that described for the intercellular hyphae (Fig. 110), extended

into the host lumen (Fig. 113).

7. Uredospores

The uredospore wall appeared completely unaltered by the treatment (Fig. 121). It was covered by the electron dense pellicle (Fig. 121) which was often slightly raised from the surface but continued unbroken over the spines (Fig. 122) and across to the stalk cell (Fig. 123).

The spines appeared unaltered by the treatment except for the central 'cavity' at the base where the material appeared to have coagulated (Fig. 122).

At the hilum region the spore appeared to be attached to the stalk cell only by the pellicle and by strands of material between the two halves of the septum (Fig. 123).

(ii) Treatment of Glut/Os fixed material with Concentrated Ammonia Solution

All specimens were embedded in epon after treatment.

During treatment with concentrated ammonia solution the dark staining usually present after Glut/Os fixation disappeared from the specimens. However, sections stained in the standard manner were still recognisable and cell walls, cytoplasmic organelles and membranes possessed normal image-contrast characteristics (Fig. 126, 128, 129). Membranes were frequently broken but had free edges instead of being formed into vesicles in the manner characteristic of poor fixation (Fig. 124, 127), indicating that this damage occurred

during treatment or subsequent embedding.

Lipid droplets, still present in the fungal cytoplasm, had lost their staining properties and were almost electron transparent (Fig.131). Ribosomes were absent but in the haustorium other granular material of the cytoplamic matrix (Fig. 130), of similar appearance to that in untreated cells (e.g. Fig. 104), could be identified.

Material remaining in the angle of contact between adjacent cells (Fig. 124) was fibrous and electron dense as in untreated material (e.g. Fig. 24).

1. Structure of the Host Walls

The host wall had a similar appearance to untreated walls (Fig. 124) both in electron density and organisation of the fibrillar material.

2. Structure of the Fungal Walls

The walls of the intercellular hyphae had a uniform granular texture but unlike untreated and Schweitzer's reagent treated material were almost completely electron-lucent and paler than the embedding medium (Fig. 124), except for a quite dense but thin surface layer.

Two layers were present in the body wall of the haustorium (Fig. 126). The outer layer, which often appeared severely eroded (Fig.127) was otherwise little altered from that of the untreated material (e.g. Fig. 60). The inner layer had lost much of its electron-scattering power (Fig. 125).

The uredospore walls were electron-lucent, except for the surrounding pellicle which could be traced over the spines and in continuity with the dark superficial layer of the stalk cells (Fig. 129). Dark linear flecks were still present in the spines (Fig. 131).

In some sections large crystals of stain were deposited on uredospore walls and spines but without obvious relationship to any definable structural detail within them (Fig. 129, 131).

(iii) Unfixed and Glut/Os fixed material treated with KOH/Acetic Acid

In unfixed material the cytoplasmic contents of the cells had completely disappeared (Fig. 132). In Glut/Os fixed material some remnants of the cytoplasm remained, although particular organelles could not be recognised, even in the uredospores (Fig. 137) which in other treatments were usually more resistant.

Material usually found in the angle of contact between adjacent cells was not present (Fig. 132, 134).

1. Host Walls

Fibrous elements were absent being apparently replaced by granular material, coarser in fixed than in unfixed specimens (cf. Fig. 133, 136). In fixed preparations the walls contained particles reminiscent of those of the

disorganised cytoplasm (Fig 136), but they were absent from unfixed material (Fig. 133).

2. Fungal Walls

The fungal walls were electron dense and granular (Fig. 132, 134), more consolidated and uneven in appearance in unfixed than in fixed cells (Fig. 133, 136).

In both fixed and unfixed material the wall of the body of the haustorium consisted of only one layer (Fig. 135) which was in each case comparable in general features with the walls of the intercellular hyphae which had received the same treatment (Fig. 133, 136).

In fixed specimens the wall of the uredospore was extremely granular and electron dense after treatment but was perforated at the sites of the two germ pores (Fig. 137). The wall surrounding the perforations tapered in thickness towards the internal openings which were approximately equal in diameter (2.5-3µm) to the conspicuous mottled plugs, now completely absent, which occupied the potential germ pores in untreated spores (Fig. 106). However, in addition to material corresponding to the mottled plugs extra wall material had been removed. On closer examination of sections of untreated spores a slight decrease in electron density of the wall was apparent in zones corresponding to those of the material that was missing in the treated spores (cf. Fig. 137, 148).

Spines, either developing within sockets or mature and

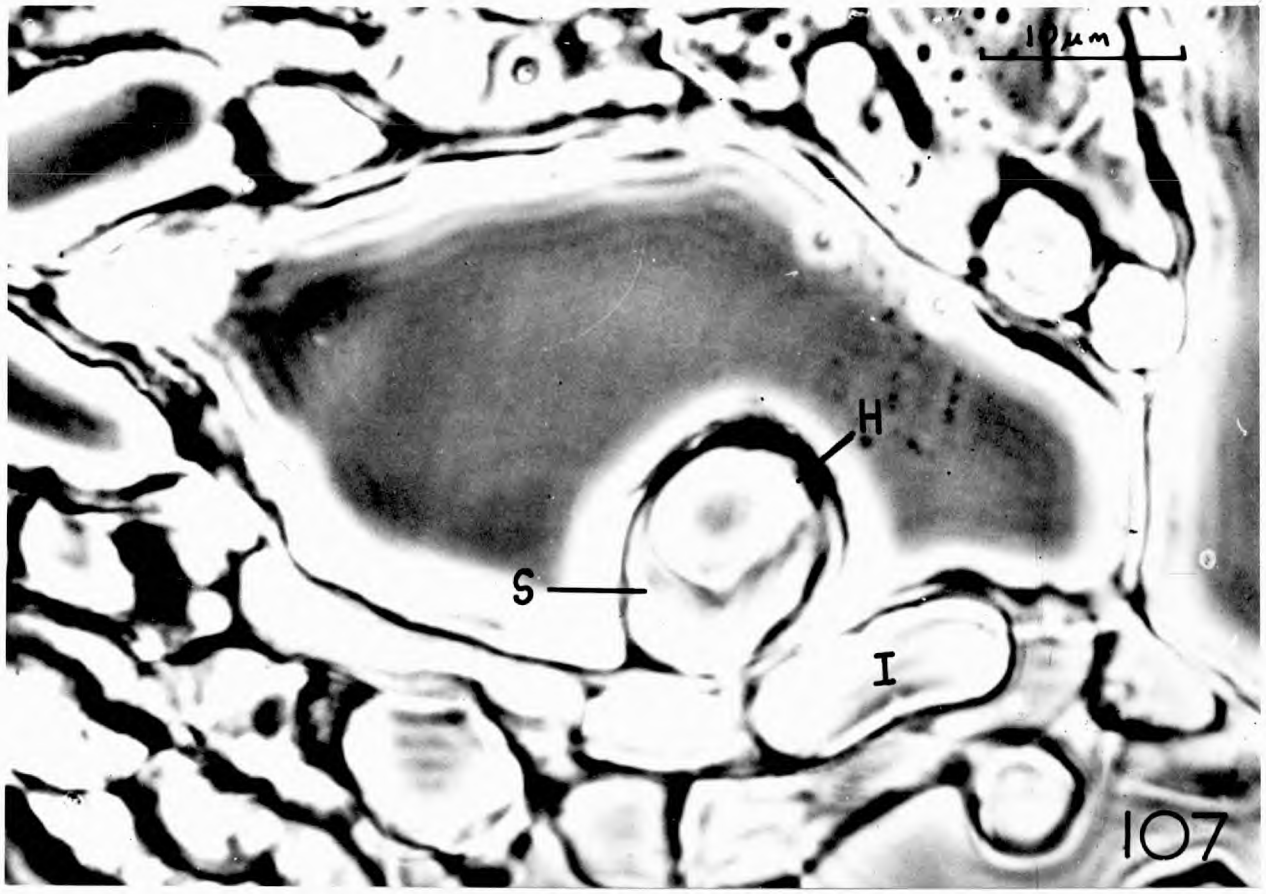
fully elevated to the surface of the spore, were severely affected by this treatment (Fig. 138-140). Remnants of the spines, in the form of a pale reticulum, remained in the base of the sockets (Fig. 139) or in mature spores were found at points in the surface (Fig. 140).

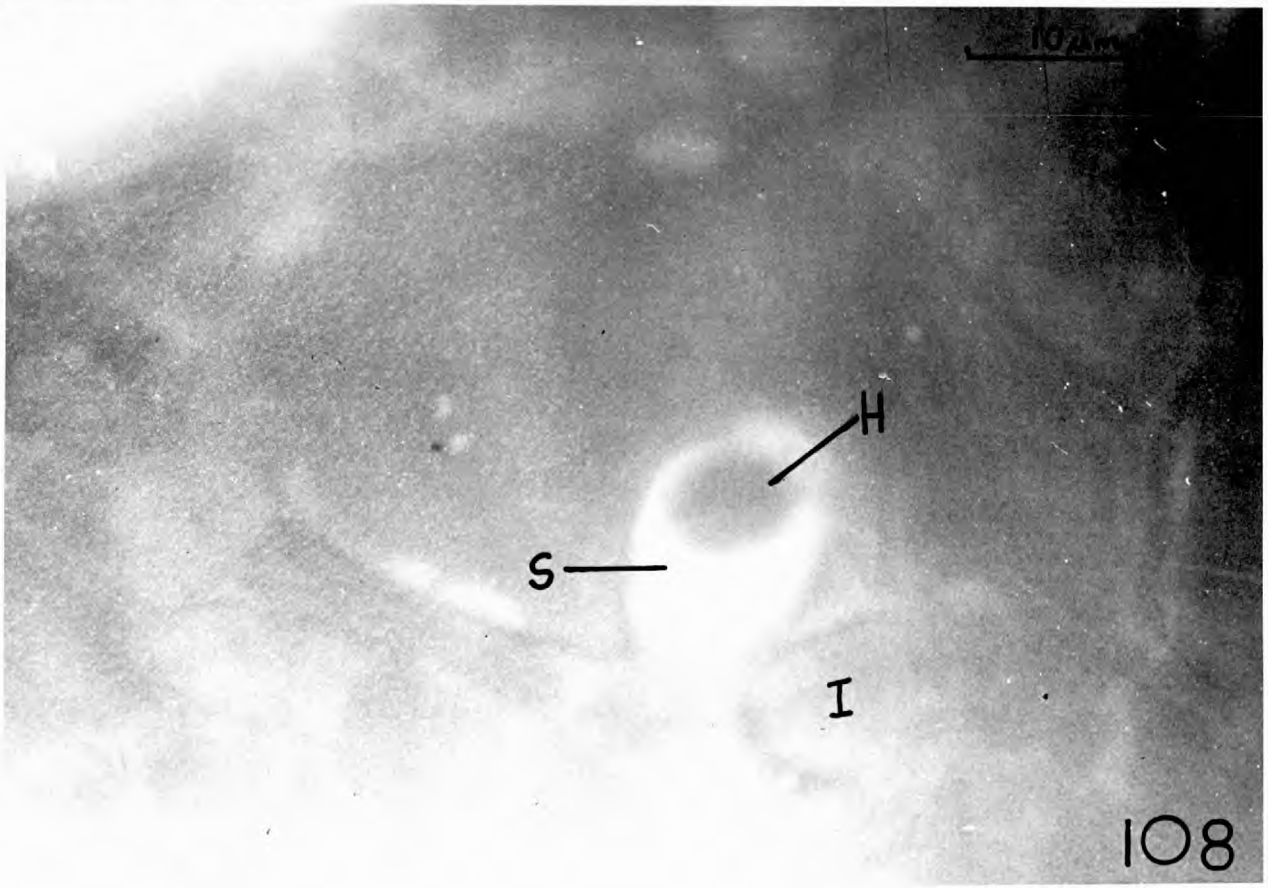
The thin electron dense pellicle, although not apparently completely destroyed, was disrupted and dispersed fragments were visible within the intercellular spaces (Fig. 137).

In unfixed material following KOH/acetic acid treatment the uredospores were completely dissolved.

107. Light micrograph of a haustorium (H) surrounded by a large sheath (s). Anoptoral phase. A/G. 1523. 177.

108. Same field as Fig. 107 viewed under U.V. brightfield. Section mounted in 0.001% aniline blue. A/G. 1523. 182.


10μm
H
S
I
107


10μm
H
S
I
108

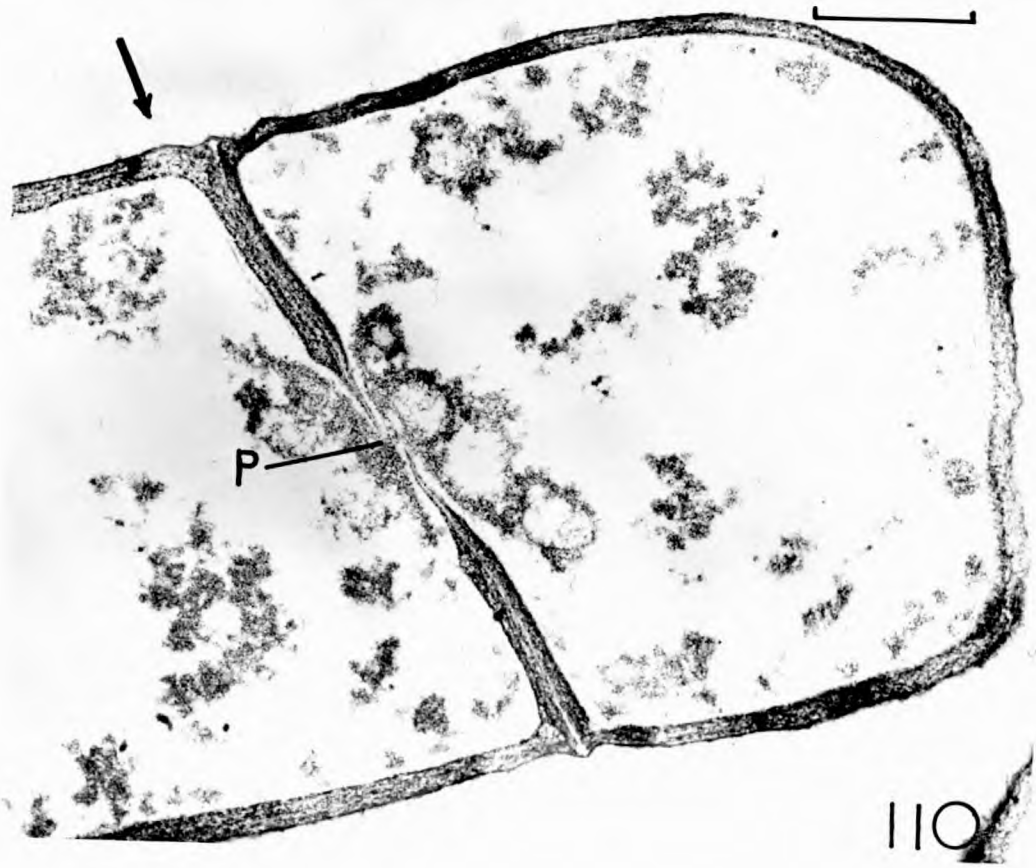
Figures 109-123 are of material extracted with Schweitzer's reagent.

109. Section showing the intercellular hyphae (I). F.A.A. 1775/1. 7355.

110. Intercellular hypha cut through the region of the septal pore (p). Note the fibrils (arrow) attached to the pellicle, the latter continuous over the edge of the septum, also the absence of wall material in this region. F.A.A. 1775/1. 7369.



109

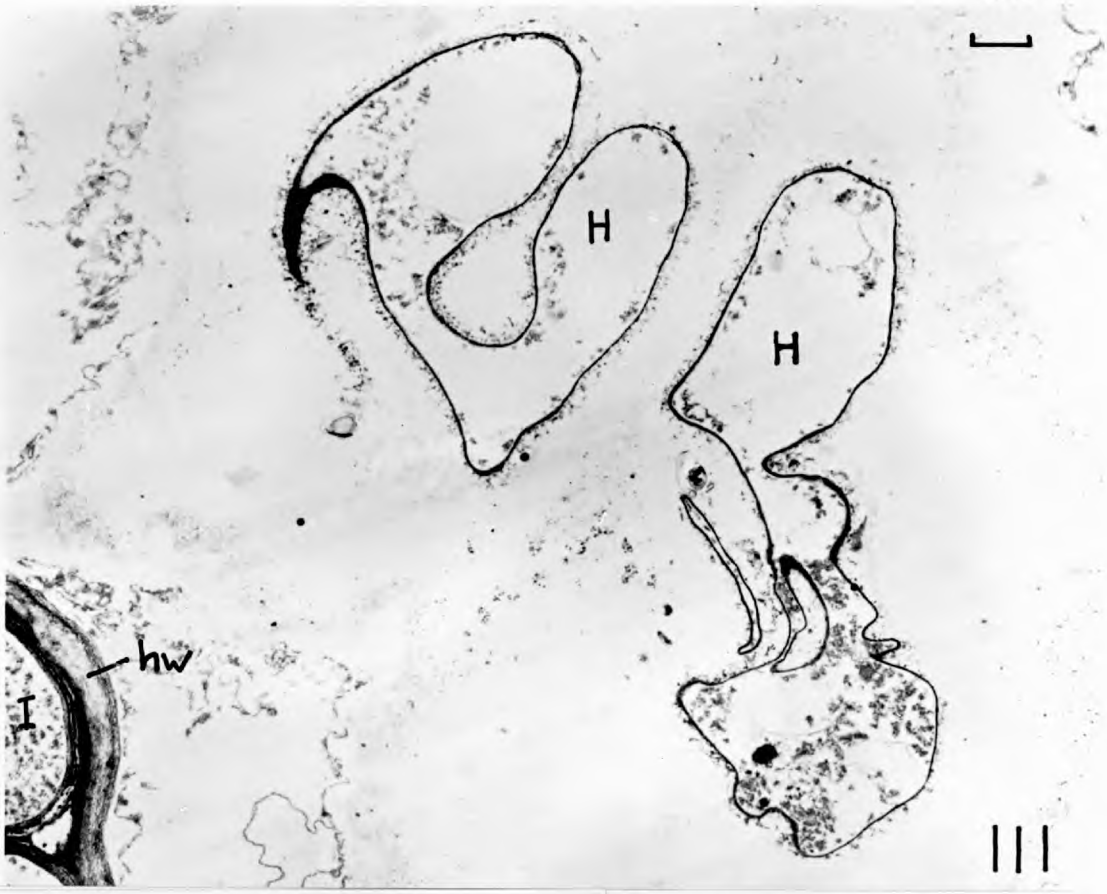


110

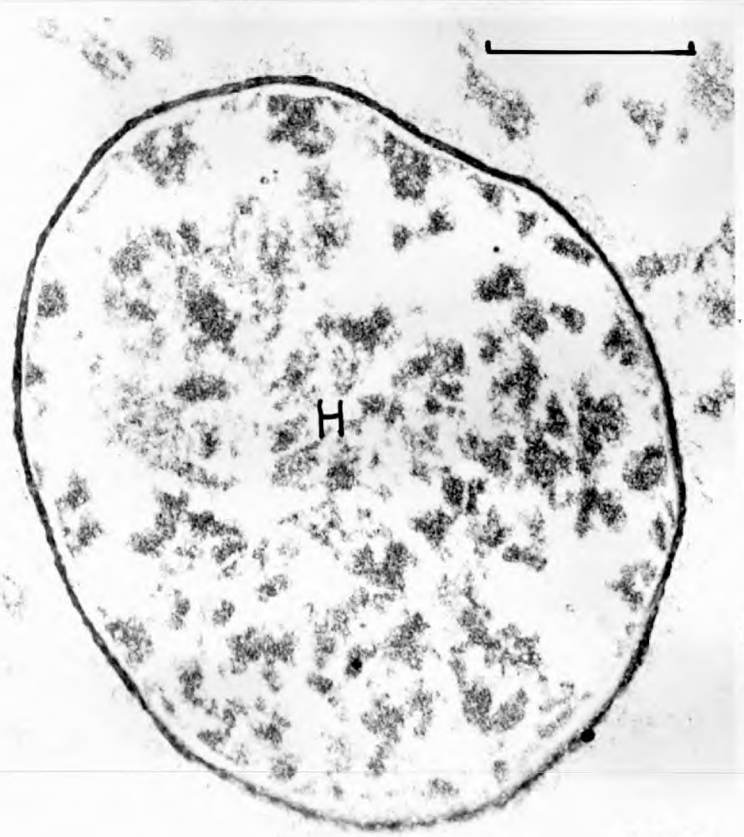
111. Section through the body region of a haustorium (H) within a host cell (hw). F.A.A. 1775/1. 7376.

112. Section of a haustorium showing the remains of the cytoplasm. F.A.A. 1775/1. 7363.

113. Detail of the body wall of a haustorium consisting of only one, electron dense layer. Note the fibrils (arrow) attached to its outer margin. F.A.A. 1775/1. 7367.



111



112

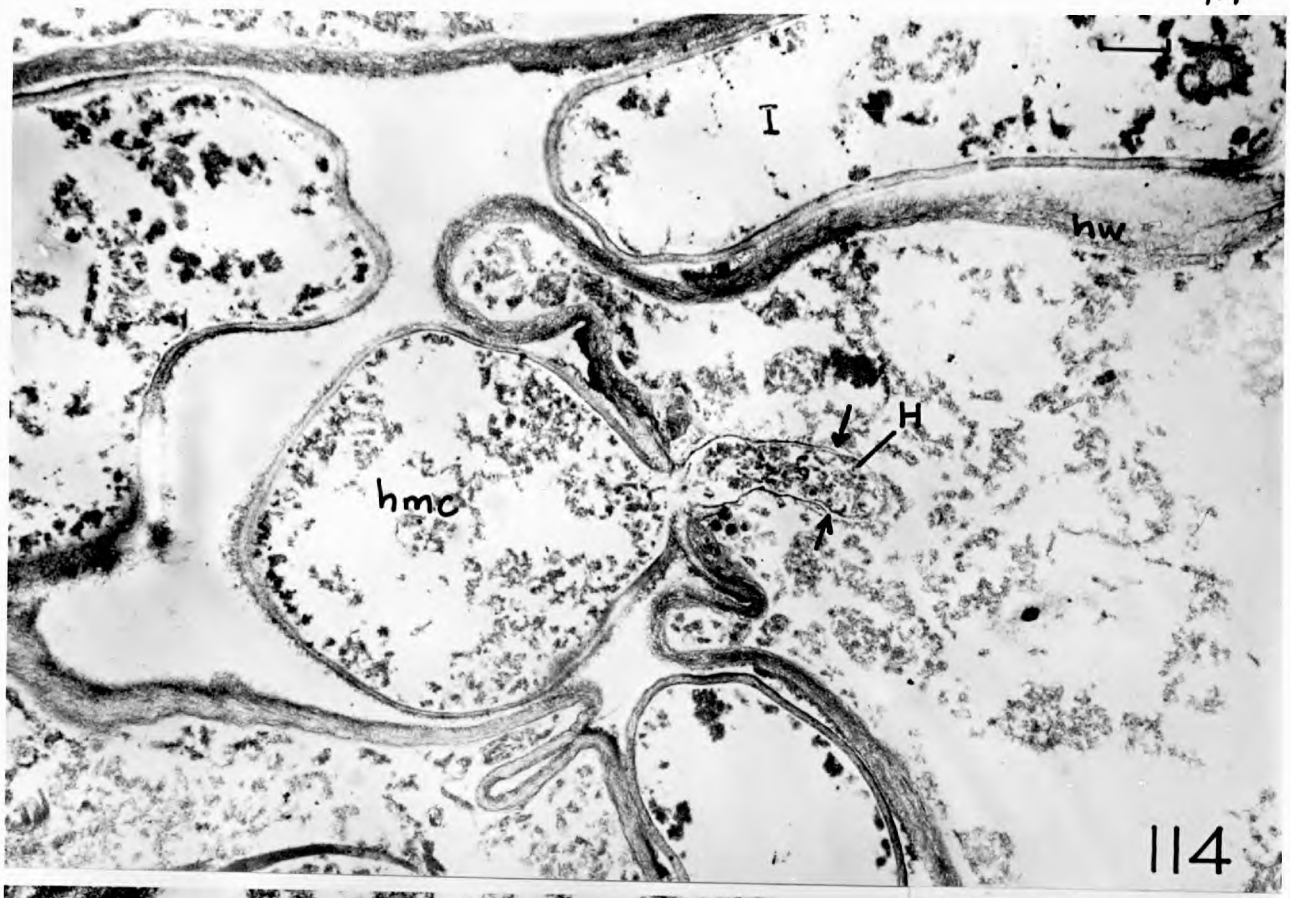


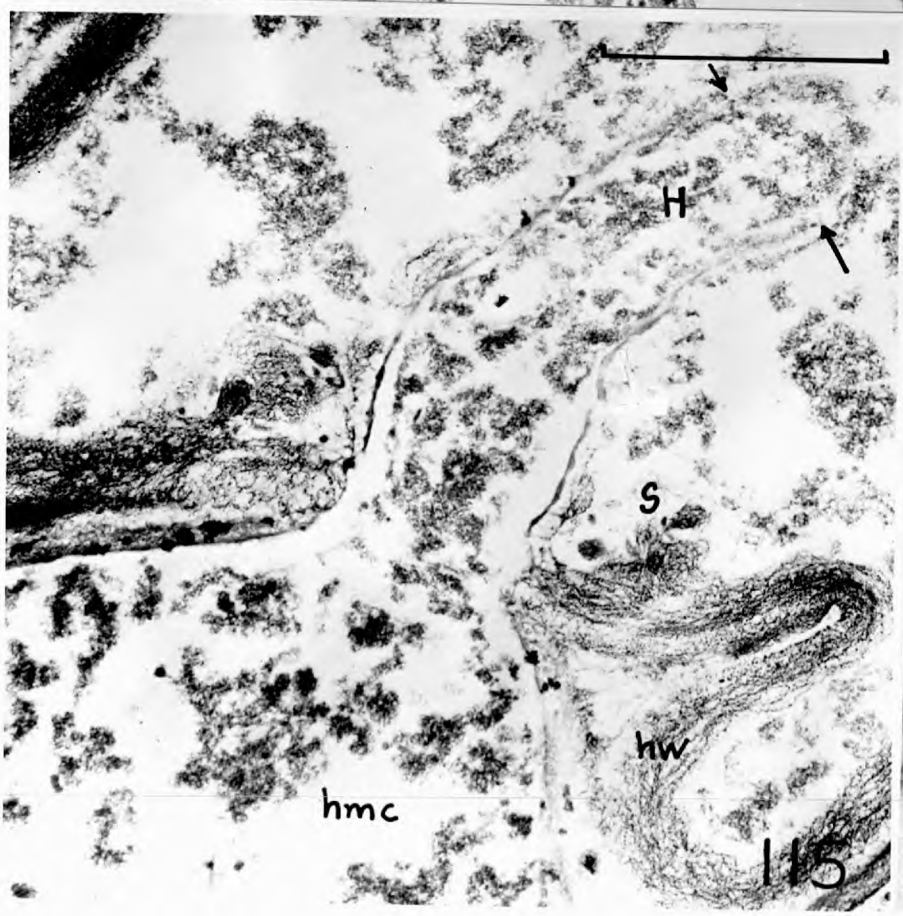
113

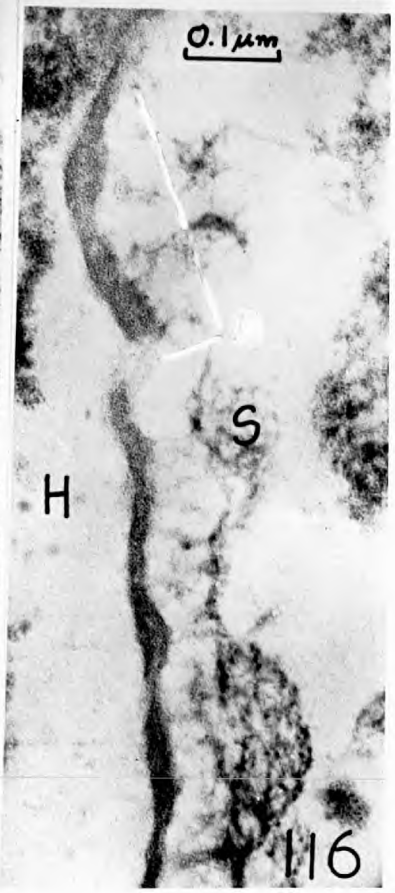
114. Section median to the penetration pore. Note the general similarity in electron density between the fungal and host cell walls and also the termination of the stem wall (arrows) surrounded by a hood of material from the host and fungal cells. F.A.A. 1775/3. 10530.

115. Section serial to Fig. 114 showing the termination of the stem wall (arrows) and the sheath material (s). F.A.A. 1775/3. 10525.

116. Detail of Fig. 115 showing the sheath (s) of fibrillar material around the neck wall and bridges of material connecting the two regions. F.A.A. 1775/3. 10526.


I
hw
H
hmc
114

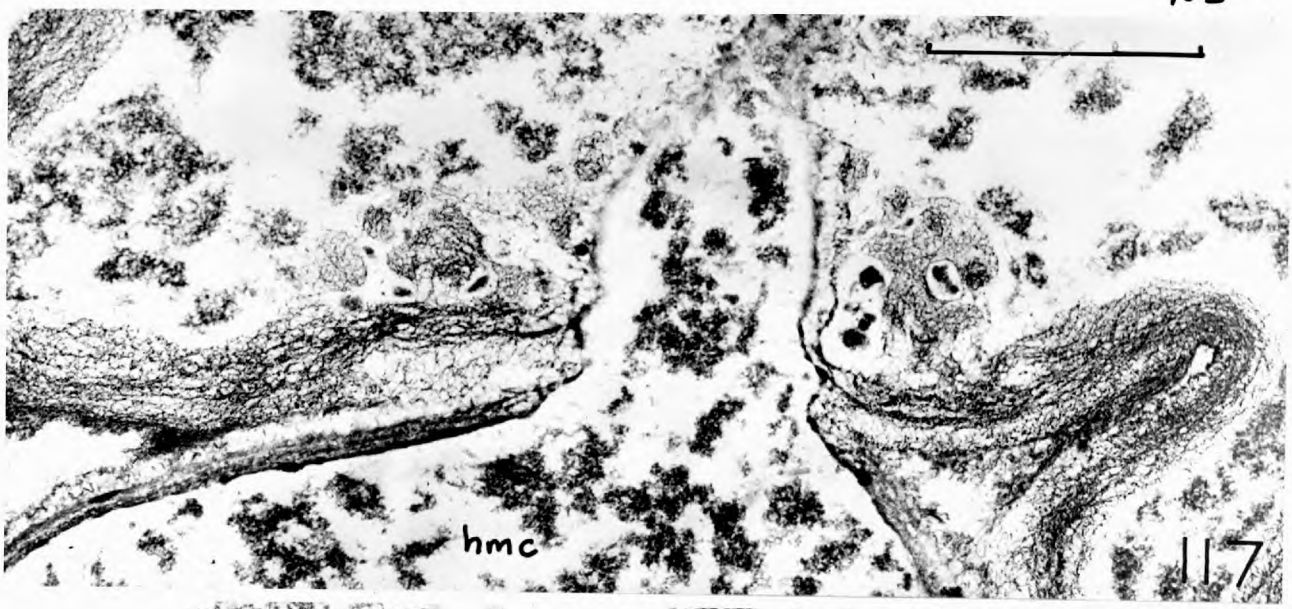

H
S
hw
hmc
115

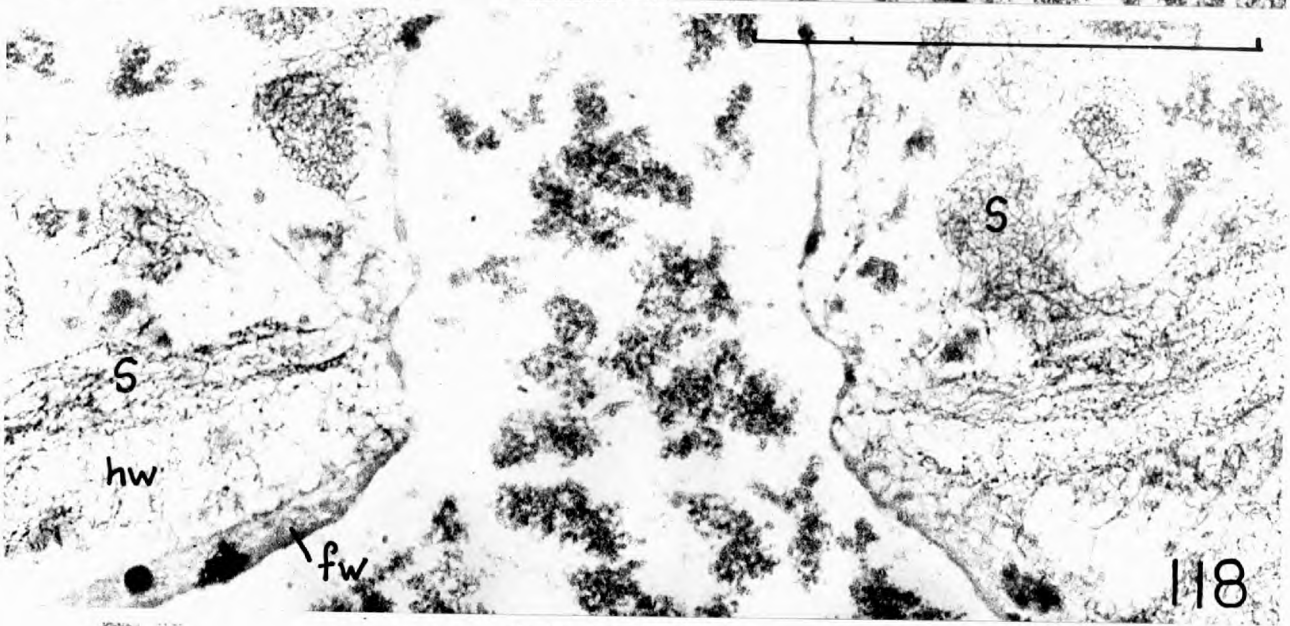

0.1 µm
S
H
116

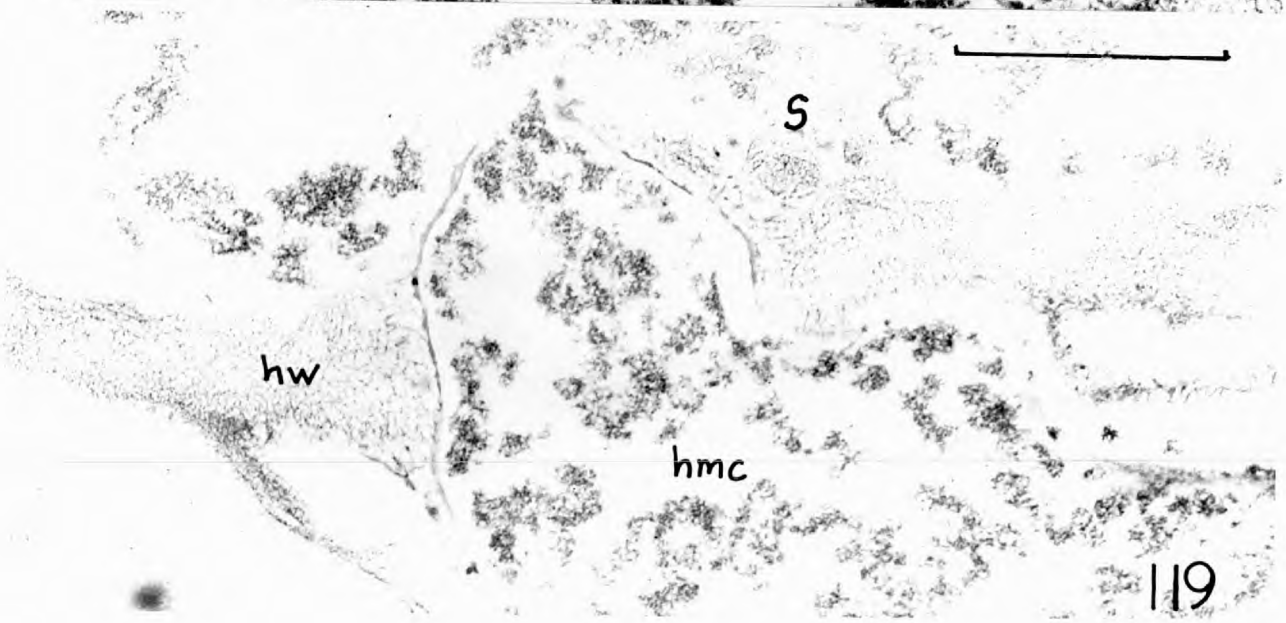
117. Section median to the penetration pore. No swelling of the h.m.c. wall is apparent. There is a reduction in the amount of fibrillar material within the host wall immediately surrounding the penetration pore. F.A.A. 1775/3. 10515.

118. Detail of the penetration pore showing the structural relationship between the sheath (s) and the remains of the host wall (hw). F.A.A. 1775/3. 10523.

119. Detail of the inner layer of the h.m.c. wall passing through the penetration pore where the layer appears to be less diffuse and is of a greater electron-opacity and also appears to be structurally bonded to the host wall (hw) and sheath (s). F.A.A. 1775/5. 10540.

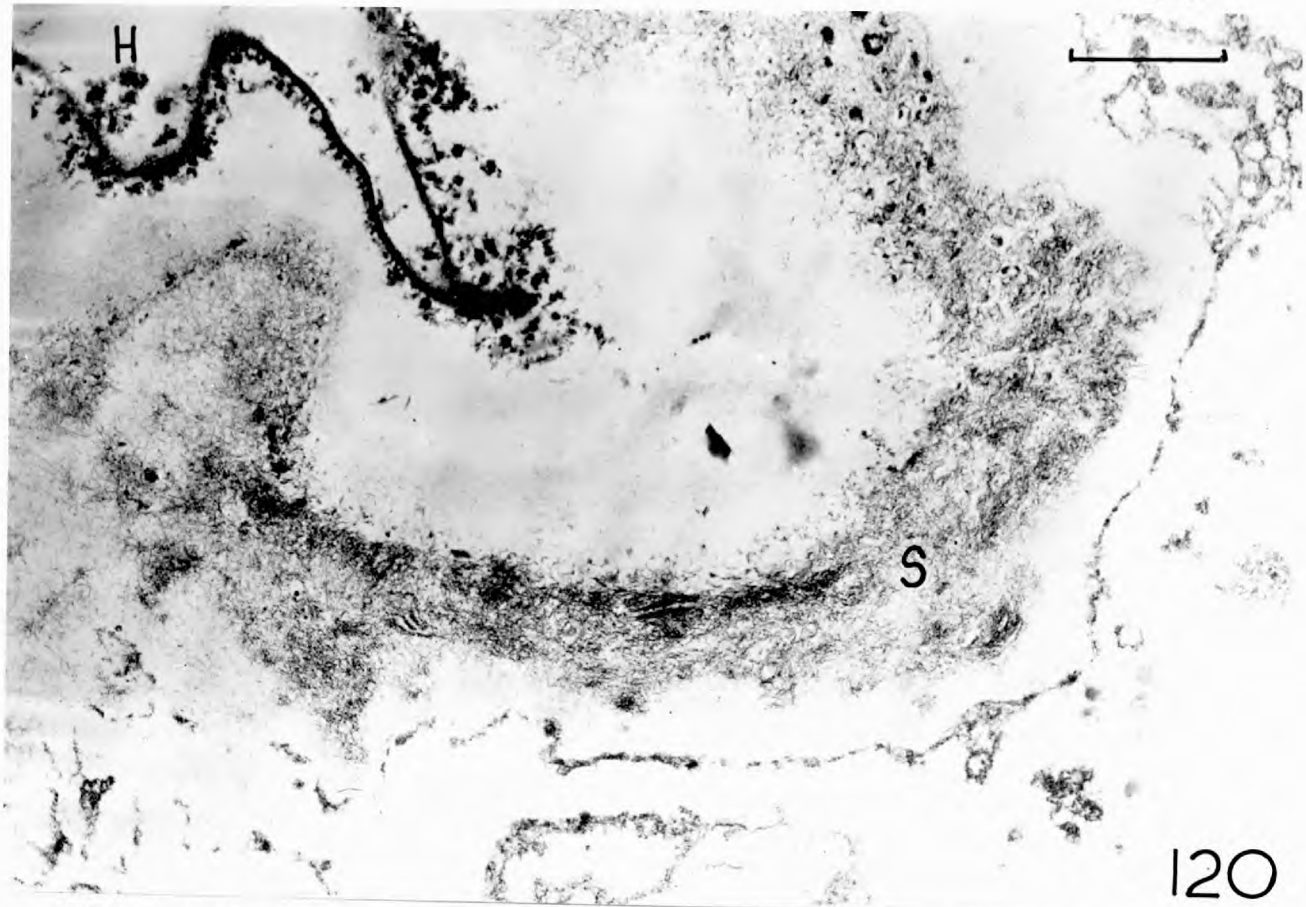

hmc
117


S
S
hw
fw
118

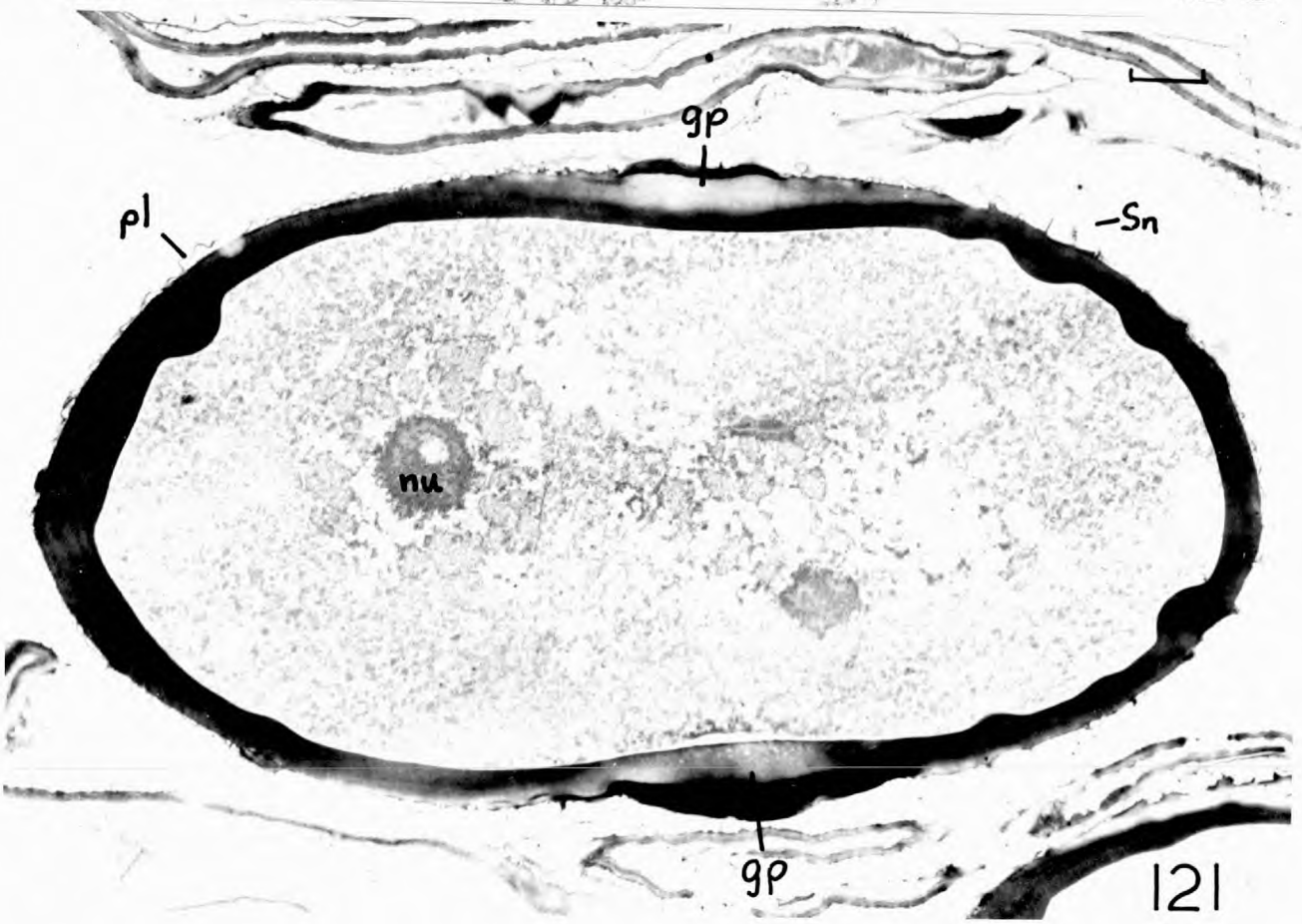

S
hw
hmc
119

120. Haustorium with a large sheath (s) of two distinct layers, a fibrillar zone surrounding and electron-lucent region. F.A.A. 1775/2. 7383.

121. Section of a uredospore median to the germ pore(gp). Its wall appears to be unaltered by the treatment. F.A.A. 1775/1. 7361.



120



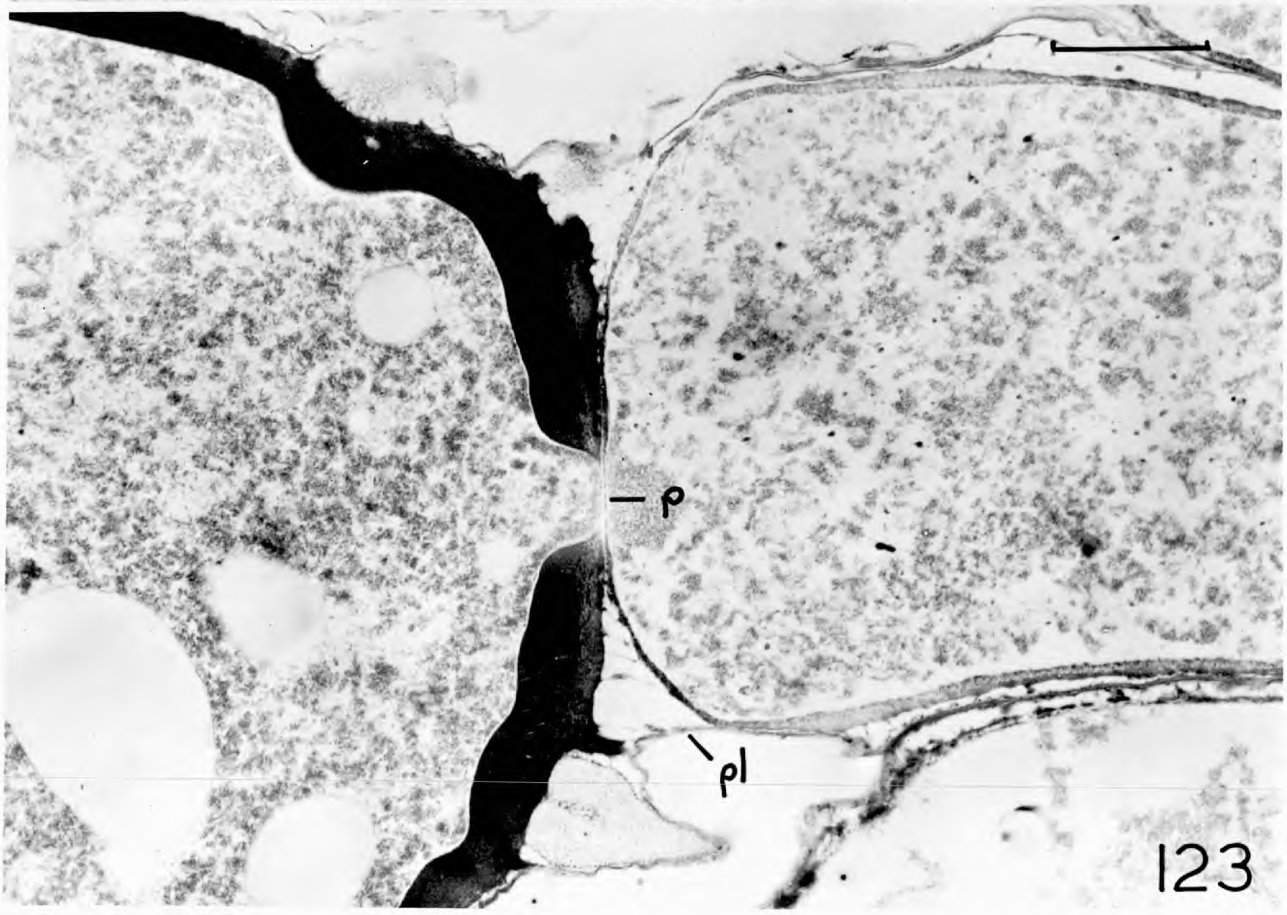
121

122. Section through a spine, apparently unaffected by the treatment apart from the material in the base which appears to have coagulated (cf. Fig. 96). F.A.A. 1775/1. 7375.

123. Section through the hilum region of a uredospore. Note the threads of material between the spore and the stalk cell and the continuity of the pellicle (pl) from one cell to the other. F.A.A. 1775/1. 7381.



122

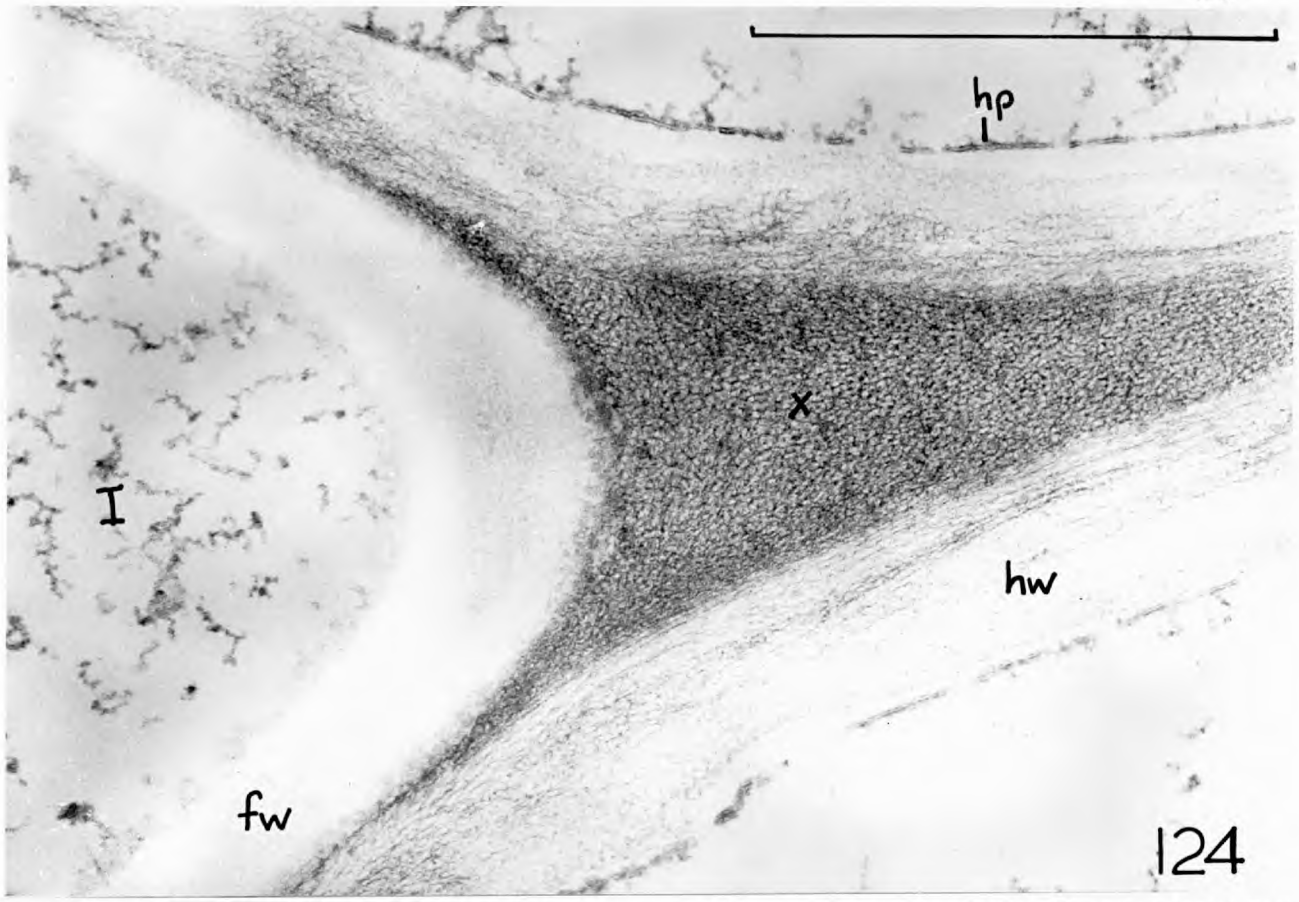


123

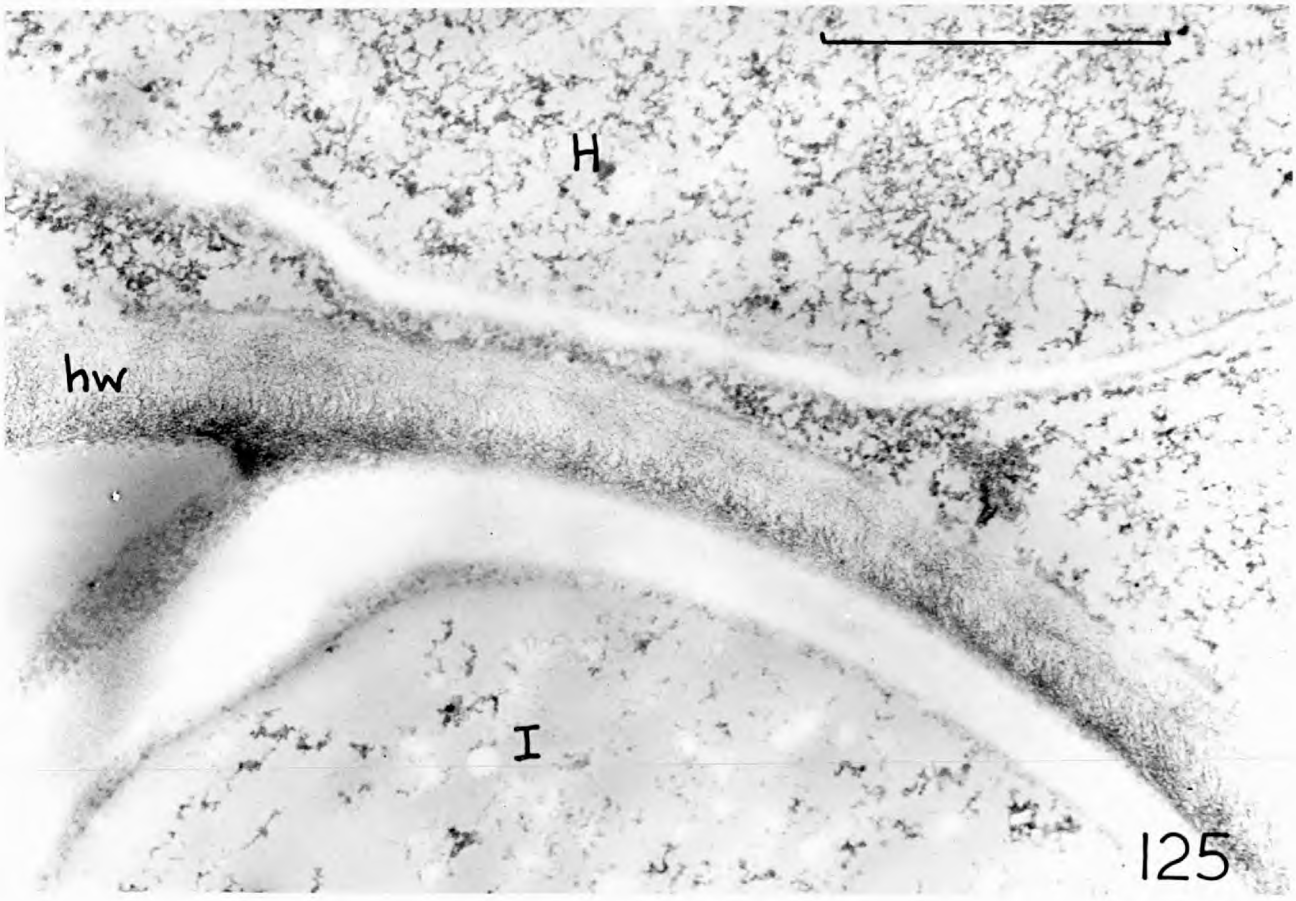
Figures 124-131 are of material extracted with concentrated ammonia solution.

124. Shows an intercellular hypha (I) between two host cells (hw). Note the absence of staining from the fungal wall (fw); the intercellular material (x) present between the cells, and the broken host plasmalemma (hp). 1871A/4. 12617.

125. Section showing a hyphal wall (I), a host cell wall (hw) and the body wall of a haustorium (H). Note the similarity between the haustorial and hyphal wall. 1871A/5. 12623.



124

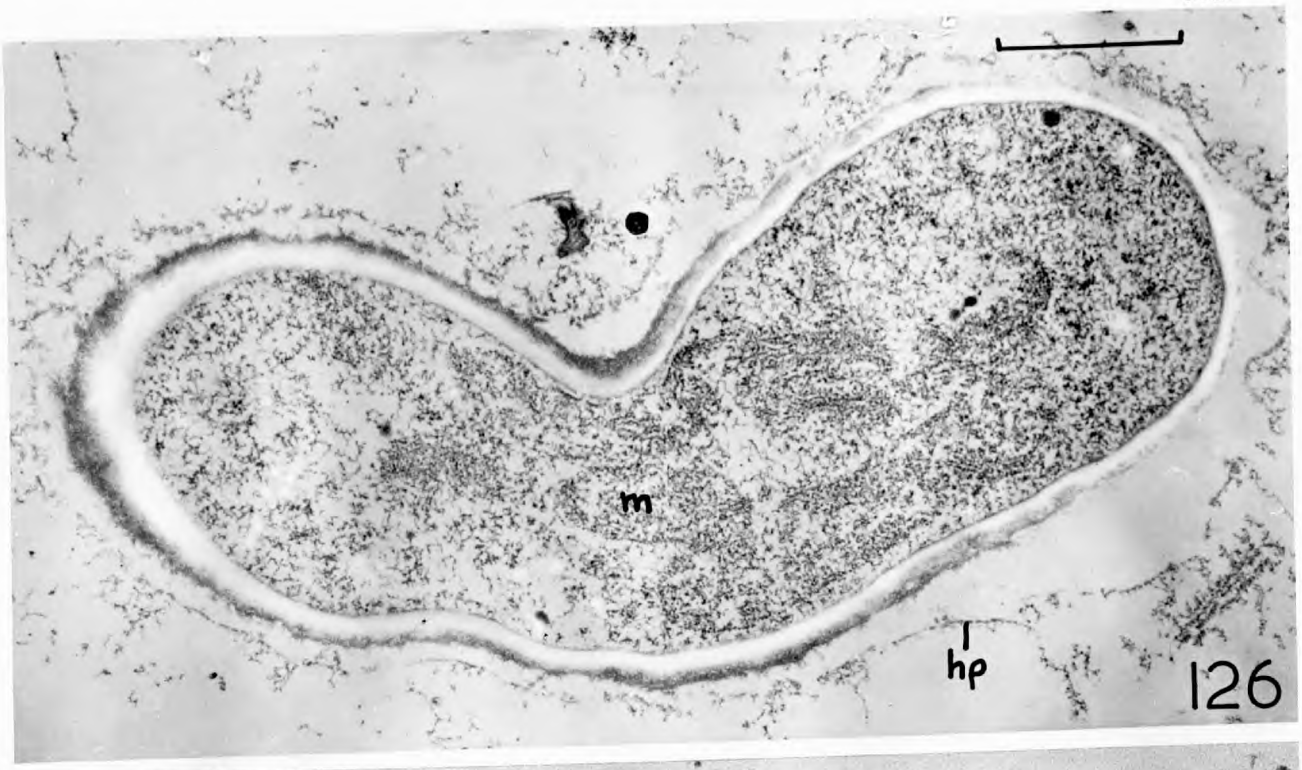


125

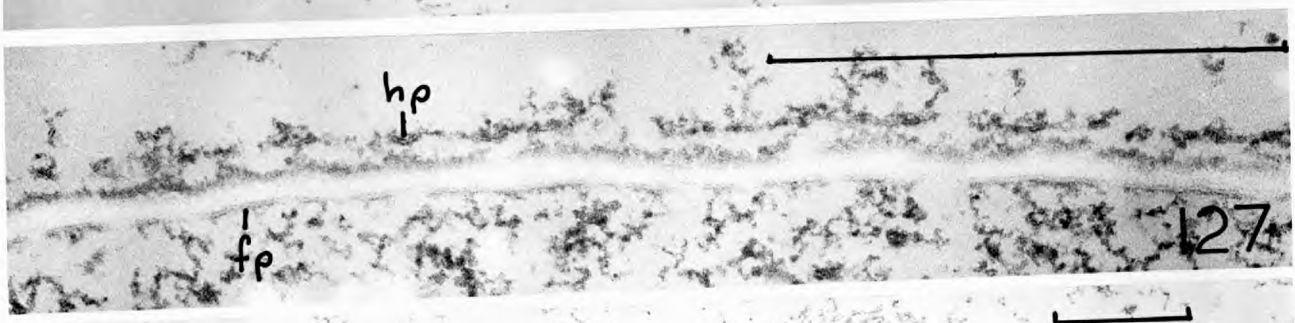
126. Haustorium showing the remains of a mitochondrion (m) within the body region. Note the surrounding disrupted host plasmalemma (hp) and the two layers of the body wall. 1871A/4. 12615.

127. Detail of the body wall of Fig. 128 showing its two layers, the outer one of which appears somewhat eroded. 1871A/4. 12613.

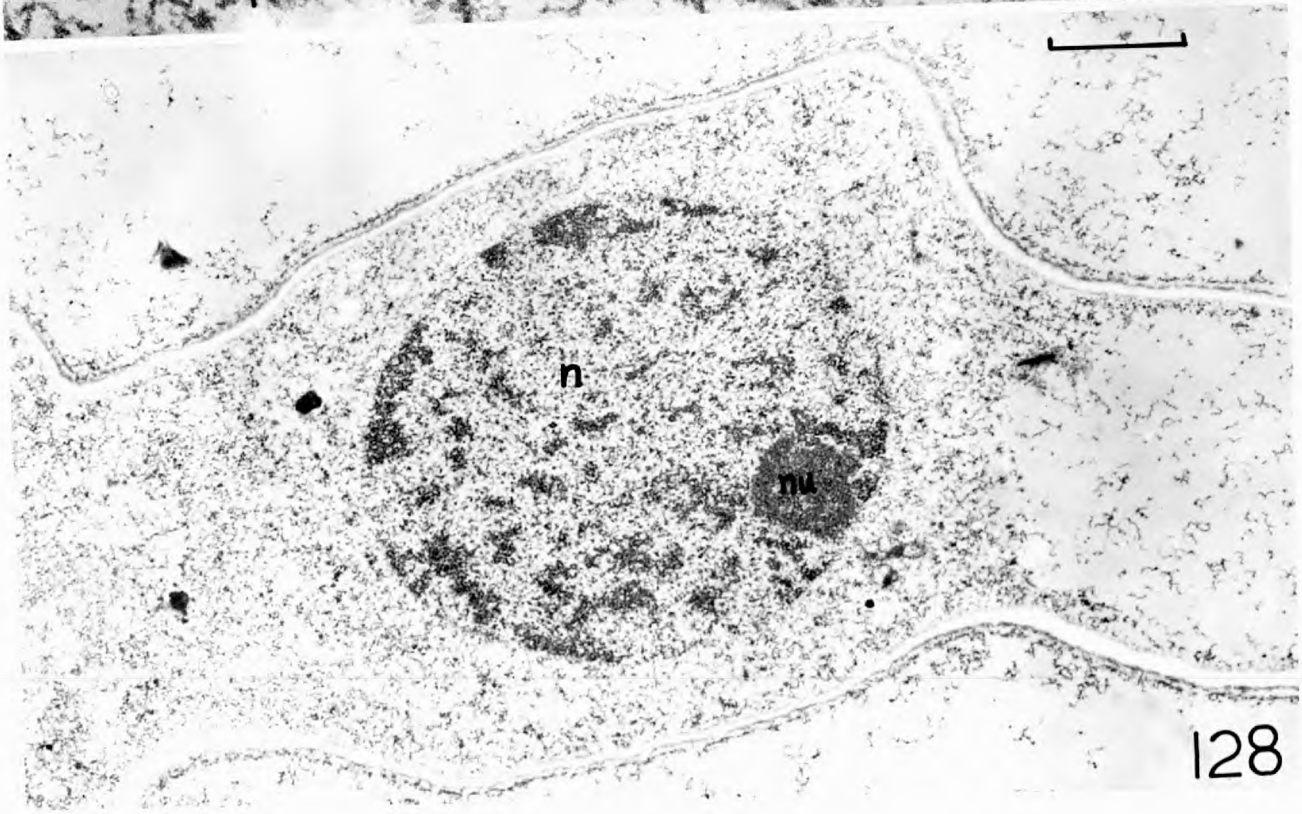
128. Haustorium with a large central nucleus (n) containing a nucleolus (nu). 1871A/4. 12614.



126



127

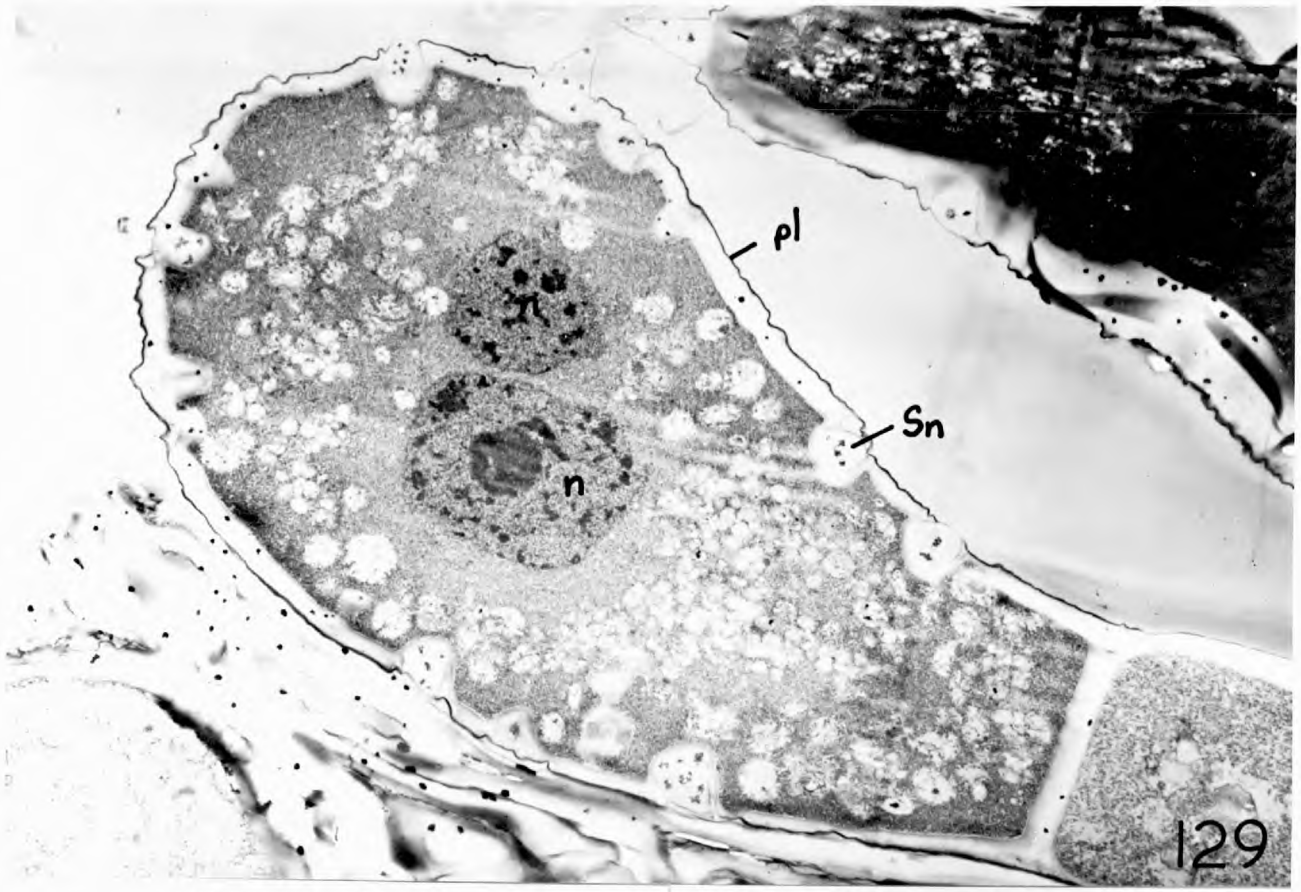


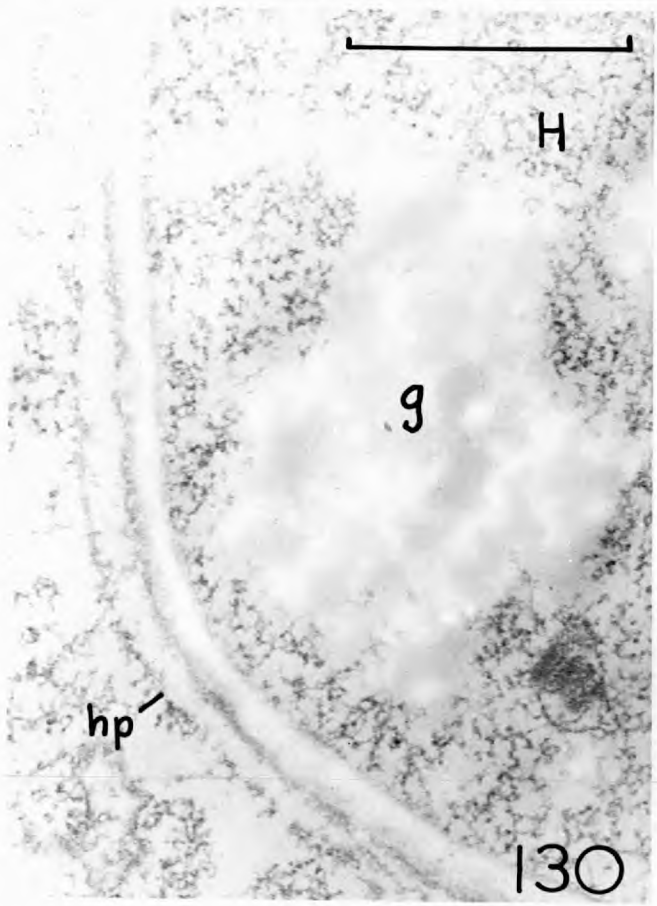
128

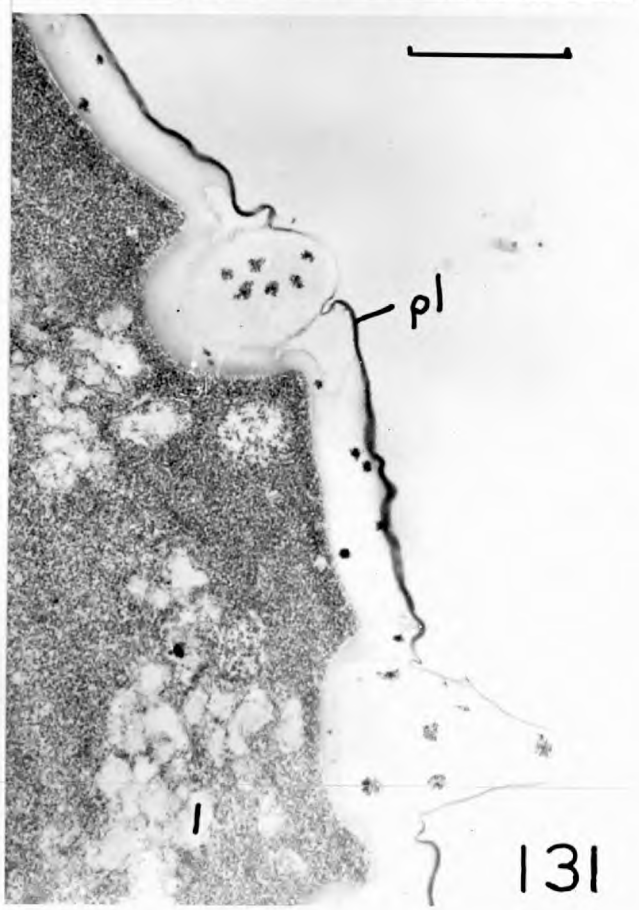
129. Section through a uredospore. Note the density and continuity of the pellicle (pl) and the deposit of large electron dense crystals restricted to the cell walls and spines. 1871A/4. 12602.

130. The body region of a haustorium containing a large aggregate of pale, mottled material (g). 1871A/4. 12616.

131. Detail of Fig. 129 showing the continuity of the pellicle (pl) over the surface of the spore and spines, also clear patches, probably of lipid (l). 1871A/4. 12601.


pl
Sn
n
129

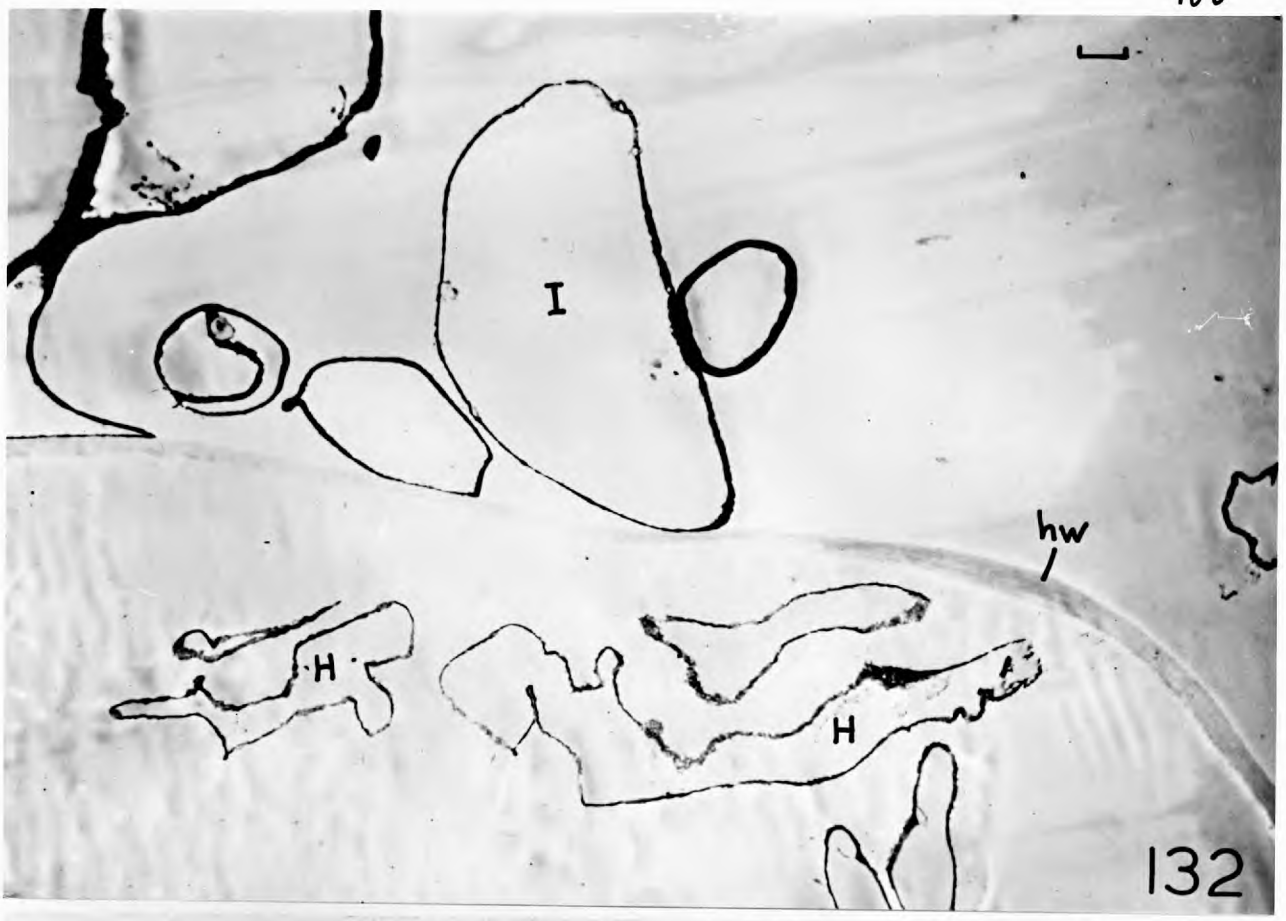

H
g
hp
130


pl
l
131

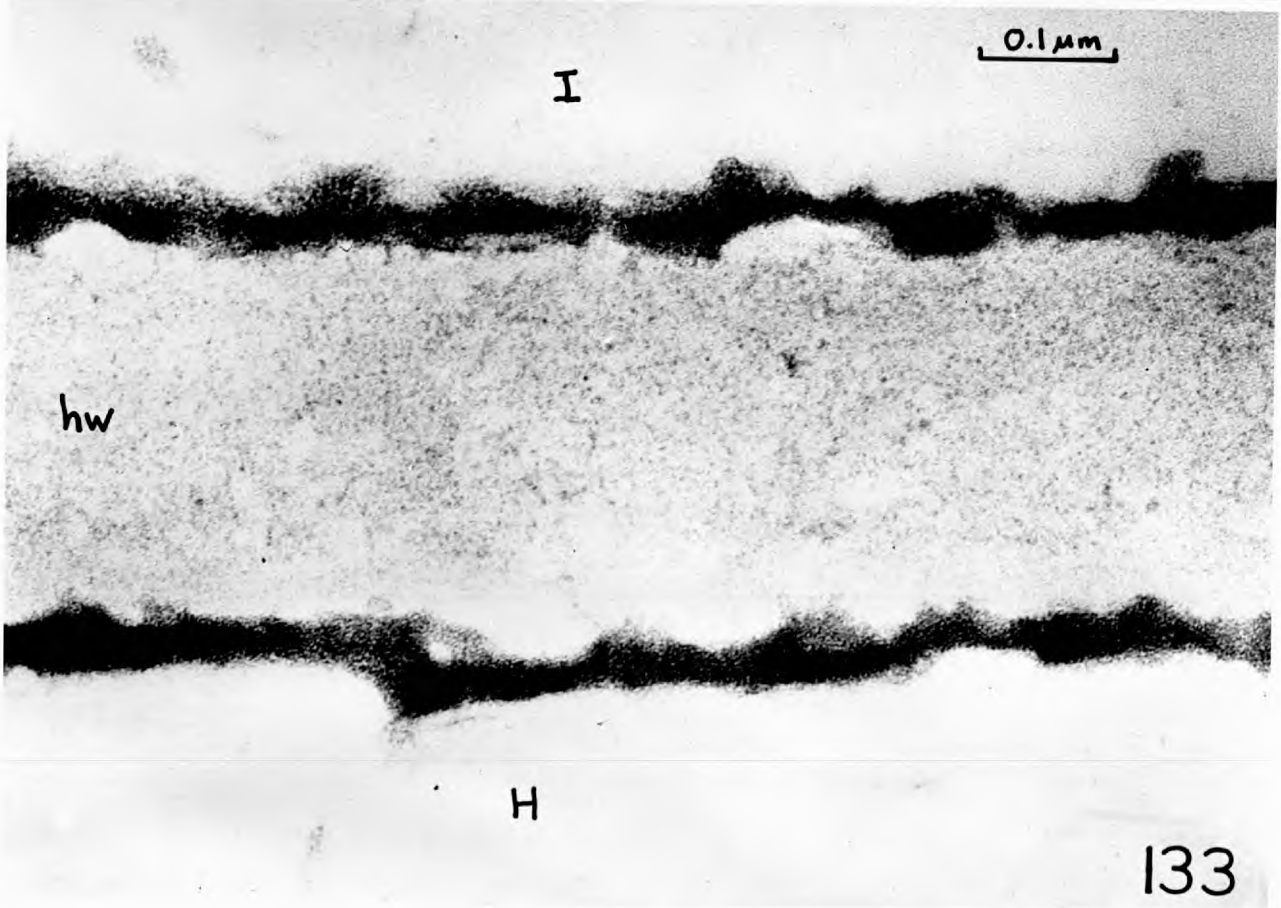
Figures 132-140 are of material autoclaved in KOH and treated with dilute acetic acid.

132. Low power showing the intercellular hyphae (I) and haustoria (H). Unfixed. 2079A/2. 14382.

133. Section through a hyphal wall (I) and the body wall of a haustorium (H) adjacent to the host wall (hw). Note the similarity between the two outermost walls and the lack of fibrillar material within the host wall. Unfixed. 2097A/2. 14384.



132

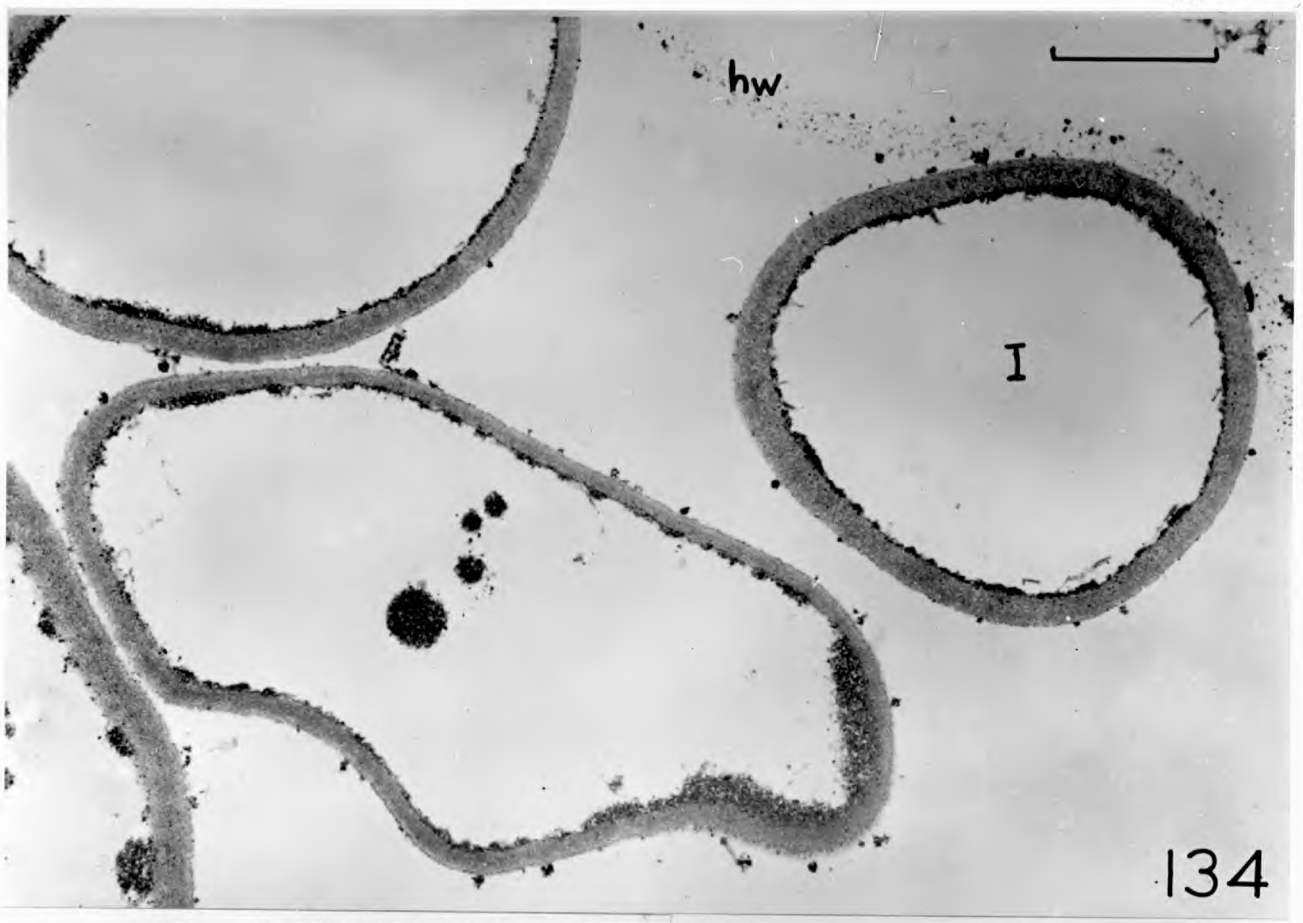


133

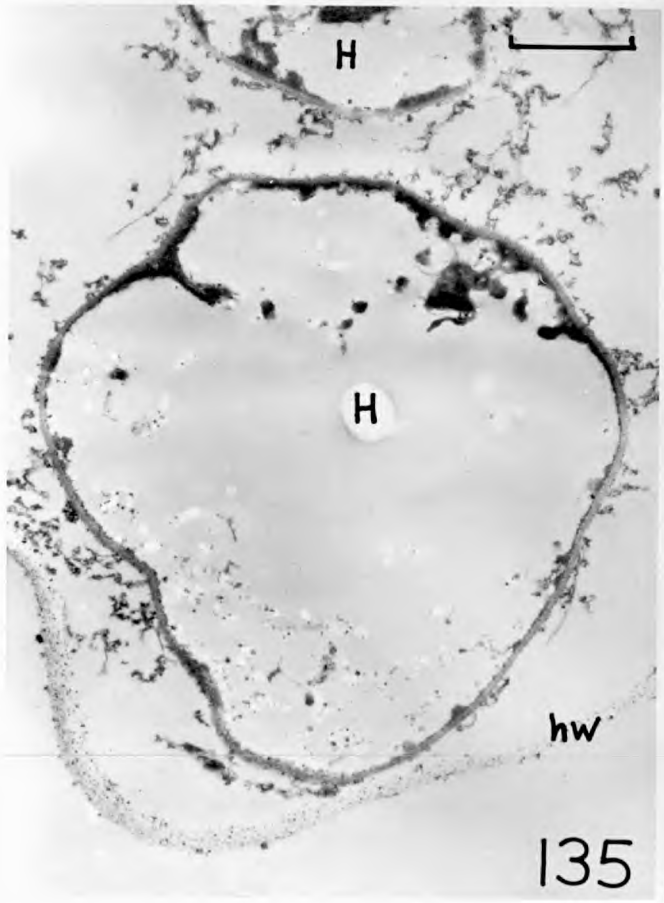
134. Low power of intercellular hyphae (I). Some remnants of the cytoplasm are present within the cells. 1873B/2. 12643.

135. Section through the body region of a haustorium, the body wall consists of only one layer. 1873B/2. 12653.

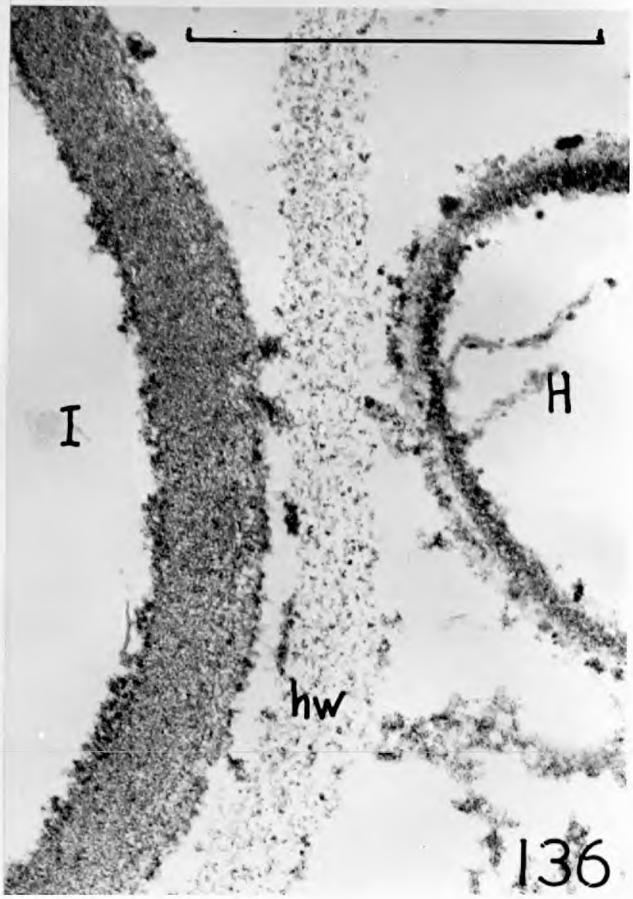
136. Comparable micrograph to Fig. 133. Note the granularity in the host wall (hw) and the material contained within of a similar form to material in the cell lumen. 1873B/3. 12654.



134



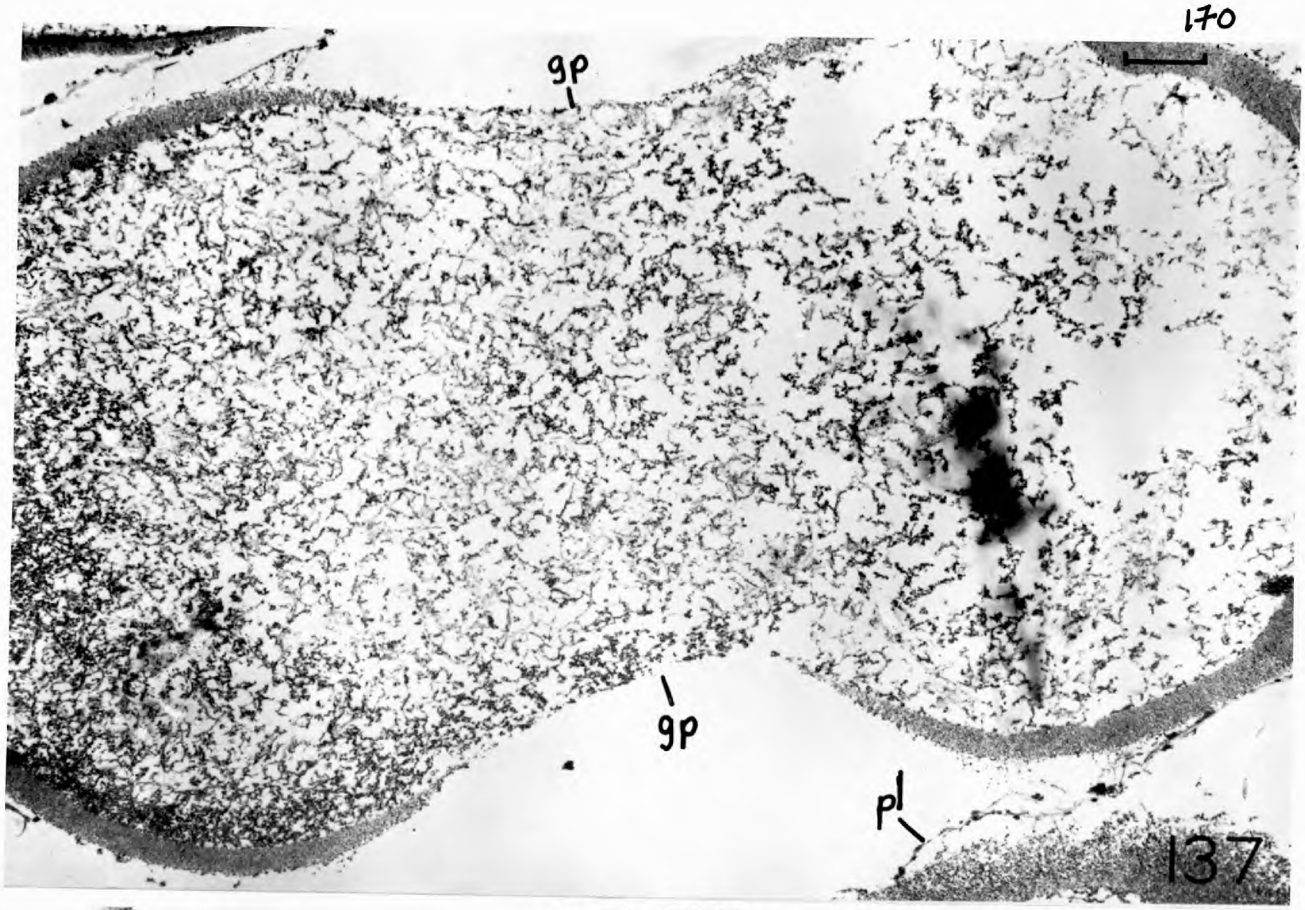
135



136

137. Section of a uredospore median to the germ pore (gp). Note the open granular texture of its wall. The pellicle (pl) although disrupted is present within the intercellular spaces. 1873B/1. 12634.

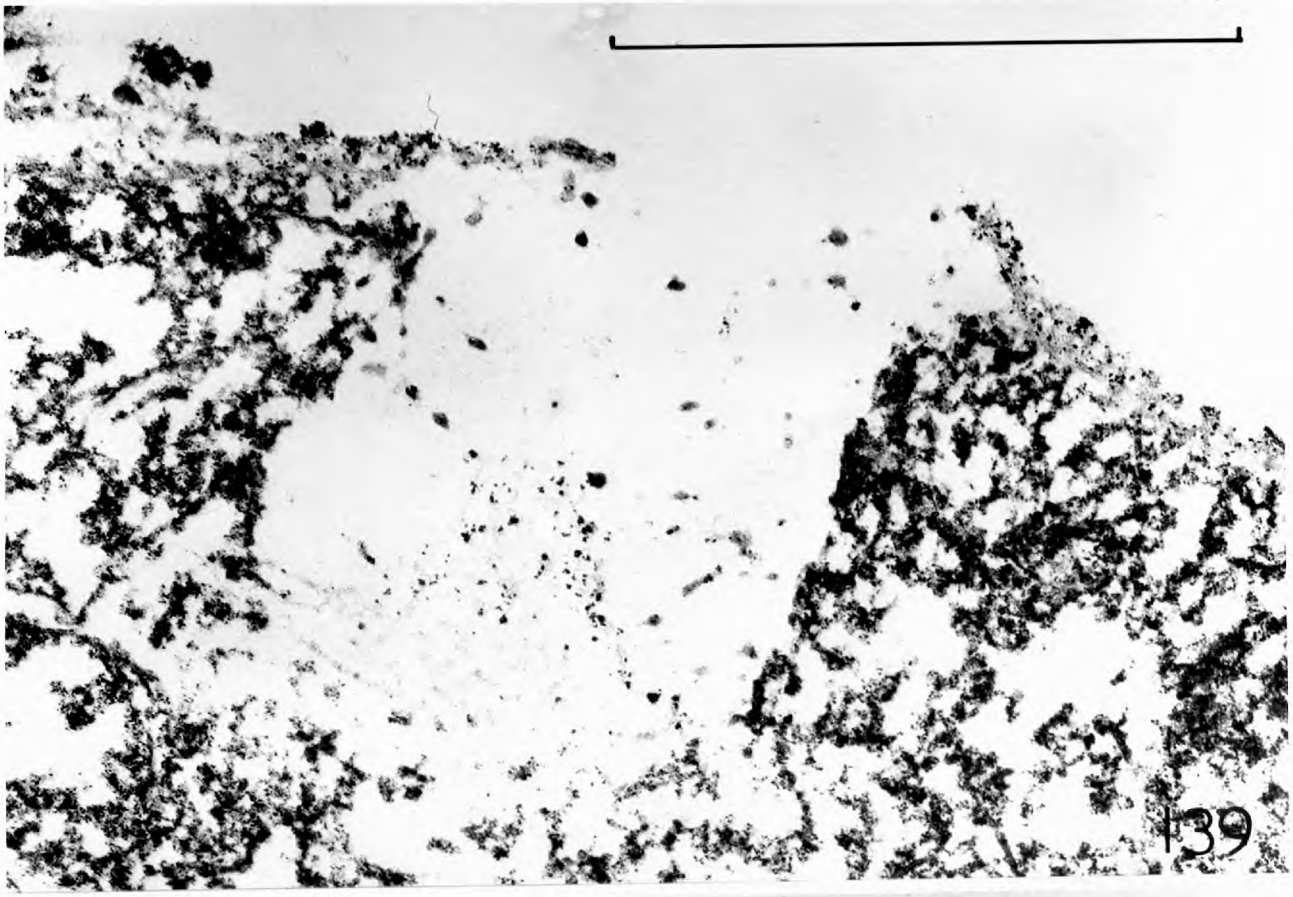
138. Section through developing spines (Sn). 1873B/1. 12636.


gp
gp
pl
137


Sn
138

139. Detail of Fig. 138 showing the pale reticulum of material at the base of the spine socket. 1873B/1. 12637.

140. Section through mature spines (Sn). Note the reticulum of pale material forming the spines and also the granular nature of the spore wall. 1873B/1. 12631.


139


Sn
140

6. UREDOSPORE GERMINATION AND STOMATAL PENETRATION

This section describes results obtained from scanning reflection electron microscopy of *in vivo* germination of uredospores prepared by method (c), (ii) (p. 56) and freeze-dried by method (c), (ii) (p.56); light microscopy and transmission electron microscopy of *in vitro* germination of spores on agar by method (a),(ii) (p.45) and transmission electron microscopy of *in vivo* germination of spores and stomatal penetration of germ tubes prepared by method (a), (iii) (p. 45).

(a) Germination

Shed, air-dried uredospores when placed in a humid atmosphere became distended, although they remained slightly flattened over the two diametrically opposed sides which were previously concave (cf. Fig. 75, 142). A germ tube usually emerged eventually from the spine-free centre of one of those sides (Fig. 145, 146).

In swollen uredospores the hilum was a spine-free flattened disk (approximately 3.5µm in diameter) in the centre of which the septal pore appeared as a small raised spot (Fig. 142).

In the initial stages of germination the plug of specialised wall material in the germ pore had become more electron-lucent and appeared partially degraded (Fig. 151).

Clear areas of the plug were interdispersed with electron dense material similar in opacity to that of the spore wall. The boundary between this plug of material and the surrounding uredospore wall was irregular, though well defined by their respective staining characteristics (Fig. 149). The plug became evaginated by the emerging germ tube which was itself covered at this time by a thin electron-lucent wall, confined to an area extending to the germ pore and passing round its margin where it appeared to terminate (Fig. 150). The cap of material from the germ pore plug was still covered externally by the pellicle (Fig. 151). Eventually, when both germ pores had reached the stage of development described above a germ tube emerged from one of them. Remnants of the plug in the pore were eventually left behind as a collar around the emerging germ tube (Fig. 146, 150). The germ tube wall was electron-lucent and appeared to lack a pellicle at first (Fig. 153), but at a later stage this superficial dense layer was present (Fig. 152). A spore with two emergent germ tubes was not observed.

In scanning electron micrographs of germinated uredospores the region of the germ pore on one side was often depressed (Fig. 143). It was assumed that these depressions were an artefact of preparation caused by retraction of the raised pellicle and contents of the protruding plug of the activated but non-utilised germ pore (Fig. 146). The hilum was readily distinguishable from such a germ pore by the ring of large spines at its margin (Fig. 141-143).

Cytoplasm moved out from the spore into the elongating germ tube in association with the insertion of a succession of one or more septa at intervals along the germ tube until eventually an appressorium was formed over a stoma (Fig. 147, 152, 155). The uredospore and older parts of the germ tube had become greatly vacuolated and were left in effect completely empty except for a few membranes, probably mainly of the plasmalemma and the vacuoles (Fig. 84, 147).

The germinating spores and the germ tubes contained numerous droplets of cytoplasmic lipid which, unlike those contained in the mature spore, were not aggregated around vacuoles (Fig. 148). The shape of the droplets was irregular and they varied considerably in size, more so than would be expected from their appearance in any one section alone. Mitochondria were small and irregular in outline but often elongated with the cristae orientated parallel to the long axis (Fig. 149). Cisternae of smooth E.R. proliferated within the cytoplasm (Fig. 153) and microbodies were also present.

Numerous microtubules running parallel to the length of the germ tube were observed (Fig. 153, 154). These were often in the vicinity of nuclei which contained large nucleoli (Fig. 154) and were judged to be in an interphase condition. No stages of dividing nuclei were identified.

Large aggregates of granular material were seen in the germinating spore (Fig. 148) but in the germ tubes aggregates of this type were smaller in size and had less affinity for stain (Fig. 153). This material appeared to be essentially

similar to granular material observed in the intercellular hyphae (Fig. 26), the haustoria (Fig. 55) and the mature ungerminated uredospores (Fig. 104).

(b) Stomatal Penetration

A swelling of the germ tube occurred over a stoma, a septum was formed which delimited the appressorium as a terminal cell of varied shape. Appressoria were surrounded by a thin electron-lucent wall (Fig. 155, 156). A penetration peg was produced from the appressorium against the stoma (Fig. 156). The wall of the penetration peg was about half the thickness of the wall of the appressorium and slightly more electron-opaque (Fig. 158). This wall appeared to be formed inside the wall of the appressorium, but like the new wall of the developing germ tube (Fig. 151), was a localised deposit extending for only a short distance from the margin of the pore and did not appear to form a lining to the whole appressorium (Fig. 156, 158).

The penetration peg followed closely the contours of the guard cells and penetrated between them, frequently thrusting the lips of the guard cells aside (Fig. 155).

The guard cell walls appeared to be unaffected by the penetrating peg and were similar to those of non-penetrated stomata (cf. Fig. 67, 155). The outer margin of the guard cell wall was covered by a cuticle of greater electron

opacity than the underlying wall layers (Fig. 158). The cuticular layer continued over the surfaces of the guard and accessory cells exposed to the intercellular sub-stomatal cavity (Fig. 155).

Within the sub-stomatal cavity of the leaf the penetration peg was expanded and, beginning at the point where it lost contact with the guard cell walls, it carried a deposit of intercellular material which was continuous over the apex of the peg (Fig. 157). The penetration peg had become pulled away from contact with the guard cells, probably as a result of a differential contraction of host and parasite cells during fixation, but the intercellular material which surrounded the peg had remained adherent to its surface. This deposit was covered by a thin electron dense layer which appeared to be continuous with the outer layer of the wall of the penetration peg (Fig. 157, 159, 160). This intercellular deposit was placed where it could potentially have formed a seal in the stomatal apperture between the guard cells and the penetration peg (Fig. 157).

The expanded penetration peg formed a septum, the new cell forming the sub-stomatal vesicle (Fig. 159). The septum appeared to have formed in a similar way to the true septae in the intercellular hyphae (see p. 77). However, one major point of difference was apparent; the half-septal layer on the sub-stomatal vesicle side was part of a continuous secondary deposit which formed an inner layer of wall

about equal in thickness to the original wall of the penetration peg. The wall of the sub-stomatal vesicle became about as thick as that of the appressorium, though of greater electron opacity (Fig. 159).

In the section examined, the only one available passing through this region, the central region of the septum was occupied by a thickened part of the wall on each side, covered by the plasmalemma of the adjacent cell, and containing sequestered membranes (Fig. 160). The section was probably not median to this structure and it was impossible to determine if it contained a central pore. However, the profile did not possess the characteristics of the margin of a 'dolipore' such as found in the septa of Basidiomycetes generally. In particular the parenthasome, a hood of cytoplasmic membrane, was absent from the area (Fig. 160). An array of small vesicles of uniform size, appearing as though in the process of fusion laterally, was situated close to the surface of the septum flanking the central structure. Although no corresponding group of vesicles was present on the other side of the septum a single large vesicle was present at one point (Fig. 160).

Large vacuoles in the appressorium confined the cytoplasm to the infection peg and regions adjacent to the stoma (Fig. 156). Lipid droplets, mitochondria and small vacuoles were numerous in both the appressorium and sub-stomatal vesicle (Fig. 156-159). Microbodies were grouped near to

the septum between the infection peg and the sub-stomatal vesicle (Fig. 160). The granular material observed in the germinating spore (Fig. 151) was absent from the appressorium and the sub-stomatal vesicle (Fig. 158, 159).

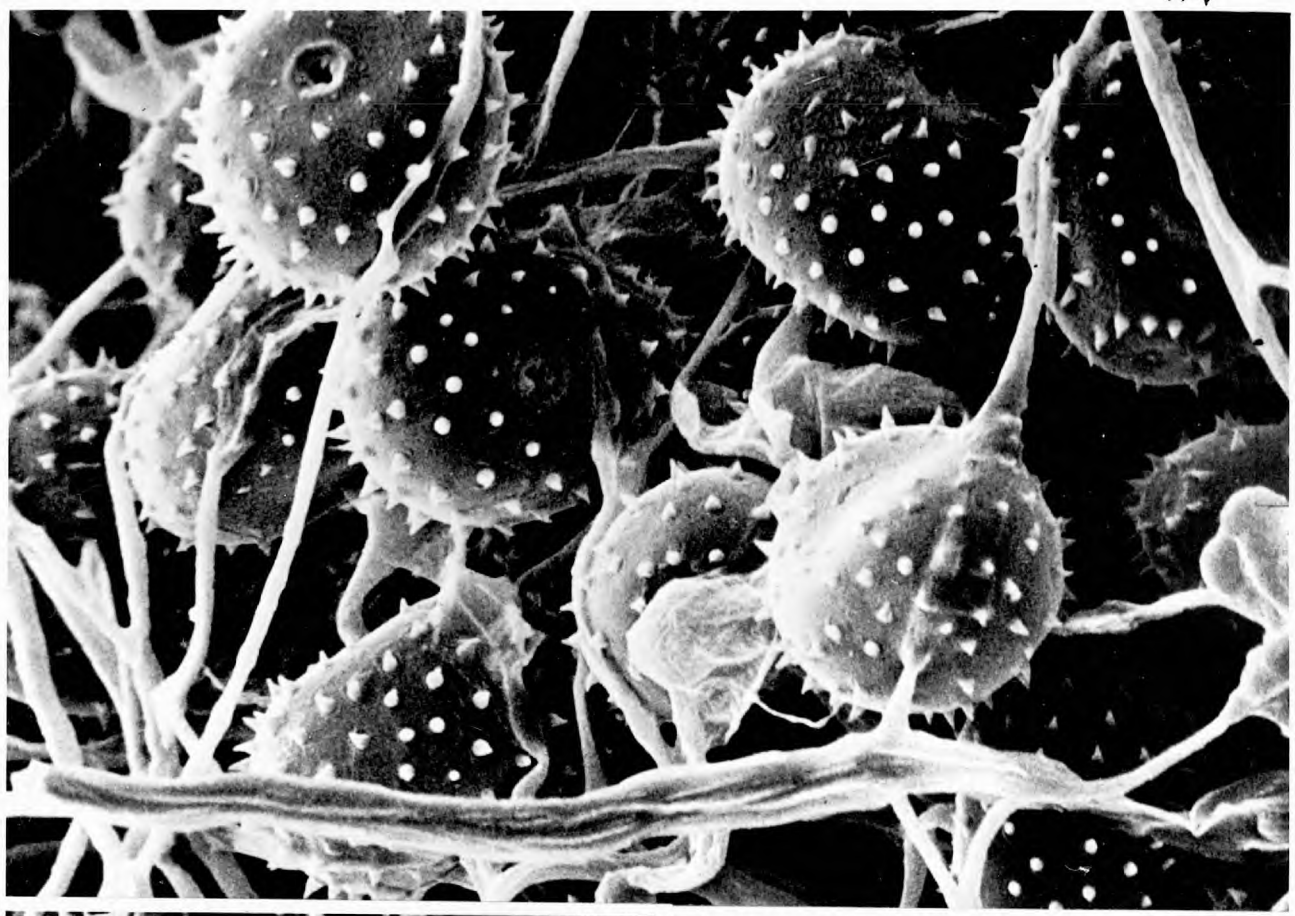
From these sub-stomatal vesicles the hyphae were produced which ramified the intercellular spaces of the leaf and from the tips of which h.m.c. were formed.

Figures 141-145 are scanning reflection electron micrographs.

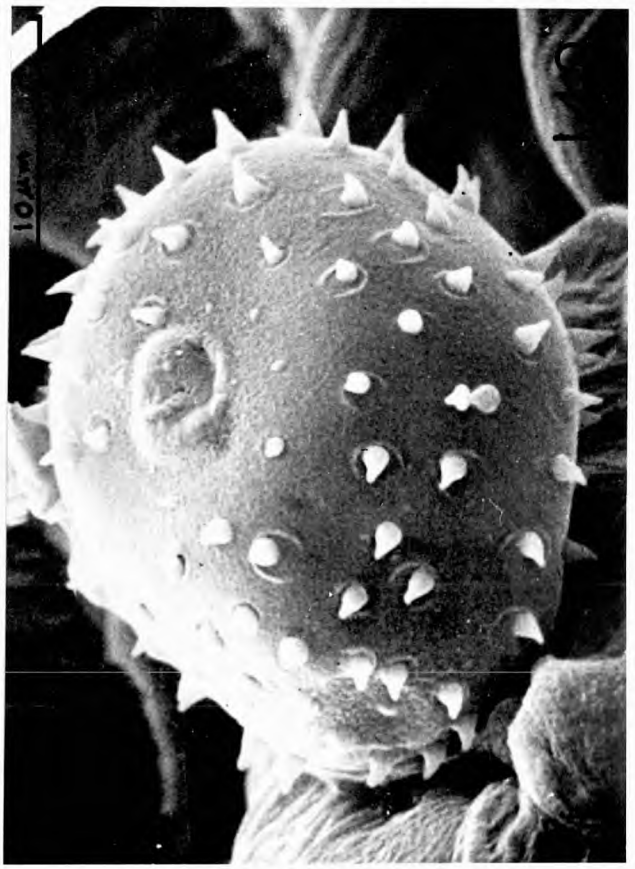
141. Germinating uredospores on a leaf surface. Note the collapse of the germ tubes where they are in contact with the spore surface. 1802. B41.

142. Swollen uredospores showing a germ pore (top centre) and the hilum region (extreme left). 1802. D5.

143. Germinating uredospores, the region of the non-utilised germ pore has collapsed and invaginated. 1802. B39.




10μm
14

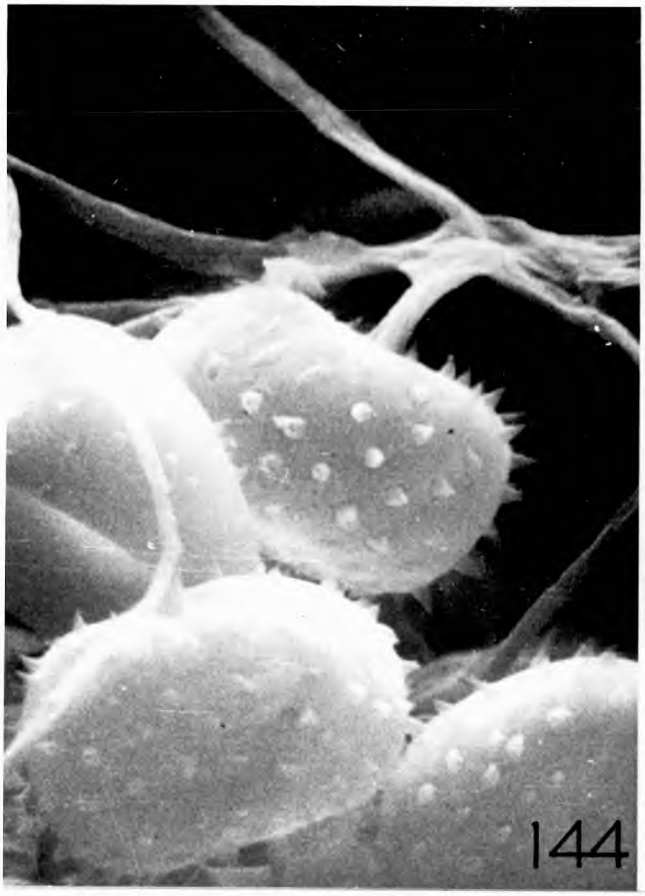

10μm
1

144. Uredospore showing possible emergent germ tube. Angle 51°. 1802. IC D19A.

145. Same uredospore as in Fig. 144 but viewed from 16° showing emergent germ tube. These two figures illustrate the usefulness of specimen movement on this microscope. 1802. IC D22A.

146. Light micrograph of a germinating uredospore on 2% tap water agar after 4h. Cytoplasm is still in contact with the opposite but non-utilised germ pore, which although swollen has not ruptured the pellicle (pl). -. 734.

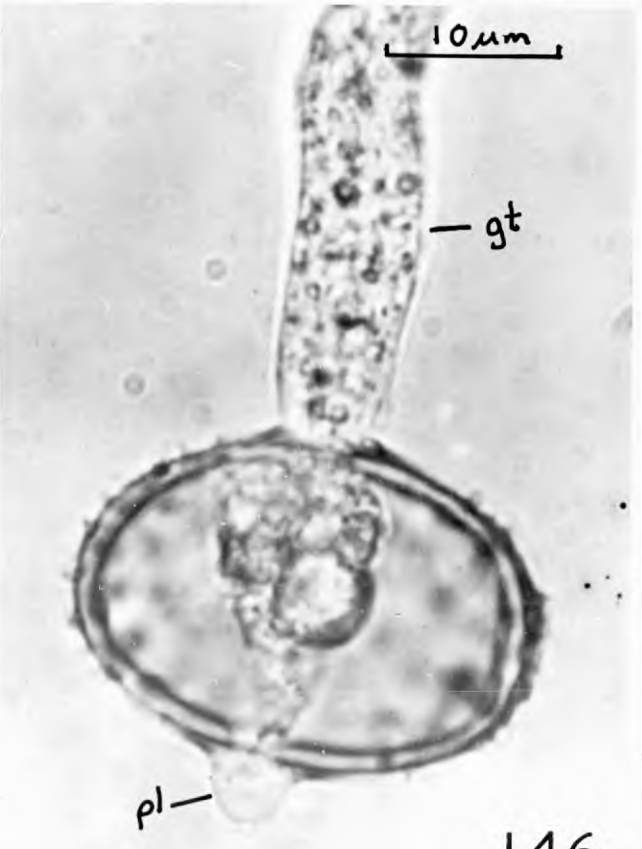
147. As for Fig. 146 only a uredospore at a later stage showing the cytoplasm cut off from the empty germ tube (gt) and spore by a septum (top centre). -. 735.



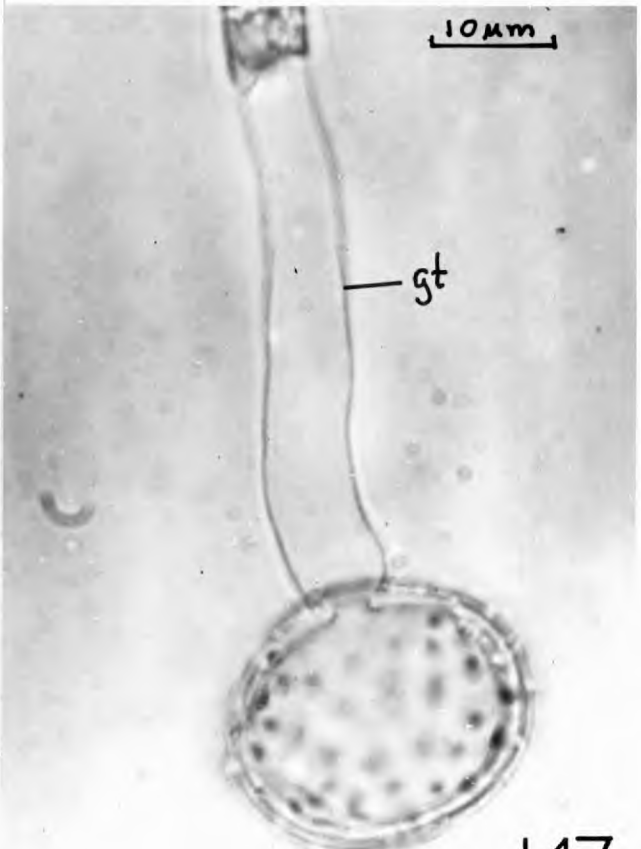
144



145



146



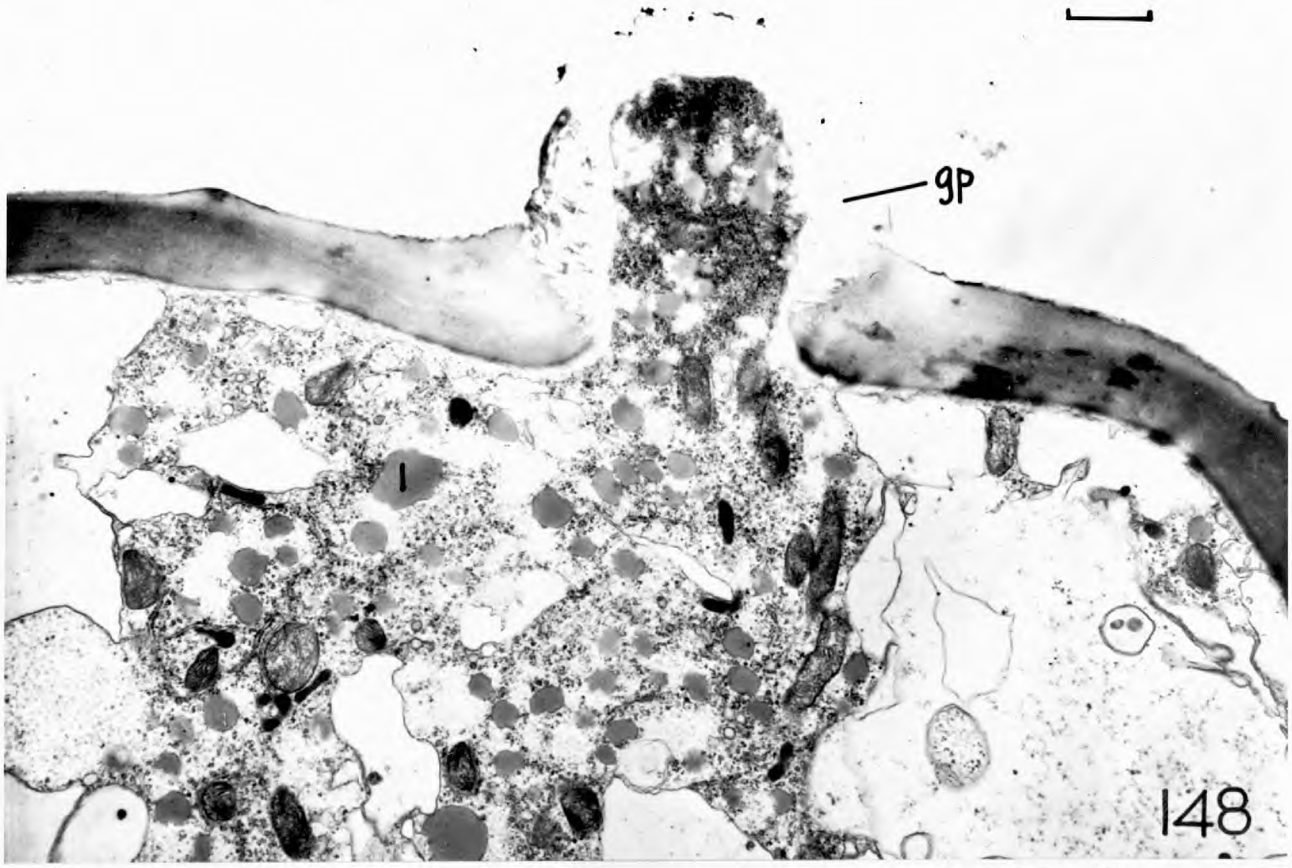
147

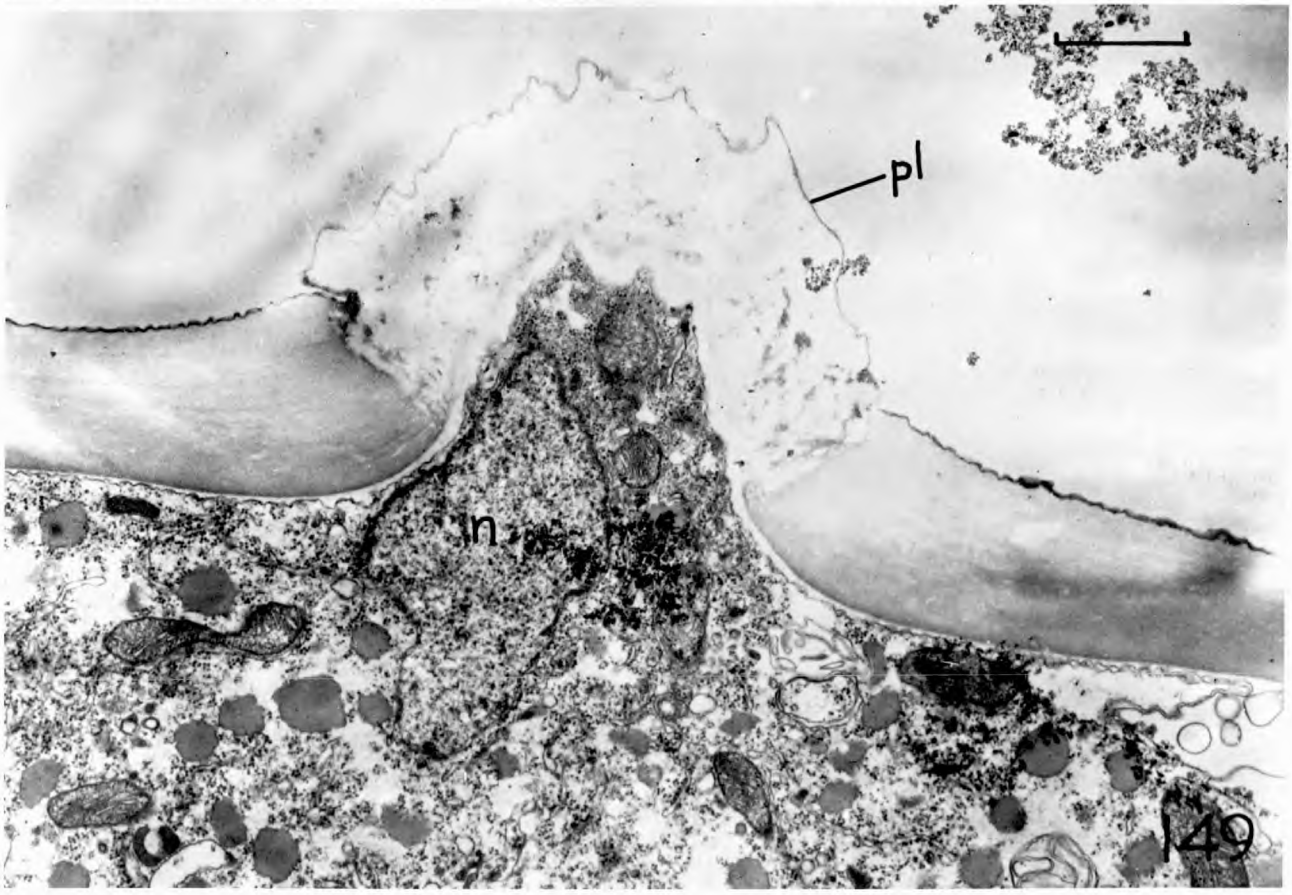
Figures 148-151, 153, 154 are of uredospores which have been placed on 2% tap water agar for 4h.

Figures 152, 155-160 are of material taken from leaf disks 24h after inoculation with uredospores.

148. Section through a germinating uredospore median to the germ pore (gp). Note the aggregations of granular material (g) within the cytoplasm. 1708/2. 10047.

149. A germinating uredospore sectioned off median to the germ pore. Note the continuity of the pellicle (pl) over the everted plug of material from the germ pore. 1708/6. 10054.

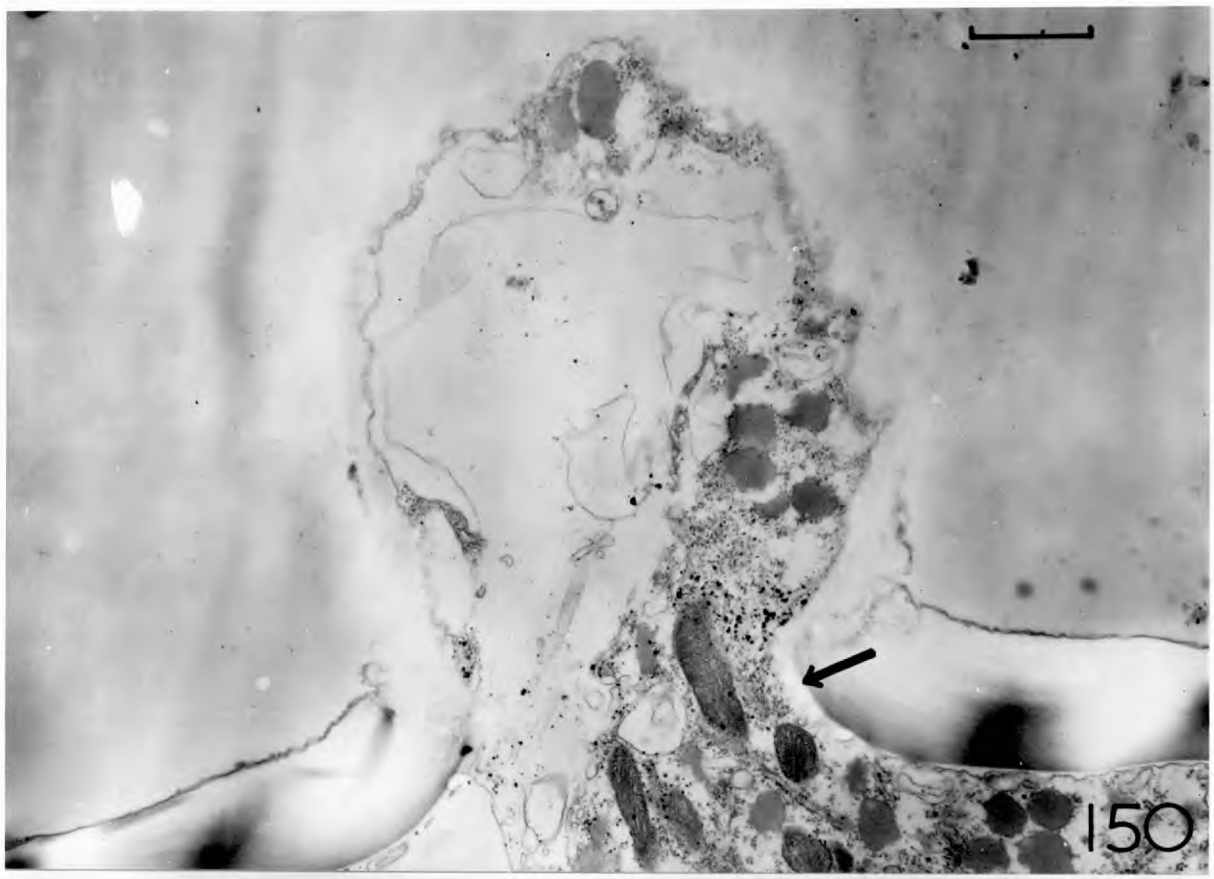

gp
l
148


pl
n
149

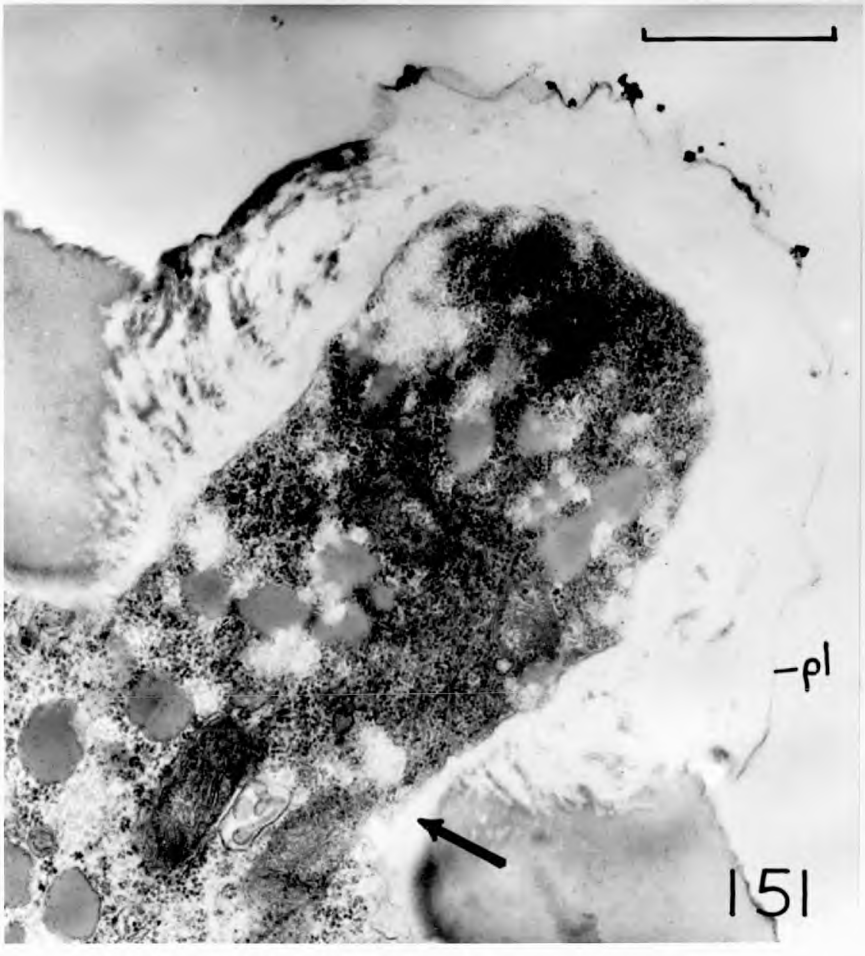
150. Section through a germ pore showing a deposit of electron-lucent wall material (arrow) starting within the original spore wall. Note the absence of a pellicle around the distal part of the emergent germ tube. 1708/6. 10060.

151. Detail of Fig. 148 to show the wall of the germ tube starting within the spore wall (arrow) and lining the evaginated plug to the germ pore. 1708/2. 10048.

152. Section through a septate germ tube (gt) on the surface of a leaf (Ep). Note the presence of a pellicle. 1541A/18. 10061.



150



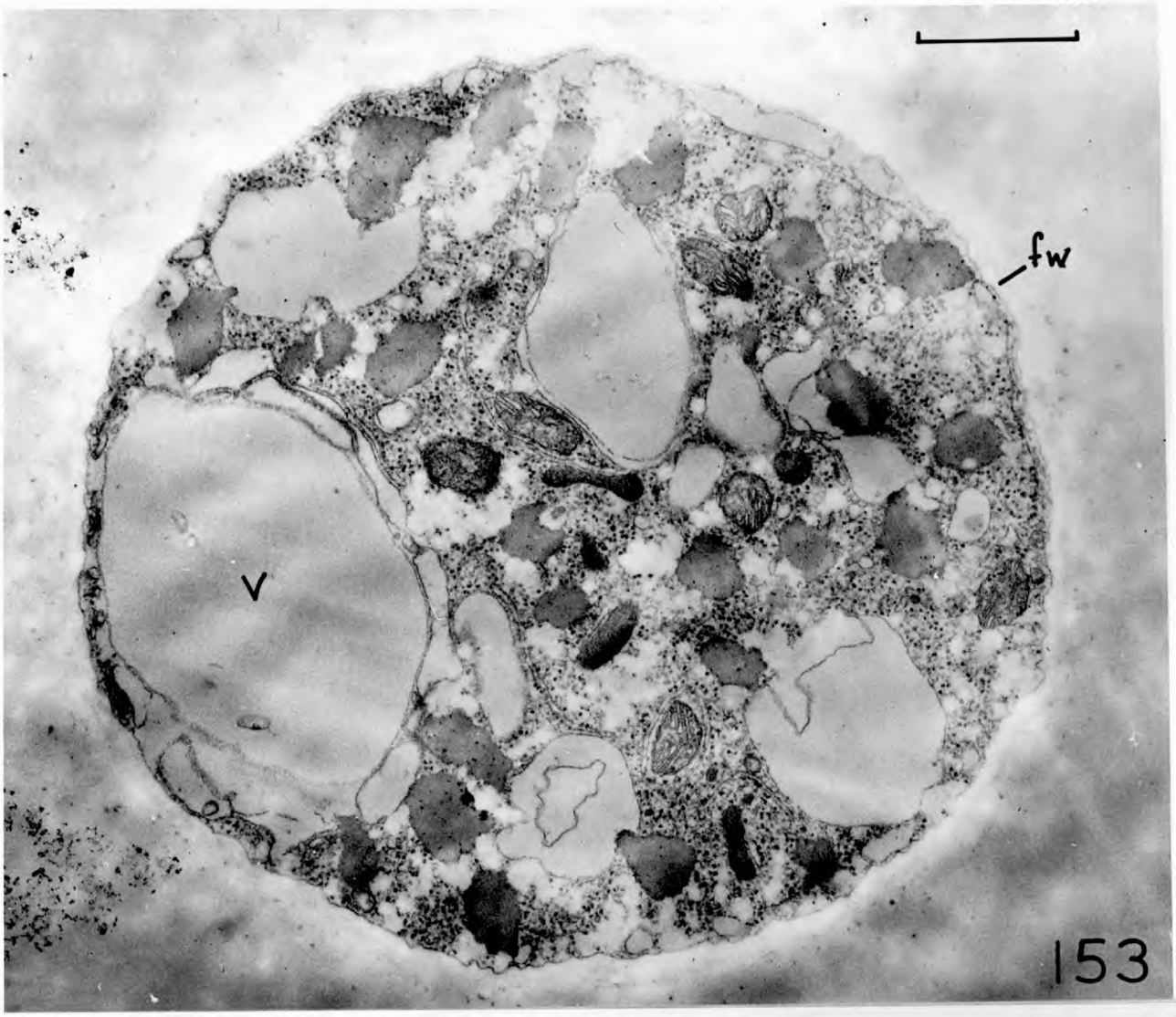
151



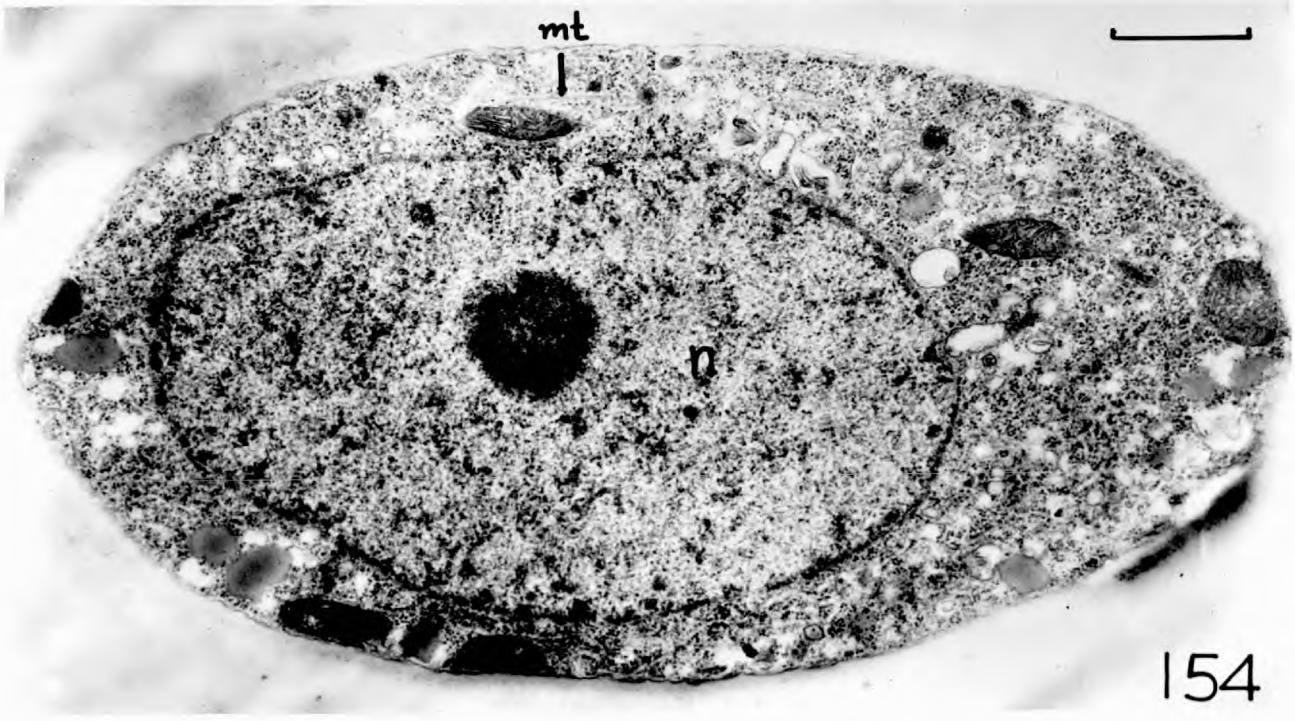
152

153. Cross section of a germ tube with an electron-lucent wall (fw) not covered by a pellicle. Note the reduction in size and opacity of the aggregates of granular material (g). 1708/6. 10059.

154. Oblique section through a germ tube with a nucleus (n) and large central nucleolus. 1708/6. 10058.



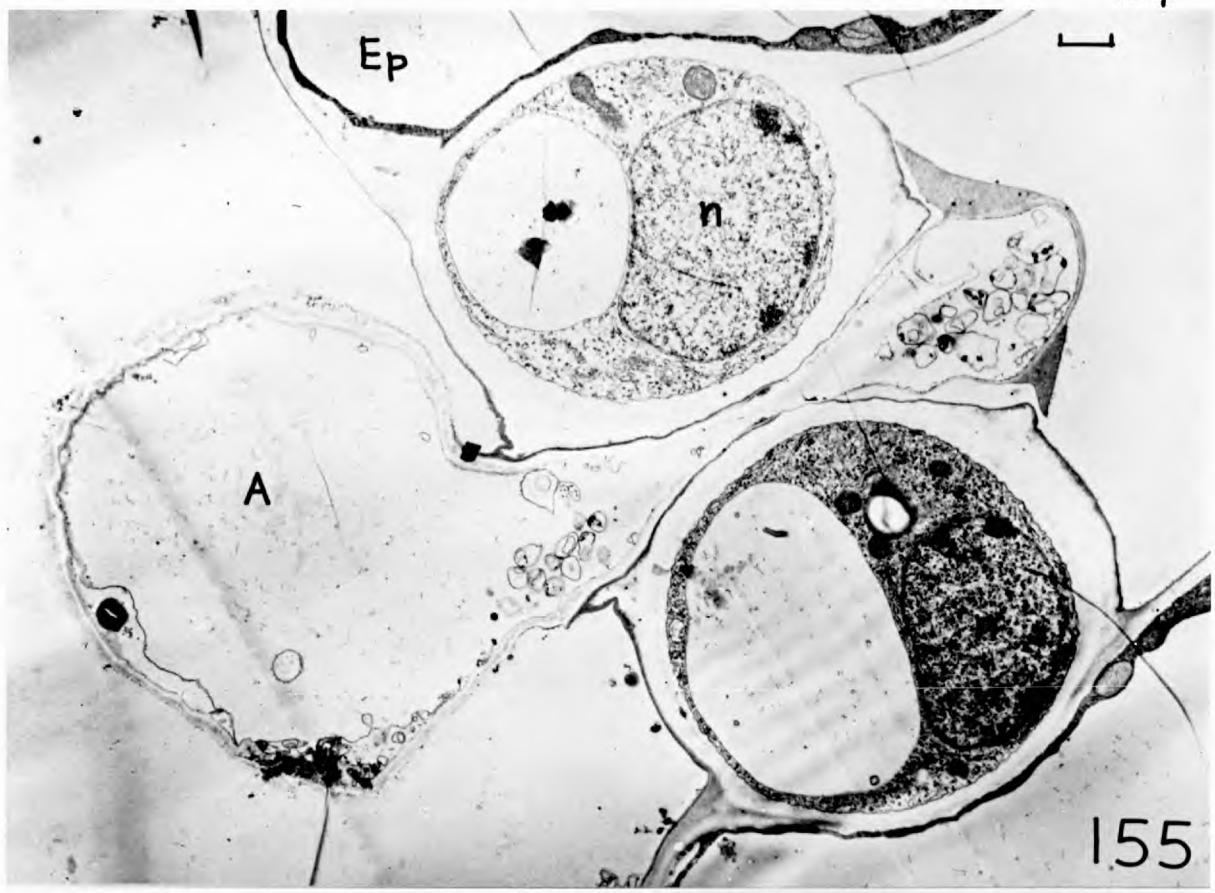
153



154

155. Section through an appressorium (A) over a stoma. Note that the lips of the guard cells have been pushed away from their normal position. 1541A/7. 8819.

156. Appressorium over a stoma with a large vacuole in the 'upper' part. Note the increase in opacity of the wall of the penetration peg in comparison with that of the appressorium. 1541A/24. 10070.



155

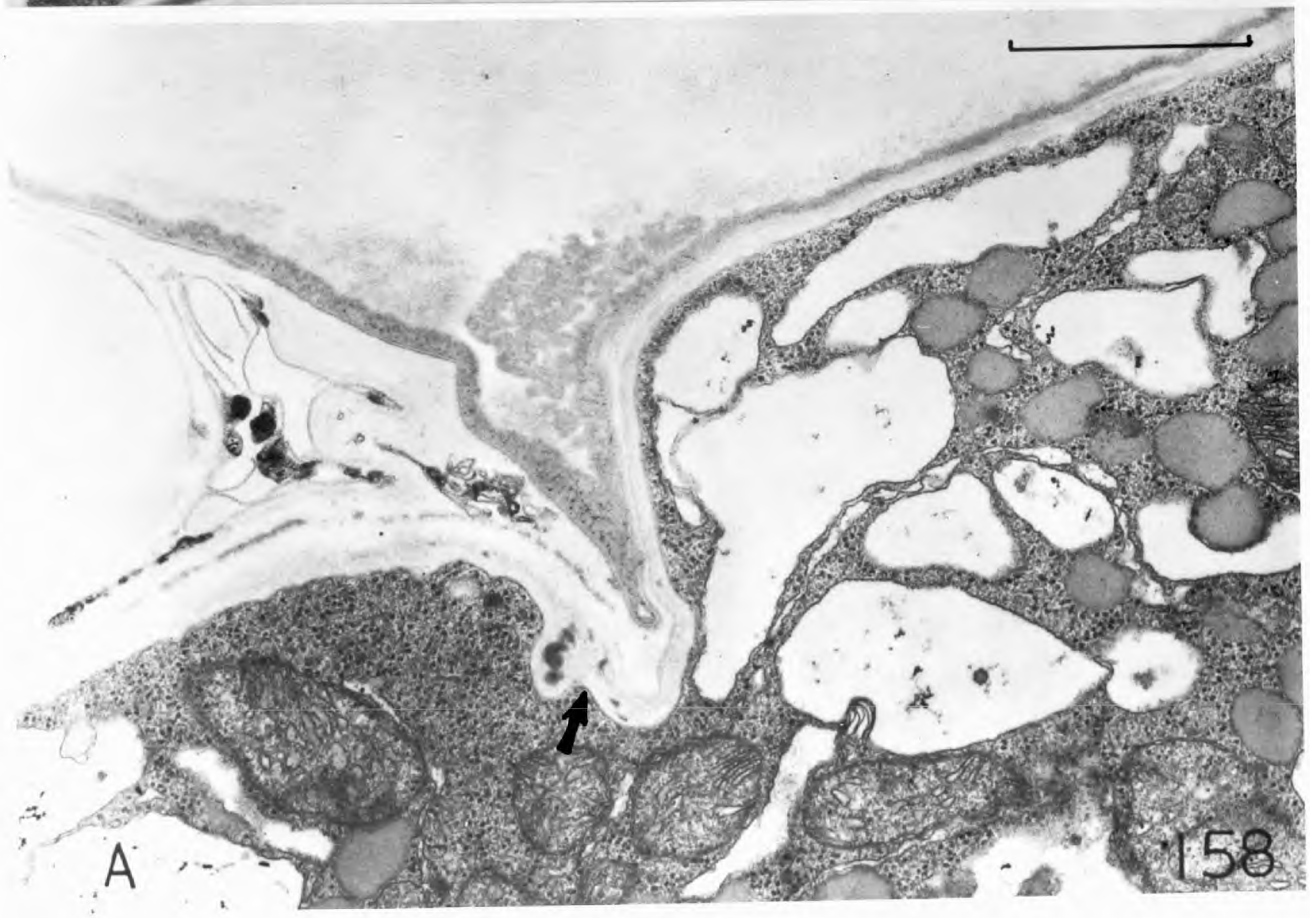


156

157. Section through the penetration peg within the sub-stomatal cavity (Sc). Between the fungal wall and the guard cells and surrounding the tip of the peg is an accretion of material with an electron dense boundary. 1541A/24. 10071.

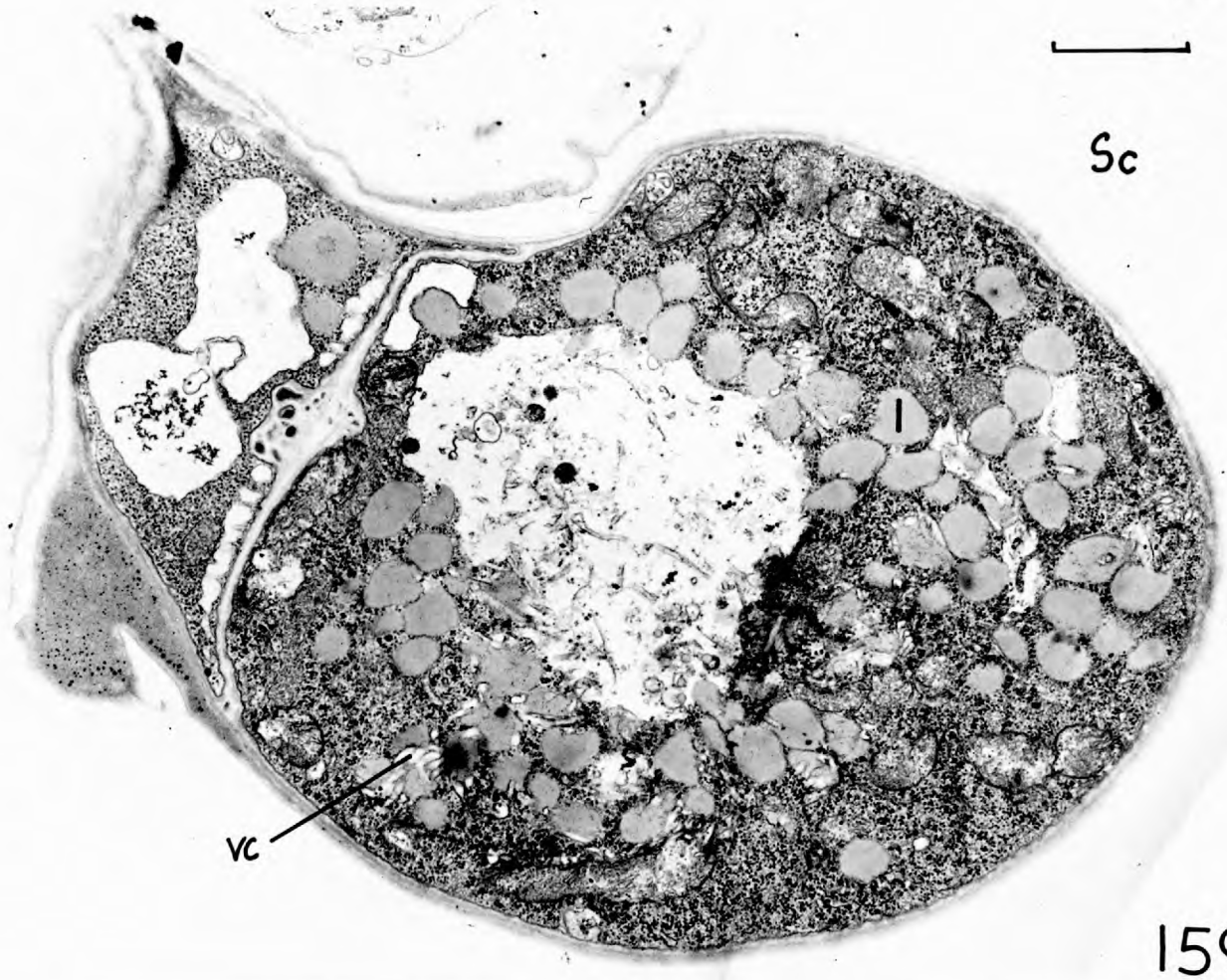
158. Detail of the appressorial and penetration peg wall in close contact with the outer margin of the guard cell wall. Note the point at which the penetration peg wall begins (arrow) and its thinner but more electron-opaque wall. 1541A/24. 10073.


Sc
VC
157

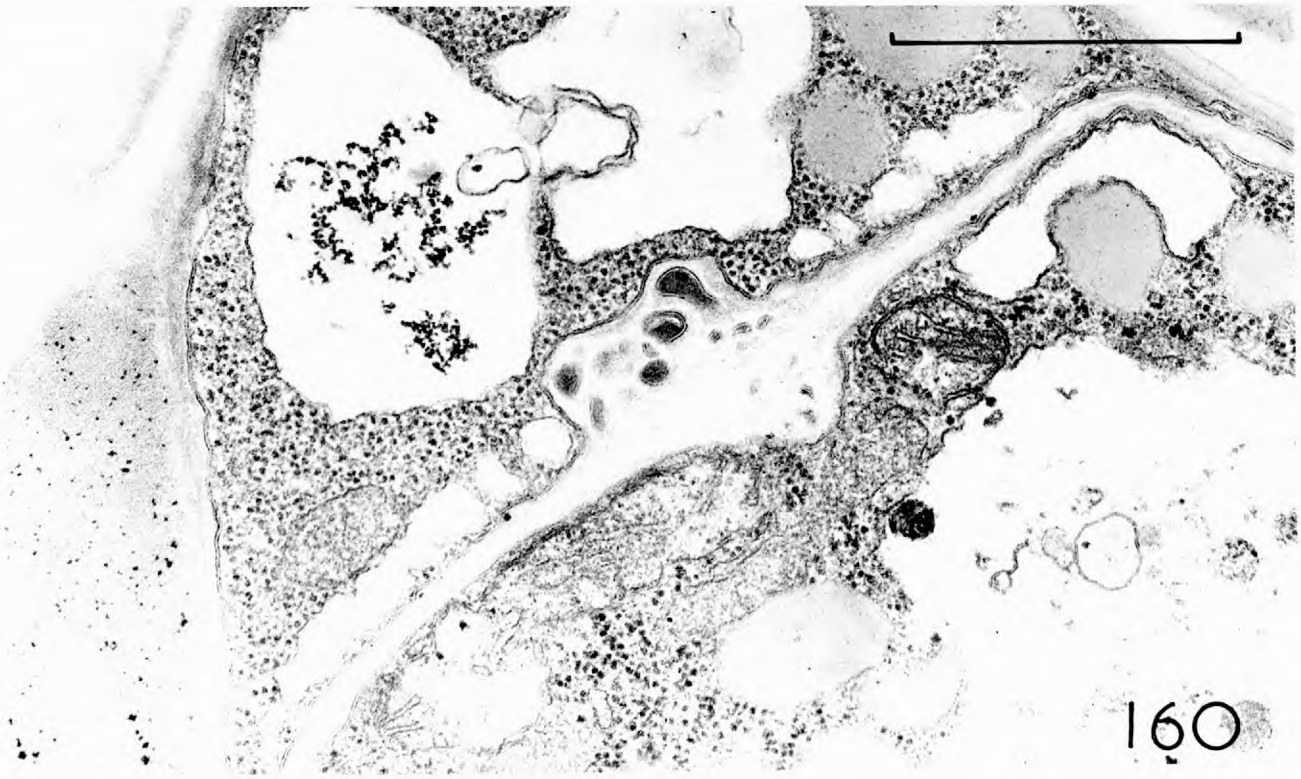

A
158

159. Section through a sub-stomatal vesicle containing a large central vacuole with broken membranes and tubular vesicles (vc). The wall has three layers; an outer bounded by a thin electron dense line is composed of the material forming an expanded region at its base; a middle layer, the original wall of the penetration peg, and an inner layer which is a continuation of the septal wall. 1541A/27. 10076.

160. Detail of Fig. 159 showing a central thickening to the septum containing sequestered membranes. Note the row of vesicles, as if in the process of fusion, lateral to the central thickening. 1541A/27. 10077.



159



160

7. YOUNG INFECTIONS

In this section young haustoria developing in cells upto 24h after inoculation are described. This material, from leaf disks, was prepared by method (a), (iii) (p. 45).

Within 24h germ tubes with appressoria had penetrated the stomata to form sub-stomatal vesicles from which infection hyphae developed and produced h.m.c. which penetrated host cells forming haustoria.

(a) The Fine Structure of the Cytoplasm

(i) Host Cells

Chloroplasts and mitochondria were as described for other invaded cells (Section 2 p. 73), although starch grains in the chloroplasts were fewer and smaller in size (Fig. 161). The chloroplast envelope was often convoluted and the folds contained large amounts of stroma devoid of lamellae (Fig. 161, 165).

Rough host E,R. with a decoration of polysomes arranged in spirals proliferated in the region of the haustoria (Fig. 165).

There were densely stained deposits in contact with the cytoplasmic side of the host plasmalemma in some but not all cells (Fig. 161). In invaded cells this deposit was present over the whole of the host plasmalemma, including the area associated with the haustoria (Fig. 163, 165). These

deposits were formed of electron dense particles, rarely larger than ribosomes, from which they could be distinguished by their greater electron opacity, greater variability in size and shape and lack of sub-structure (Fig. 162, 163). They were arranged at close although irregular intervals along the plasmalemma, frequently touching but more often at distances from one to three diameters apart (Fig. 162).

(ii) Fungal Cells

These were generally similar to those described in Section 2 (p. 75). Intercellular hyphae and the h.m.c. were well filled with cytoplasm. Vacuolation occurred on the side of the h.m.c. away from the point of penetration. Small irregularly shaped, single membrane bounded vesicle-like cisternae were present in large numbers in one haustorium, probably before extensive vacuolation had occurred (Fig. 164). Other rather larger vacuole-like vesicles with tubular inclusions were also present.

Three nuclei were observed in a section of one haustorium (Fig. 161), each containing dense regions of chromatin (Fig. 165) though no distinct nucleolus could be seen in any of the nuclei.

A lomasome was observed in one haustorium (Fig. 166). The continuity of the plasmalemma could be traced into, but not entirely round, the lomasome, which consisted of finger-like vesicles similar to those described for lomasomes from

intercellular hyphae (Fig. 31, 33).

(b) Fine Structure of the Cell Walls

The h.m.c. was similar to that described in Section 3 (p. 92) and the cell walls of the host appear to have been penetrated in much the same way (cf. Fig. 49, 163).

No definite fungal wall had been deposited in the base, although there was some superficial staining of the host wall lining the penetration pore. There were no lateral extentions of sheathing material over the host wall at the side of the penetration pore and there was little or no fibrillar sheathing material on the neck, except for a small amount at the margin of the pore and this was not extended laterally for any appreciable distance over the inner surface of the host wall (Fig. 163). In other respects the sheath deposit in this region resembled those of other haustoria examined, except that the electron opaque zone extending from the vicinity of the fungal plasmalemma, being normal in amount, occupied a proportionately greater part of the incipient sheath.

The wall of the stem was uniform in thickness and texture, though with some clear patches along its length, with the plasmalemmas of host and fungus closely applied (Fig. 163). The neck of the haustorium of Fig. 163 was intersected obliquely but there was an increase in electron density

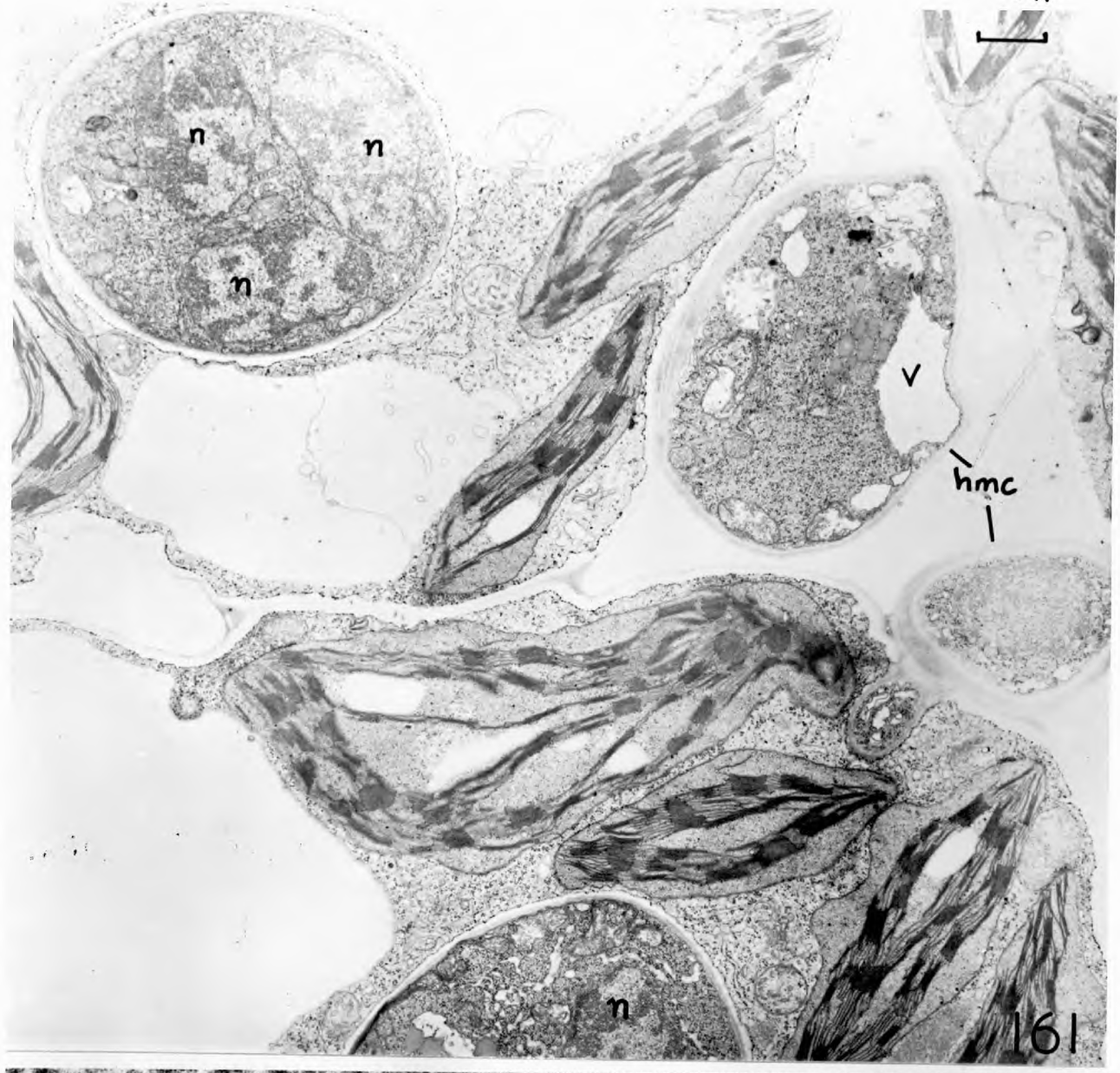
of the stem wall, cut at glancing incidence where the wall disappeared from the plane of section, consistent with the presence of a neckband in that region.

The stem of the haustorium expanded abruptly into the body which was surrounded by an electron dense layer of granular texture, about 20nm thick, adjacent to the fungal plasmalemma and separated from the host plasmalemma by an electron-lucent region of varying width (Fig. 162, 165). The latter region contained some dark granular material which was more compact and therefore more conspicuous in the narrower parts of this region. Where the electron-lucent region was wider the granular material present was mainly associated with the host plasmalemma or the surface of the electron dense layer of the haustorial wall (Fig. 162).

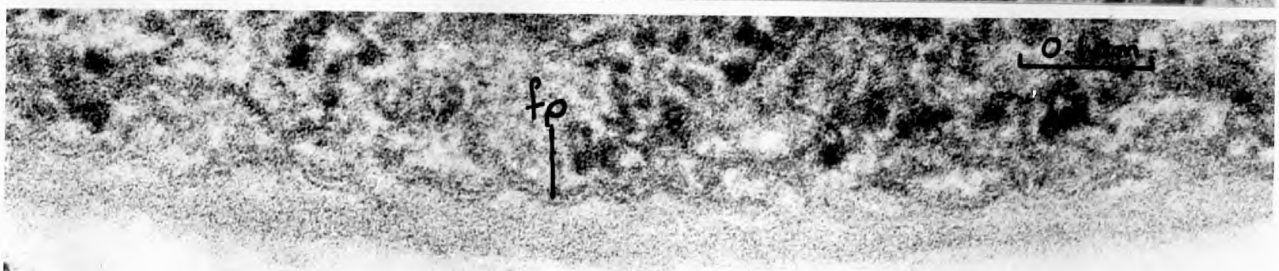
Fig. 161-166 are of material from leaf disks 24h after inoculation.

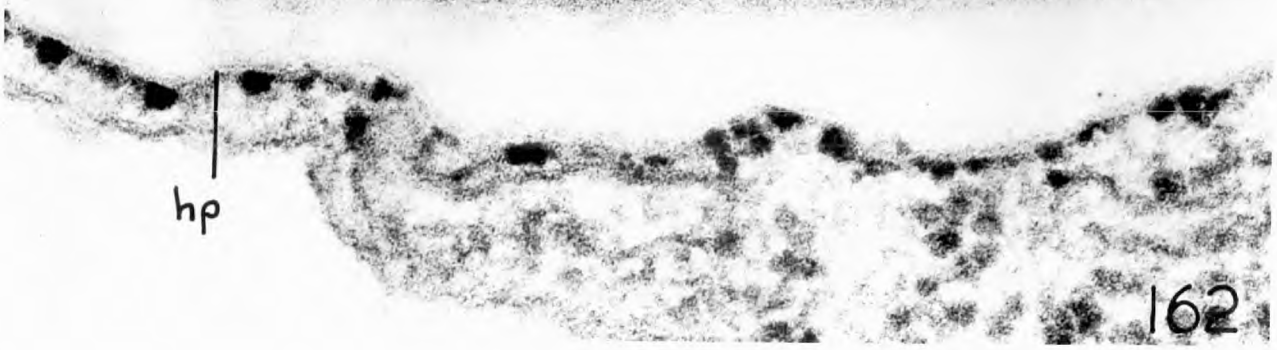
161. General view of haustoria in host cells. 1541A/27. 10081.

162. Detail of the body wall of a haustorium. Note the bridges of opaque, finely granular material between the host plasmalemma (hp) and the single layered body wall, and also the electron dense particles on the cytoplasmic face of the host plasmalemma. 1541A/27. 10090.



161

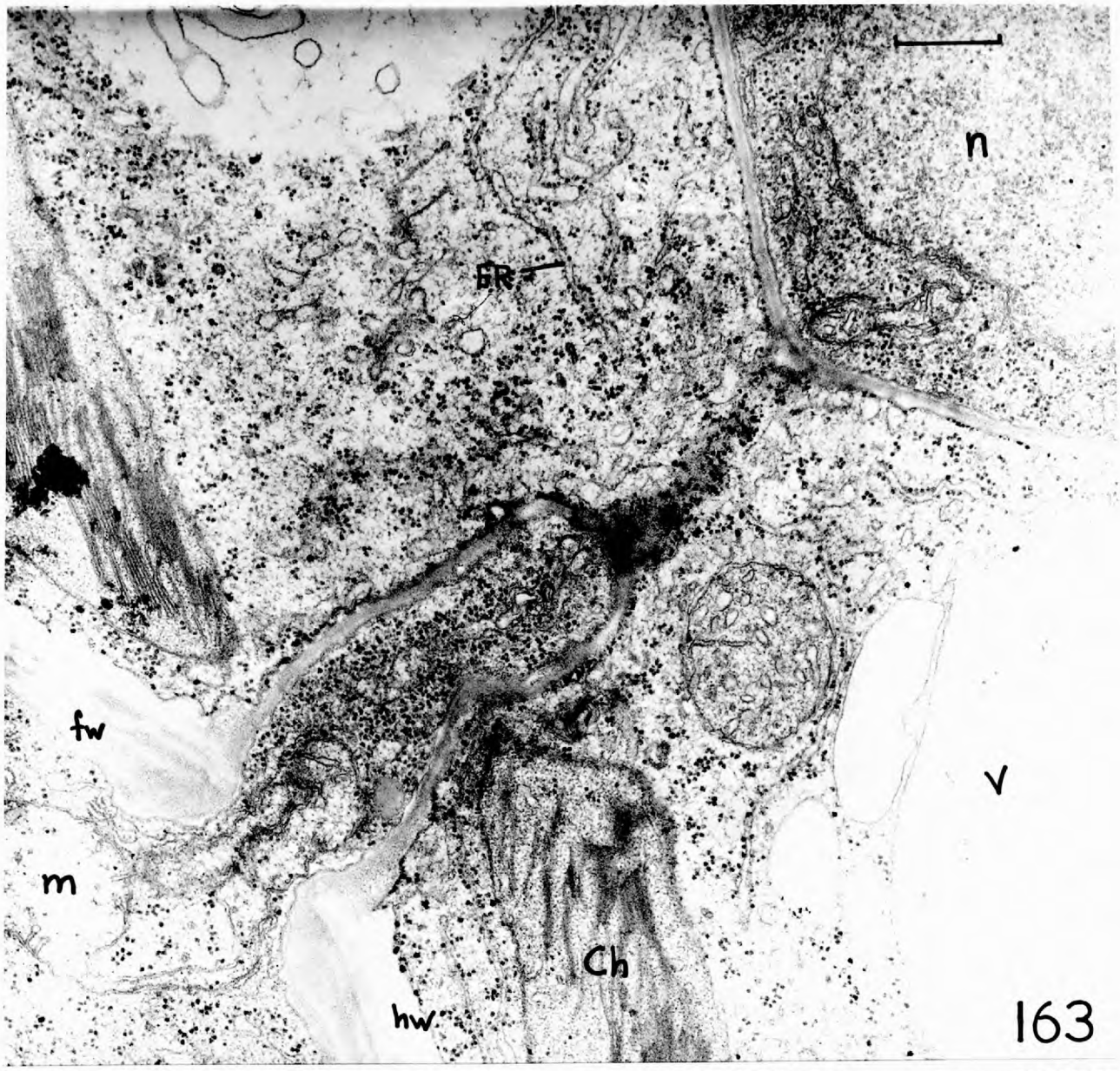


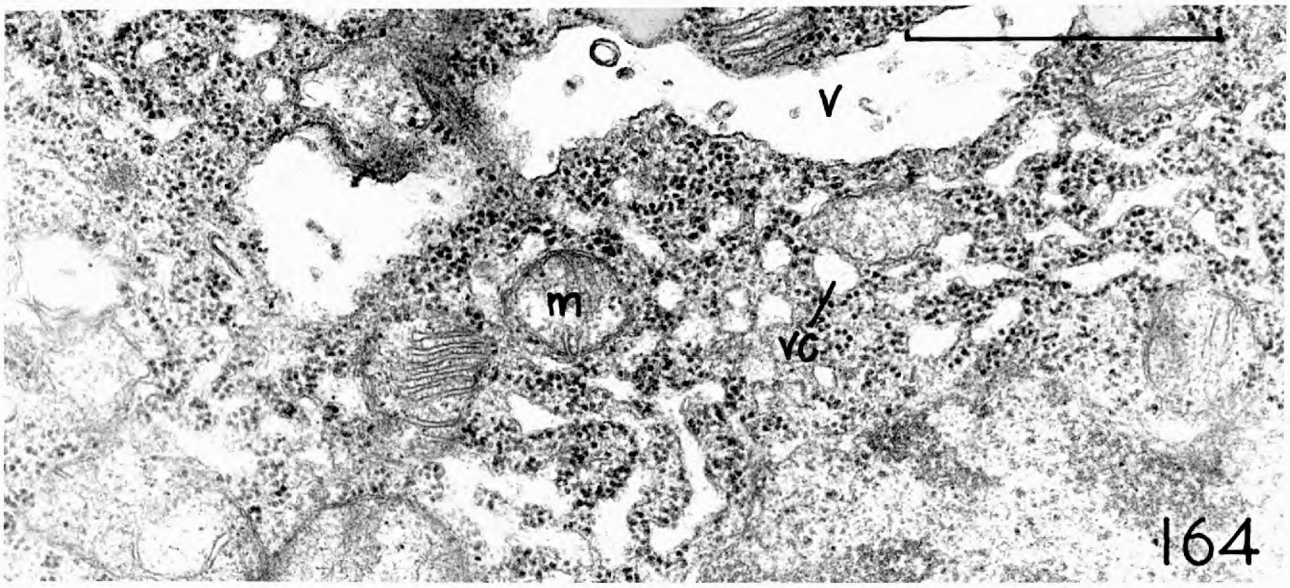


162

163. Section median to the penetration pore showing the electron dense particles on the host plasmalemma in the stem region cut at grazing incidence. Note the absence of a large sheath and the 'dimpled' mitochondrion (m). 1541A/27. 10082.

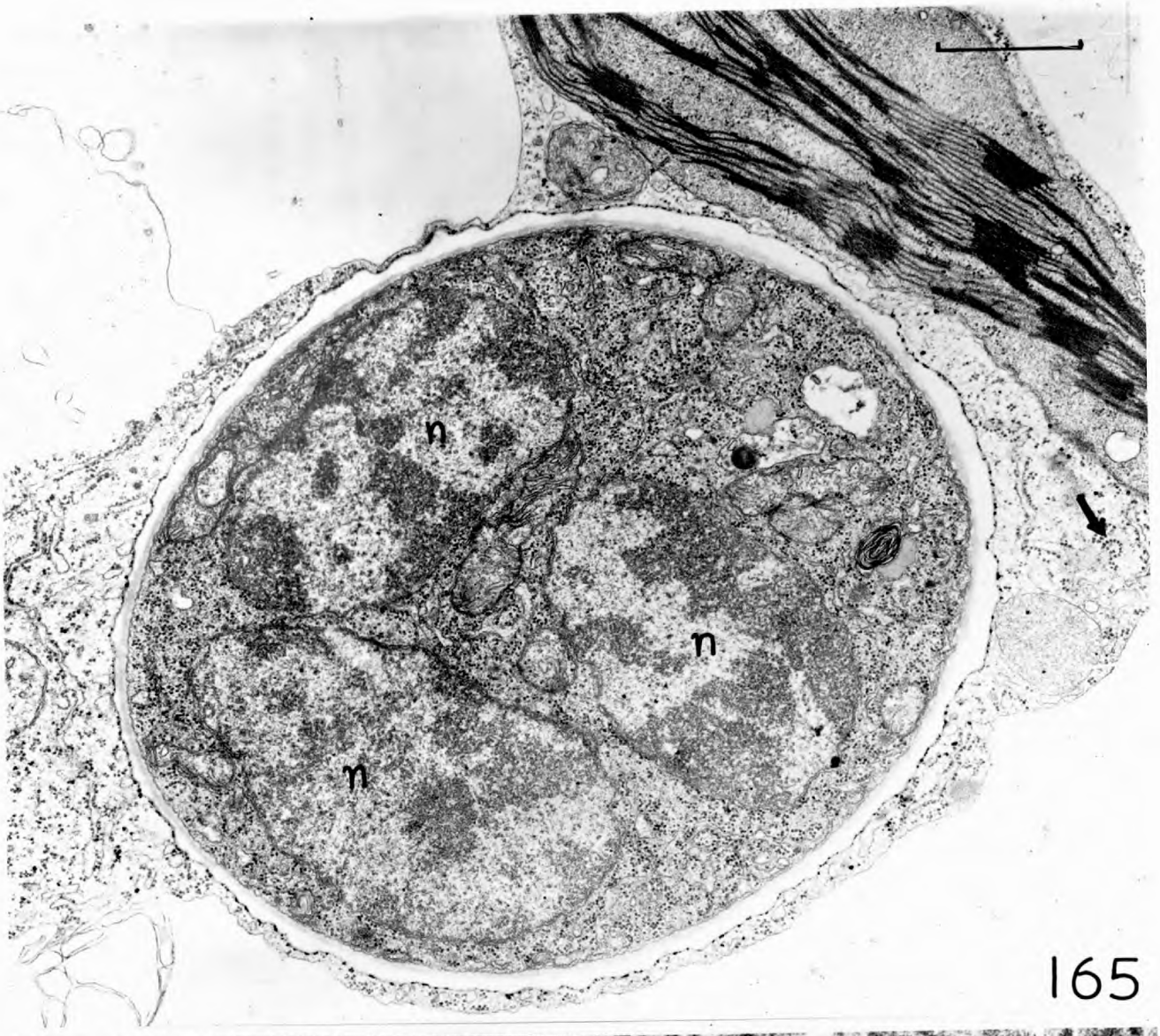
164. Section through the cytoplasm in the body region of a young haustorium showing a large vacuole-like vesicle with tubular membranous inclusions and other scattered vesicles (vc), probably expanded regions of the E.R. 1541A/27. 10086.


n
ER
fw
m
V
Ch
hw
163


V
m
VC
164

165. Section through the body region of a haustorium possessing three nuclei (n). Note the polysomes arranged in the form of spirals (arrow). 1541A/27. 10091.

166. A lomasome (lo) against the body wall of a haustorium. The fungal plasmalemma (fp) can be traced into the lomasome. 1541A/27. 10088.



165



166

8. OLD INFECTIONS

This section describes fungal and host cells at a late stage in the development of sori from which teleutospores were then being produced. At this stage the material was taken and fixed as described earlier (p. 48) from 24 day-old lesions on whole plants. The tissues of the leaf were generally chlorotic, but with green islands immediately around the sori.

(a) Host Cytoplasm

There was a general similarity between the cytoplasmic contents of cells from sori, green islands and chlorotic tissue. In many cells there was severe disruption of the cytoplasm with breakage of its contained membranes, fragmentation of the vacuole, sometimes into smaller vesicles, and a general dispersion of organelles and cytoplasm within the lumen of the cell (Fig. 167, 168). Despite the disruption of the tonoplast the plasmalemma generally remained substantially intact and still closely applied to the cell wall (Fig. 167)

Chloroplasts were often swollen but starch grains were not observed (Fig. 169). The chloroplast envelope was sometimes ruptured with a consequent loss of the stroma, though the osmiophilic droplets remained, often apparently

trapped within the surrounding network of lamellae, which however, showed various degrees of disruption sometimes with distensions of the thylakoids (Fig. 170). The osmiophilic droplets were about 250nm in diameter and often numbered about 20-30 per section of a chloroplast, they were, therefore, larger and more numerous than in the chloroplasts of cells from uninfected plants and from invaded cells of plants with younger infections (cf. Fig. 11, 12, 25, 171, 172).

Mitochondria were not larger than those in apparently healthy cells but were rounded in contour and had dense contents (Fig. 168). The cells also contained microbodies, cisternae of rough E.R. and Golgi bodies, all of which were still recognisable in the disrupted cells (Fig. 167, 168).

(b) Haustoria

Haustoria in disorganised cells possessed a body wall composed of two layers of about 85nm in total thickness (Fig. 176) which appeared similar to that found in less advanced stages of invasion and described in Section 3 (p. 97).

The cytoplasm contained large globules of lipid (Fig. 174), and lipid-like osmiophilic droplets with characteristic 'streaky' tails were present in the vacuoles. The presence of these droplets apparently overlapping areas of vacuolar membrane seen in oblique surface view (Fig. 175) was explained by the fact that the tails were small enough to allow the

accommodation of both the membrane and the tails in the thickness of one section.

(c) Teleutospores

At this stage sori produced teleutospores rather than uredospores. Teleutospores had multilayered walls, thicker than those of the uredospores and were even more difficult to fix and embed satisfactorily (Fig. 177).

Clear areas with sub-circular profiles, without a bounding membrane were arranged around the vacuoles (Fig. 177). The bodies closely resembled the lipid droplets observed around vacuoles in uredospores (e.g. Fig. 72), though they completely lacked any staining capacity.

Ribosomes were aggregated into groups of four or five and occasionally into double linear chains, the ribosomes in one row alternating with those in the other as would be the case if they were arranged in the form of a tight helix (Fig. 178).

In areas of the cytoplasm near to the wall, small, electron opaque particles about four times the size of ribosomes were present, these possessed a similar appearance to material incorporated in the inner layers of the spore wall (see below).

In a mature spore (Fig. 177) large aggregates of electron dense material in the form of compressed globules surrounded

by narrow clear zones formed a thick layer (about 0.2μm) adjacent to the spore wall. The internal preservation of the spores was such that the position of the plasmalemma could not be established.

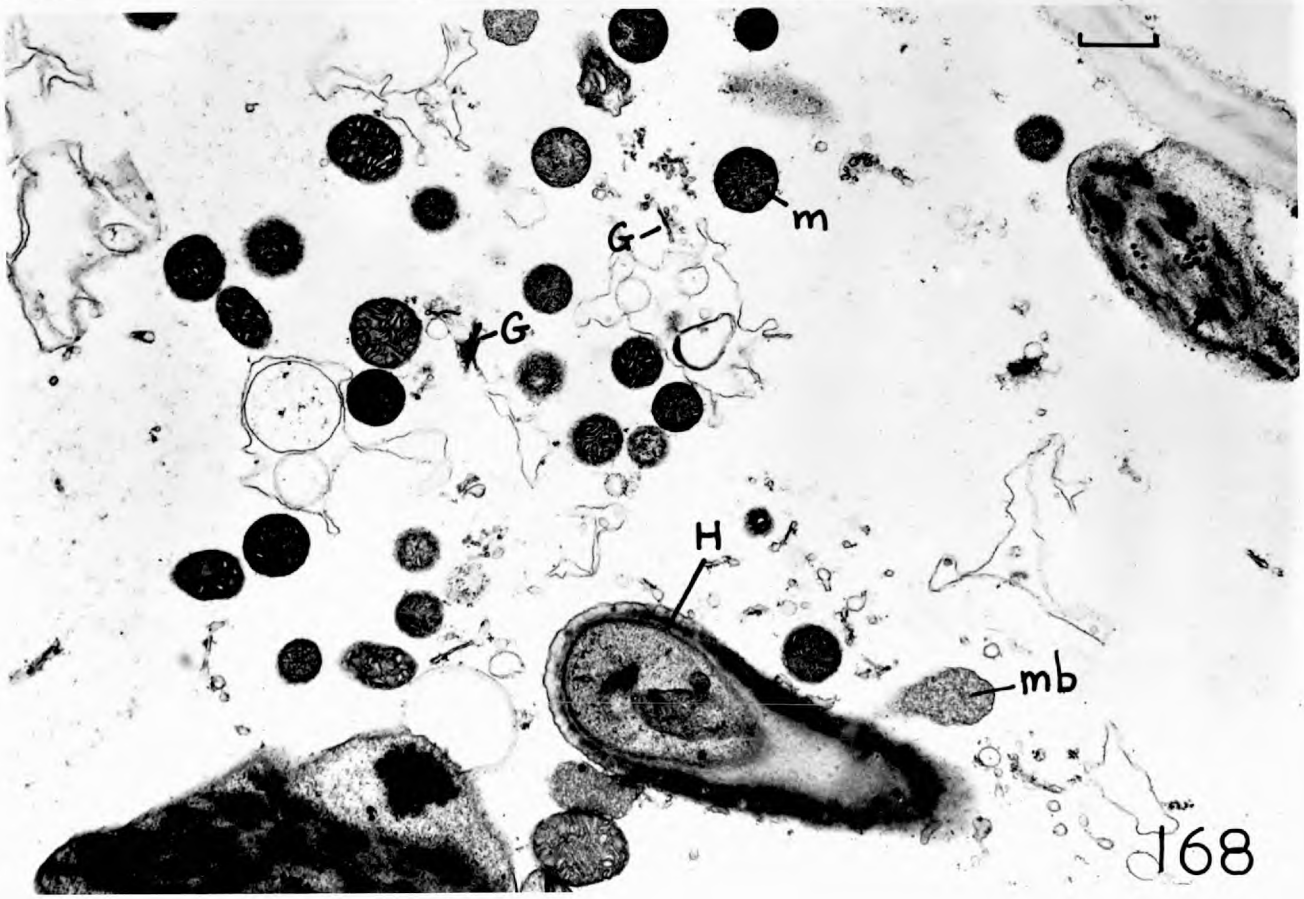
The wall appeared swollen at the apex of the teleutospore where layers of parallel fibres were loosened in texture with small, electron dense,rounded flecks distributed throughout the whole thickness of the wall (Fig. 178). These bodies were absent, or present in much smaller numbers, in other parts of the wall (Fig. 177). One or two flecks were present at the boundary of the wall and even projected into the cytoplasm (Fig. 178). The outermost layer of the wall was very electron opaque, of variable thickness, though thicker at the ends (Fig. 177). The other sub-layers of the wall, numbering as many as nine, alternated in electon opacity, with one or two of the layers containing light patches.

167. Section through necrotic cells showing breakage of internal membranes, though the host plasmalemma (hp) remains applied to the host wall. 1714B/2. 10199.

168. Cell containing haustoria (H). Note the rounded off mitochondria (m) with dense contents. Golgi bodies (G) and microbodies (mB) can be seen amongst the dispersed cell contents. 1714B/1. 10194.

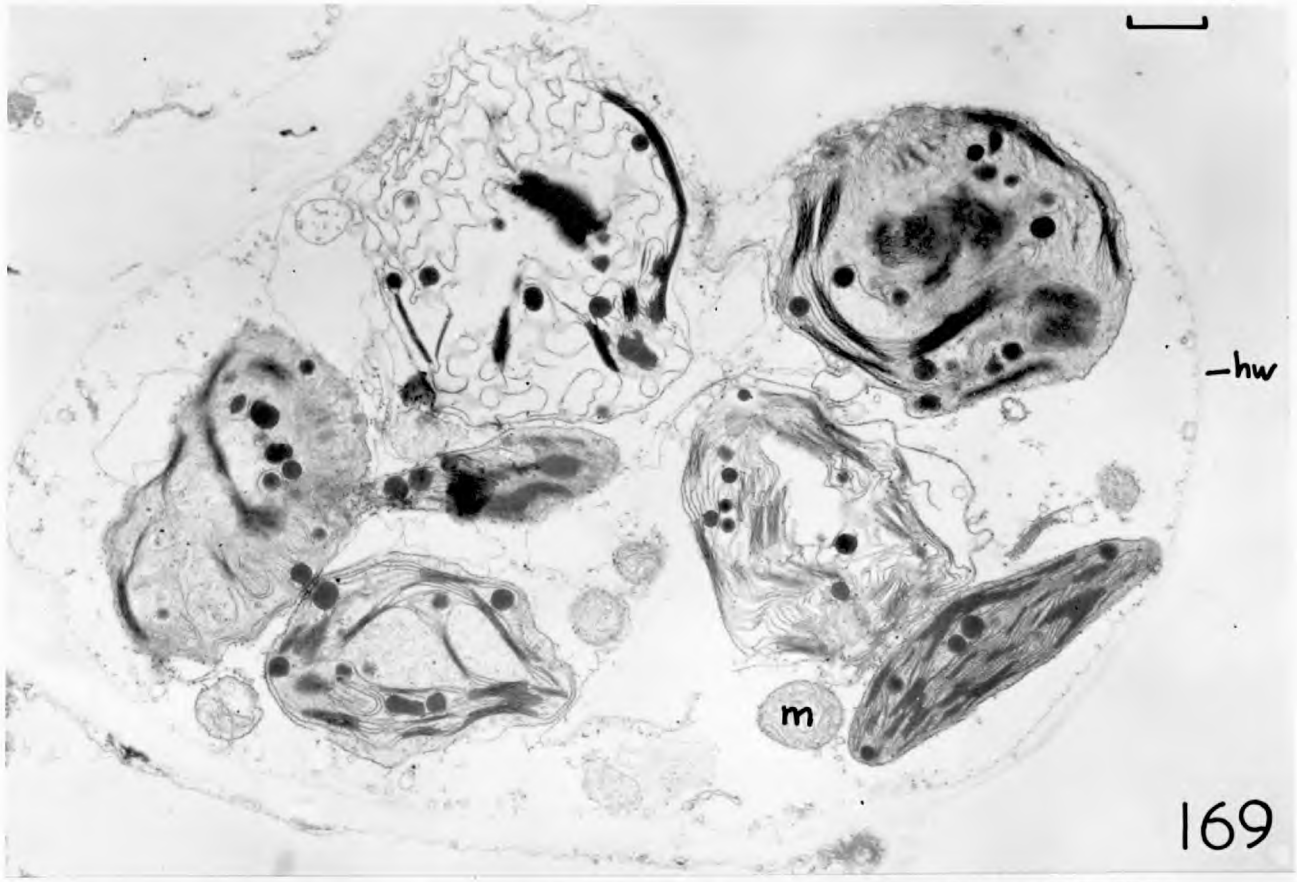
Fig. 167 - 178 are of material 24 days after inoculation with rust uredospores.


m
hp
Ch
I
167

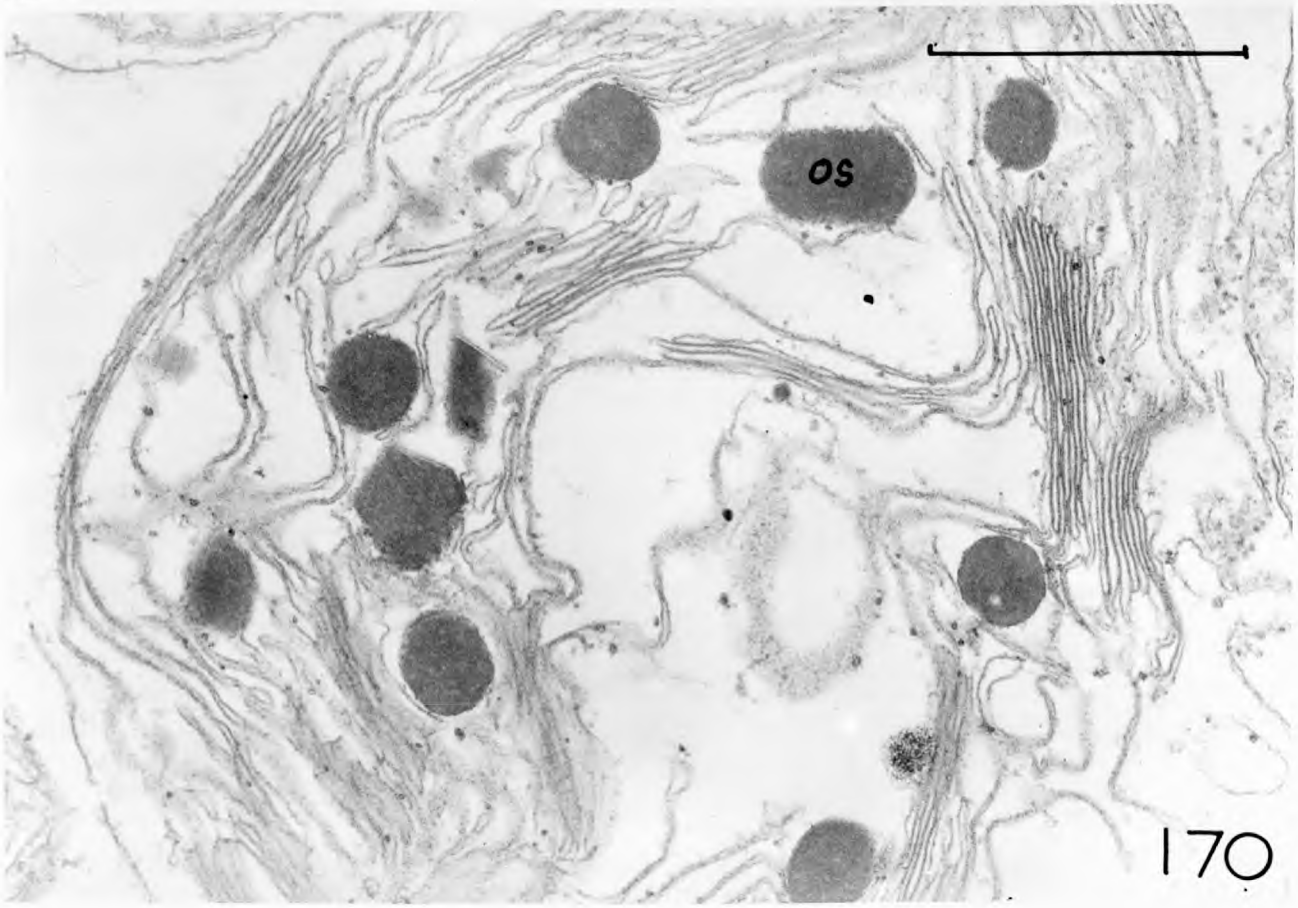

m
G
G
H
mb
168

169. Chloroplasts at various stages of disruption. 1715B/2. 10219.

170. Chloroplast without its envelope membranes and stroma although the osmiophilic droplets (os) remain surrounded by distorted lamellae. 1715B/2. 10220.



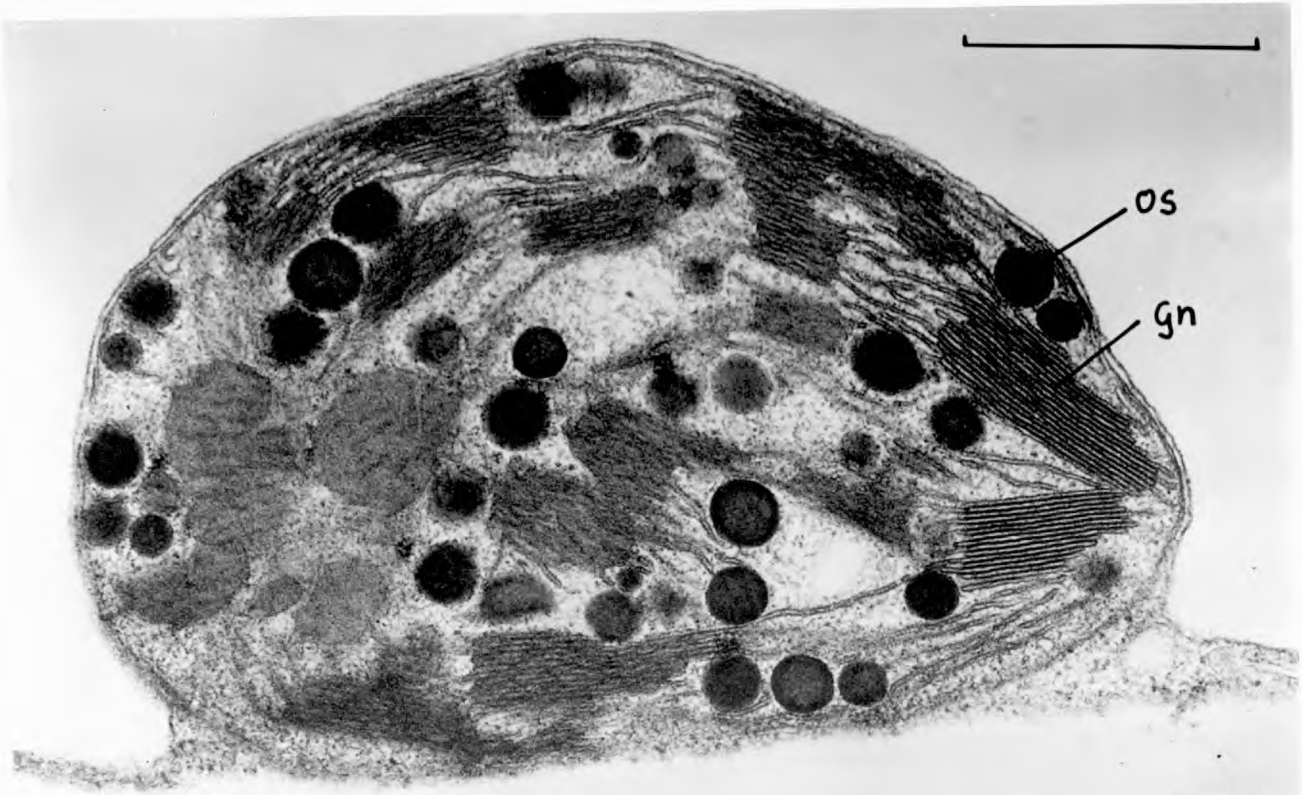
169



170

171. Chloroplasts from the chlorotic zone around the sorus. Osmiophilic droplets (os) are large and numerous. 1715A/1. 10208.

172. Chloroplast from a cell beneath a sorus. Note the absence of starch and the increase in size of the osmiophilic droplets. 1715B/1. 10218.



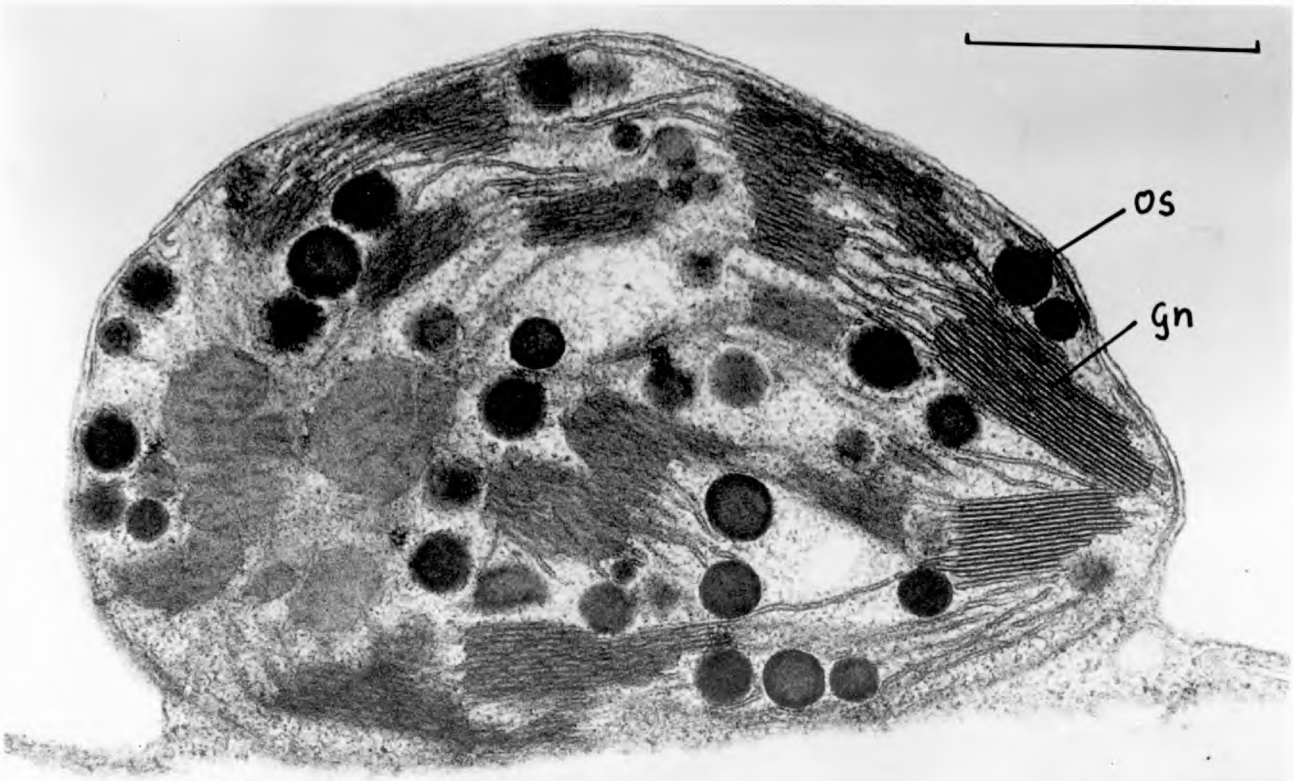
171



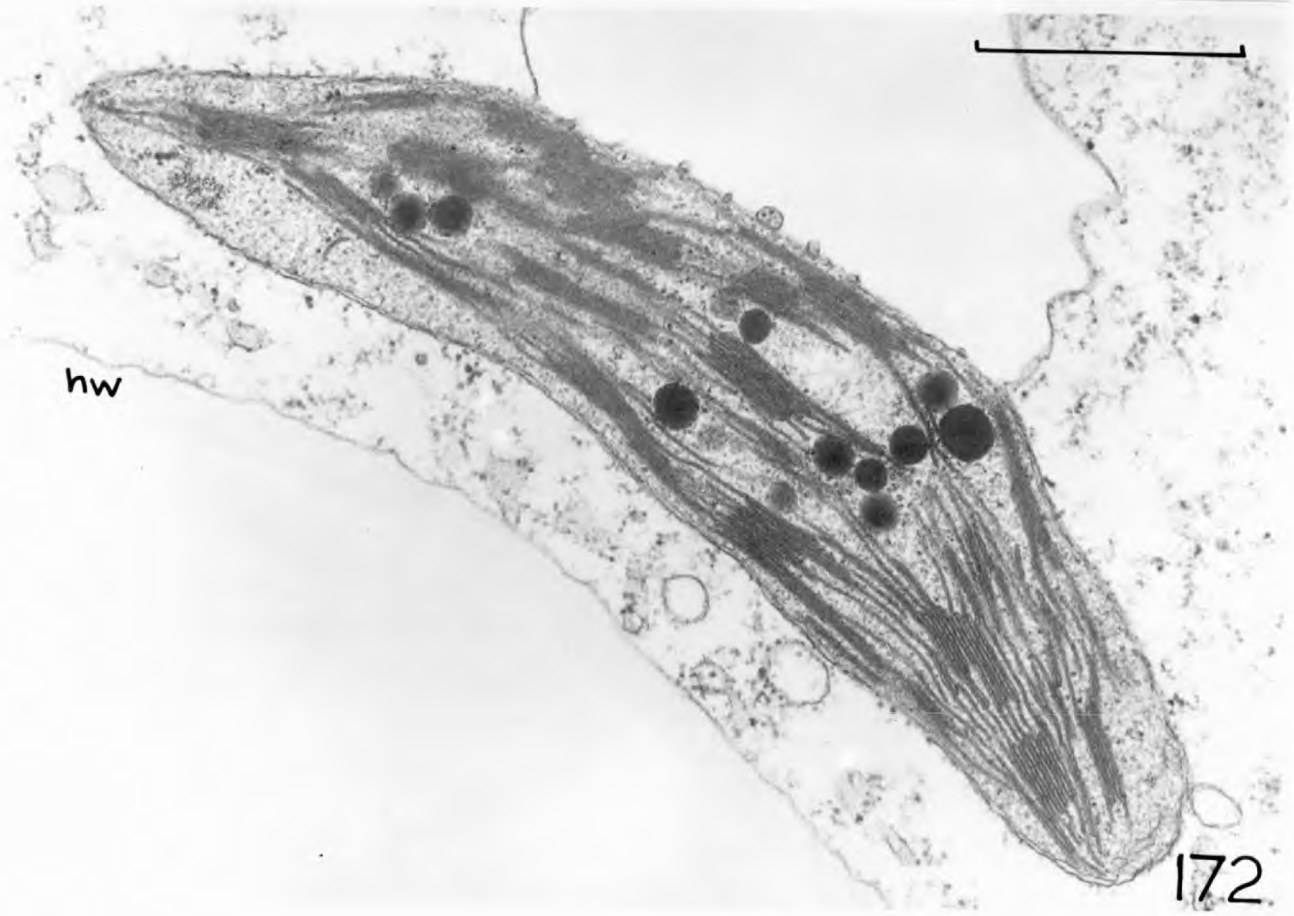
172

173. General view of a haustorium within a host cell showing little membrane breakage. 1714B/2. 10200

174. Body region of a haustorium with a large amount of electron dense globular material with streaky tails (arrow) present within the vacuole (v). 1714B/2. 10203.



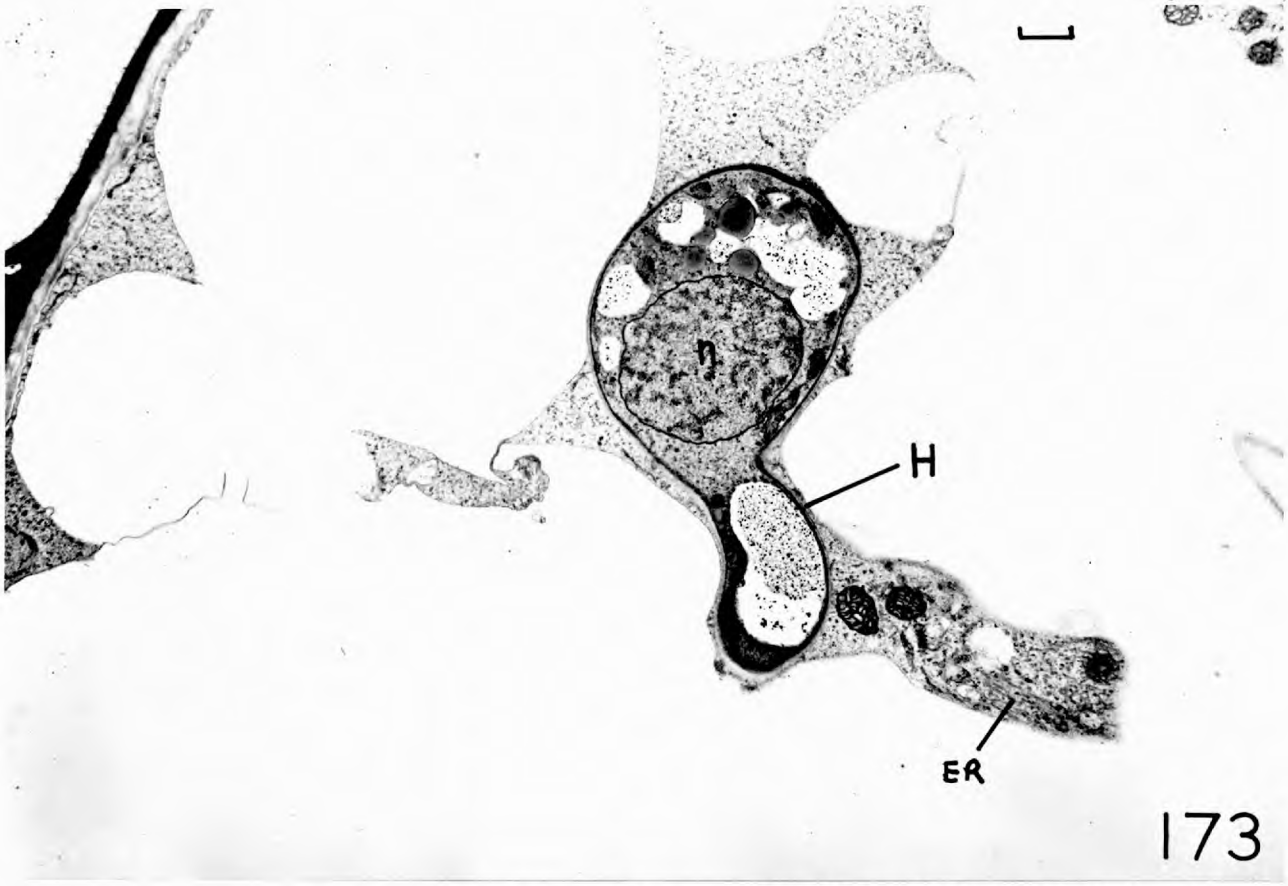
171



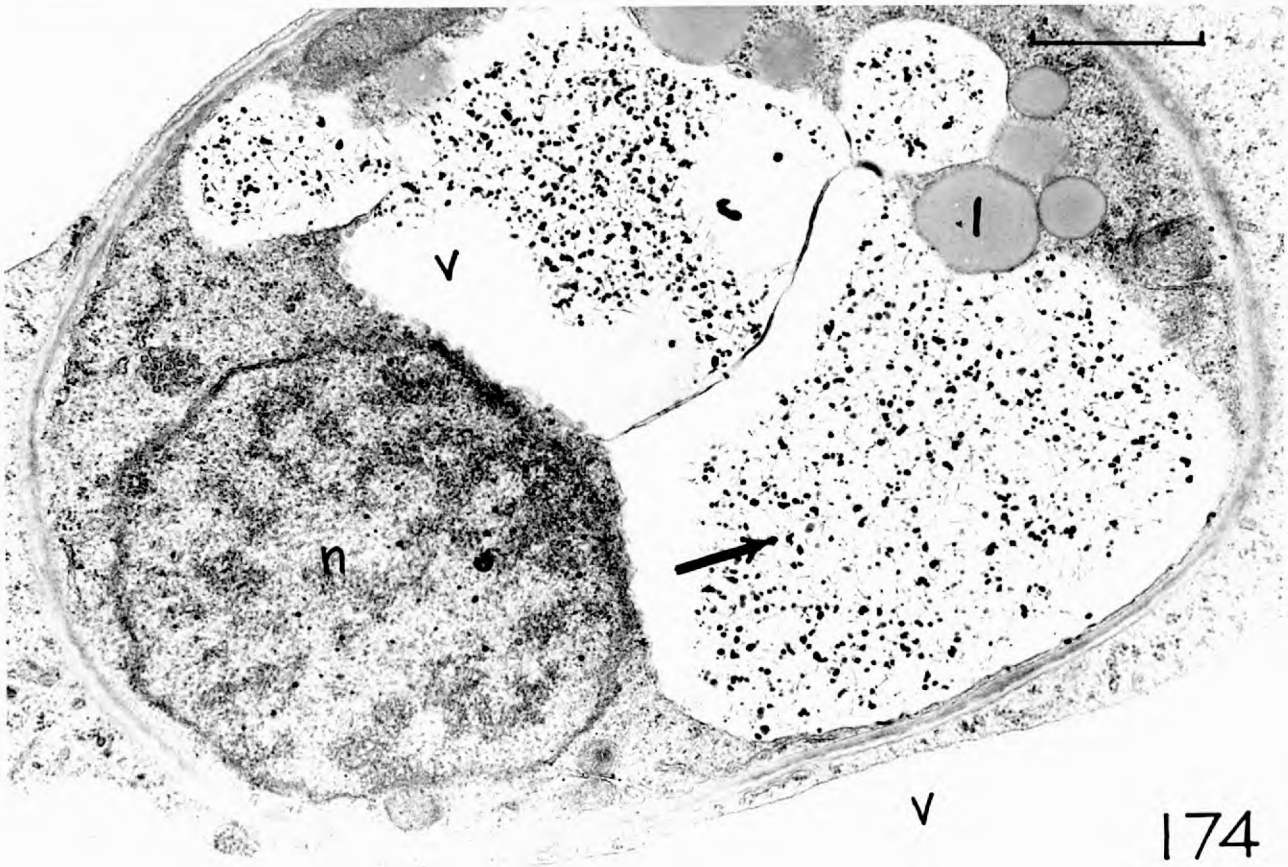
172

173. General view of a haustorium within a host cell showing little membrane breakage. 1714B/2. 10200

174. Body region of a haustorium with a large amount of electron dense globular material with streaky tails (arrow) present within the vacuole (v). 1714B/2. 10203.



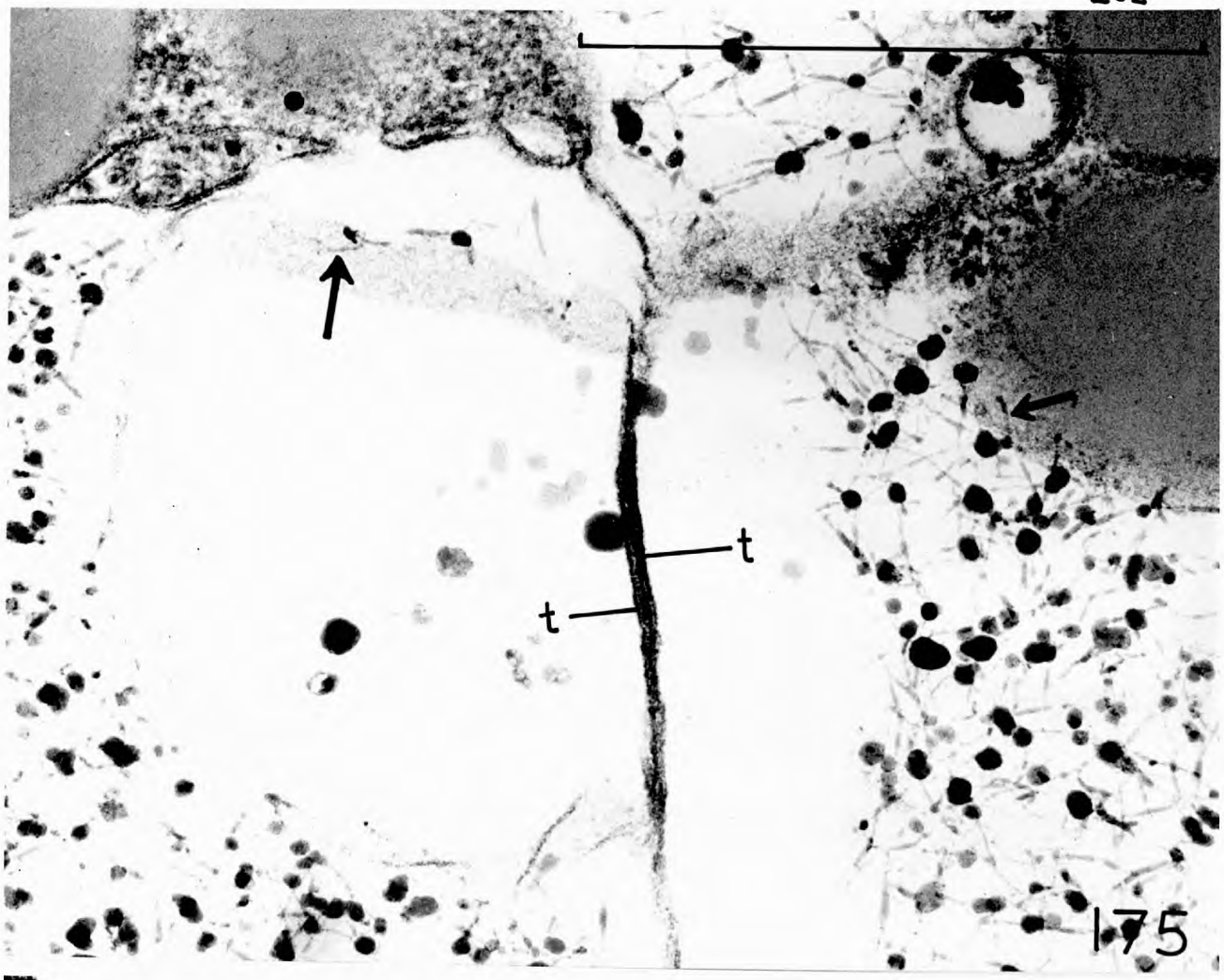
173

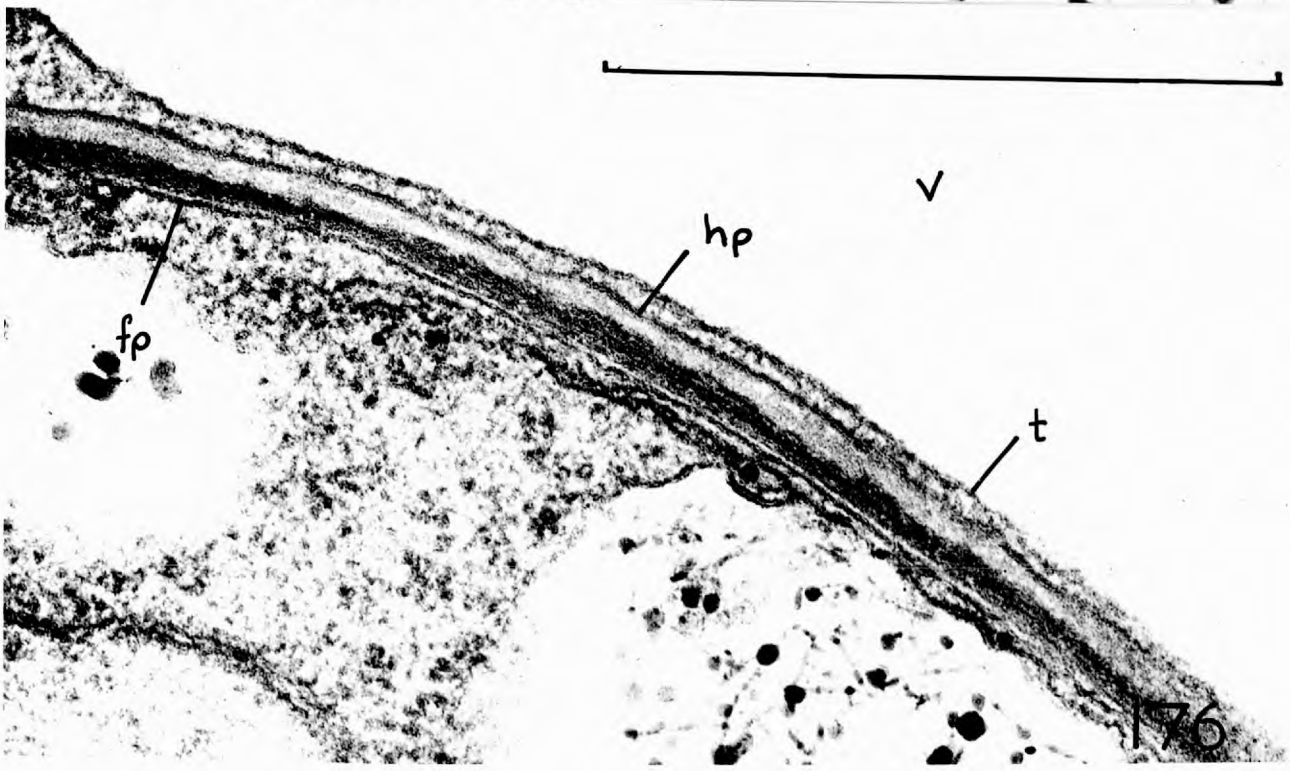


174

175. Detail of Fig. 174 to show a portion of the osmiophilic material and its streaky tails often covering cytoplasmic membrane (arrows) when cut in glancing section. 1714B/2. 10205.

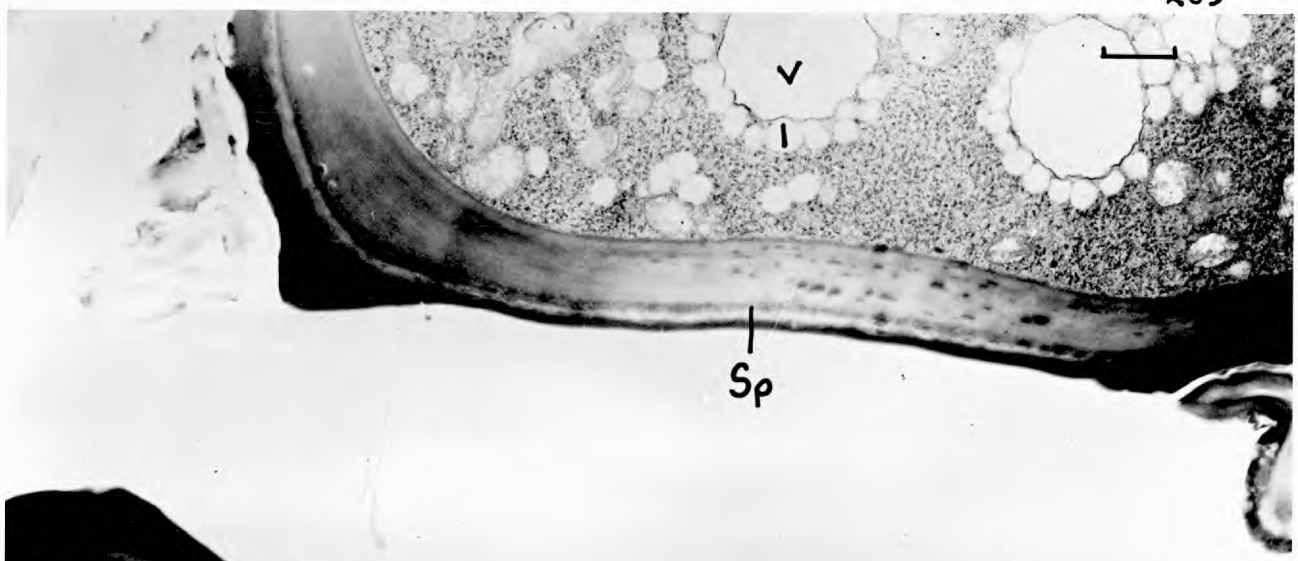
176. Detail of the body wall of a haustorium shows the usual two layers, equal in thickness but differing in electron-opacity. 1714B/2. 10201.

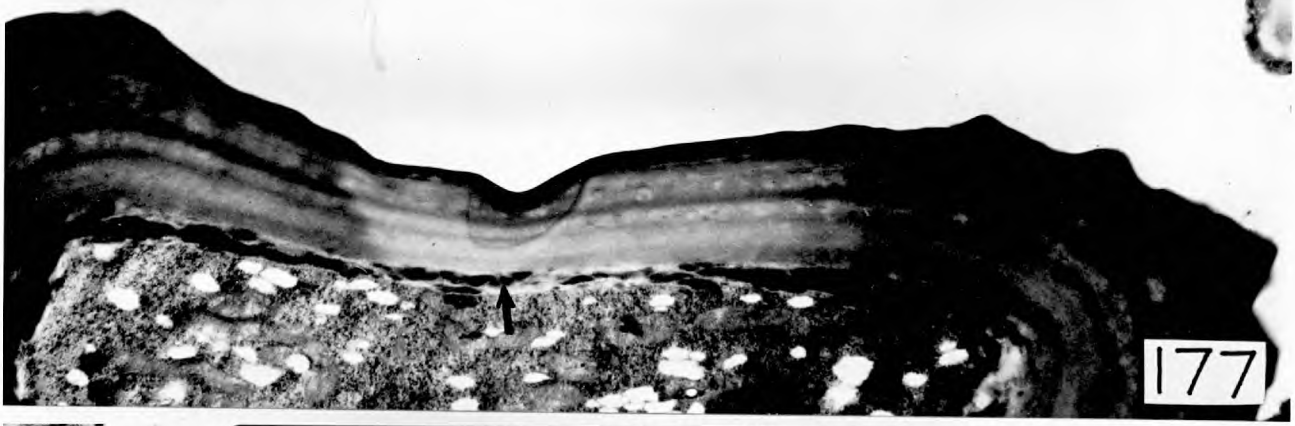

t
t
175

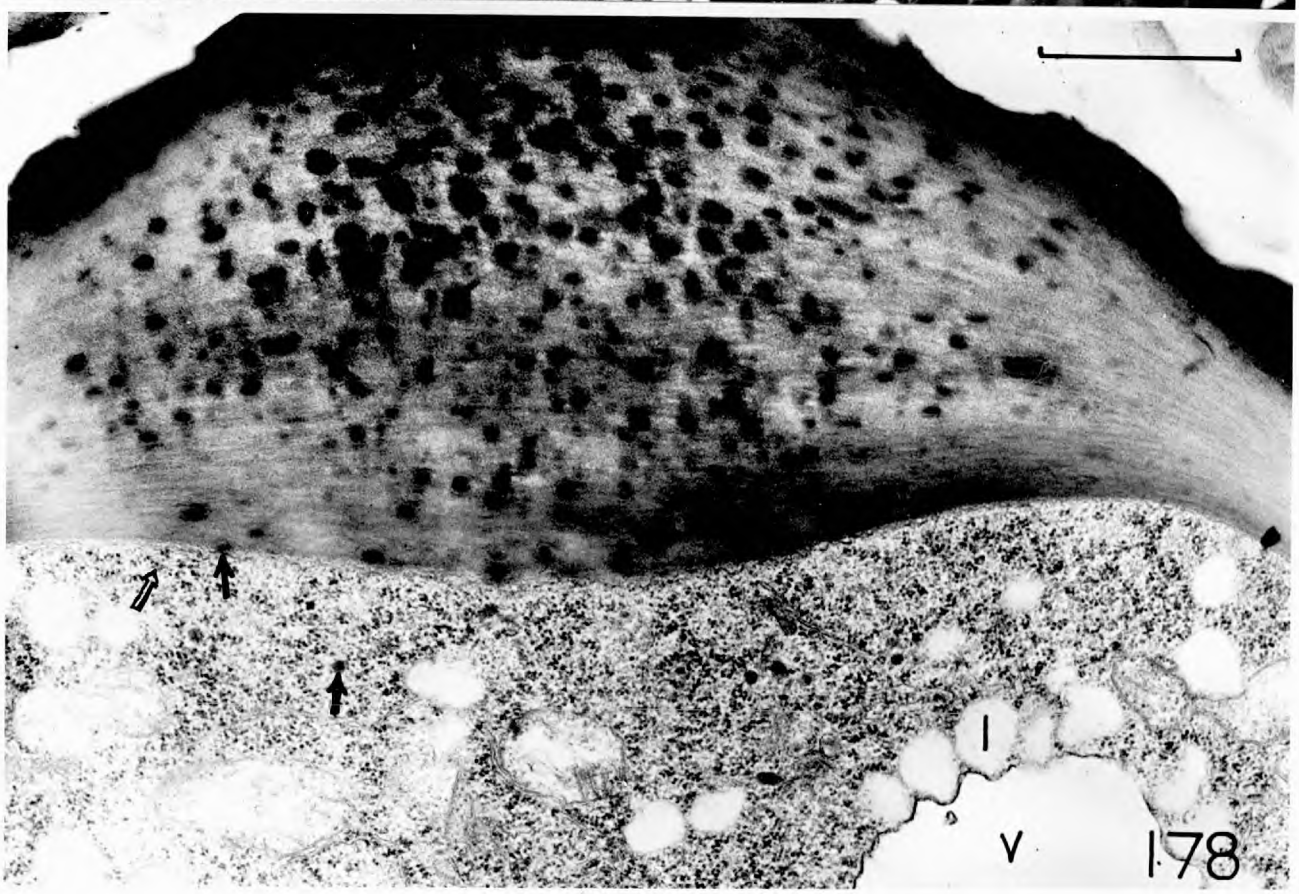

V
hp
fp
t
176

177. Section through two teleutospores (Sp) with multi-layered walls. Lipid droplets appear grouped around the vacuoles (v). The lower spore is badly fixed and embedded with consequent distortion of the cytoplasm and spore wall. Note the electron dense material on the inner face of its wall (arrow). 1714B/3. 10207.

178. Apex of a teleutospore showing the expanded fibrillar material containing electron dense particles. Note particles of a similar density in the cytoplasm (solid arrow) and the 'helical' form of the polysomes (open arrow). 1714B/3. 10206.


V
I
Sp


177


I
V
178

9. RESISTANCE

The reactions of twenty French bean cultivars to U. appendiculatus is given below. Methods were as described on p. 44 . The grade of reaction was scored on the scale (1-5) of Davison and Vaughan (1963), where

1 - immune, no symptoms

2 - necrotic fleck, no uredospores produced

3 - sori less than 300µm in diameter

4 - sori from 301 to 499µm in diameter

5 - sori greater than 500µm in diameter

CULTIVAR		CULTIVAR	
Black Prince	3	Mont d'Or	5
Bounteous	5	Perpetual	5
Canadian Wonder	4	Pinto	5
Dubble Witte	5	Pirry Marrow	4
Emerson 51	4	Red Kidney	3
Imuna	4	Red Mexican	5
Kievitsboon Koekoek (i)	4	Sunrise	5
Kievitsboon Koekoek (ii)	2 & 3	Tendergreen	4
Lightning	4	The Premier	4
Michelite	5	Top Crop	5

In the second batch of 'Kievit' (Zwaan & Zn, now unobtainable) two distinct reactions occurred on the same leaf, necrotic flecking with no uredospore production and small sori, which produced a limited number of uredospores,

surrounded by necrotic tissue.

The second batch of Kievit was selected as being the most resistant cultivar; material from both types of reaction centre was fixed and embedded as described (p. 48) ten days after inoculation.

(a) The Cytoplasm of Invaded Cells

There were no marked fine structural differences between the host cytoplasm from either type of reaction centre.

The cytoplasm and organelles of the host appeared to have reacted sharply to the presence of the fungus in a way not observed in susceptible leaves. The cytoplasm included a large number of vacuoles of different sizes with contents of varied type (Fig. 191). There was no evidence of breakage of membranes as observed in necrotic cells of susceptible leaves (e.g. Fig. 167).

The nuclei were often distorted against haustoria. Nuclear profiles frequently bore deposits of stain in the form of aggregations of small needles (Fig. 184). These deposits were preferentially accumulated on the denser parts of the nucleus and apparently confined to them, although this could not be established with certainty because the covering of stain frequently completely obscured underlying details. In contrast the more opaque parts of the nucleolus remained clear of crystals, though they were deposited over the nucleolar vacuoles.

The chloroplasts often appeared quite normal, with starch grains, osmiophilic droplets and an undistorted lamella system (Fig. 186, 189, 191). However, the chloroplasts in cells on which needle-like crystals were deposited were severely disorganised; in these plastids the membranes of the envelope were not ruptured but the thylakoids were evenly distended without breakage of membranes, the starch grains and the osmiophilic droplets still persisted apparently unchanged (Fig. 190). The needle-like crystals of stain were deposited only on what remained of the stroma between the lamellae and were orientated parallel to the latter (Fig. 185).

Mitochondria in such cells were not greatly distorted and only received apparently casual deposits of stain when crystals were plentifully deposited on the surrounding cytoplasm (Fig. 185).

Lipid droplets were frequently observed in the cytoplasm of Kievit (Fig. 179, 184), although such bodies were not normally present in healthy or diseased cells of the susceptible variety Pinto.

Cells, especially around veins, contained a large deposit of loose fibrillar material, often with sequestered membrane, between the walls and the plasmalemma (Fig. 190). This extra deposit of material also occurred in non-invaded cells (Fig. 180), but was absent from similar cells of susceptible plants (Fig. 10). Connections between adjacent cells were

maintained by extensions of the plasmodesmata passing through these deposits and filled with amorphous material continuous with that in the intra-mural parts of the plasmodesmata (Fig. 181). Cisternae of the E.R. were situated close to the termination of the extensions of the plasmodesmata. The plasmodesmata appeared to lack a defining plasmalemma and apart from obscure traces of small membrane fragments in the region of the middle lamella of the walls there was no evidence of internal membranes.

The deposit of new material against the cell wall was similar in appearance to that found in the sheaths of haustoria (e.g. Fig. 53).

(b) Fine Structure of Hyphae and Haustoria

The cytoplasm of the intercellular hyphae was often obscured by needle-like crystals of stain and a large proportion of the cells were in stages of necrosis (Fig. 183). Small vesicles were often present within the cytoplasm (Fig. 185) as observed in young haustoria (e.g. Fig. 164), also larger vacuole-like vesicles with tubular inclusions (Fig. 182) and larger bodies with numerous concentric or coiled membranes (Fig. 193).

Haustoria were found in cells of infection centres associated with either necrotic flecks or limited production of uredospores.

Apparently healthy haustoria were found in cells which were severely disrupted with heavy deposits of the needle-like crystals over the host cytoplasm (Fig. 185), although deposits of these crystals were not observed on the cytoplasm or the body wall of the haustorium (Fig. 182).

Haustoria had sheaths (Fig. 187) similar in appearance to those described in the susceptible reaction (e.g. Fig. 56) including examples where the sheath formed a collar-like extension round the stem beyond the region of the neckband (Fig. 188).

The body wall of the haustorium was similar to that of the susceptible reaction (e.g. Fig. 60) and consisted of two layers (Fig. 186), often of equal thickness, but the outer pale layer was more variable in texture and thickness. It was sometimes as much as five times as thick as the inner electron dense layer and was very often uneven in thickness in an individual haustorium. The outer layer often appeared to be divided into two finely granular regions, one against the dark inner layer and the other against the host plasmalemma with a more translucent zone between them, as if the host plasmalemma had become displaced from its original position closer to the haustorium (Fig. 189, 192). Where the proximity of two lobes of a haustorium resulted in two areas of the host plasmalemma being in contact the granular material associated with the haustorial side of these membranes was absent (Fig. 193).

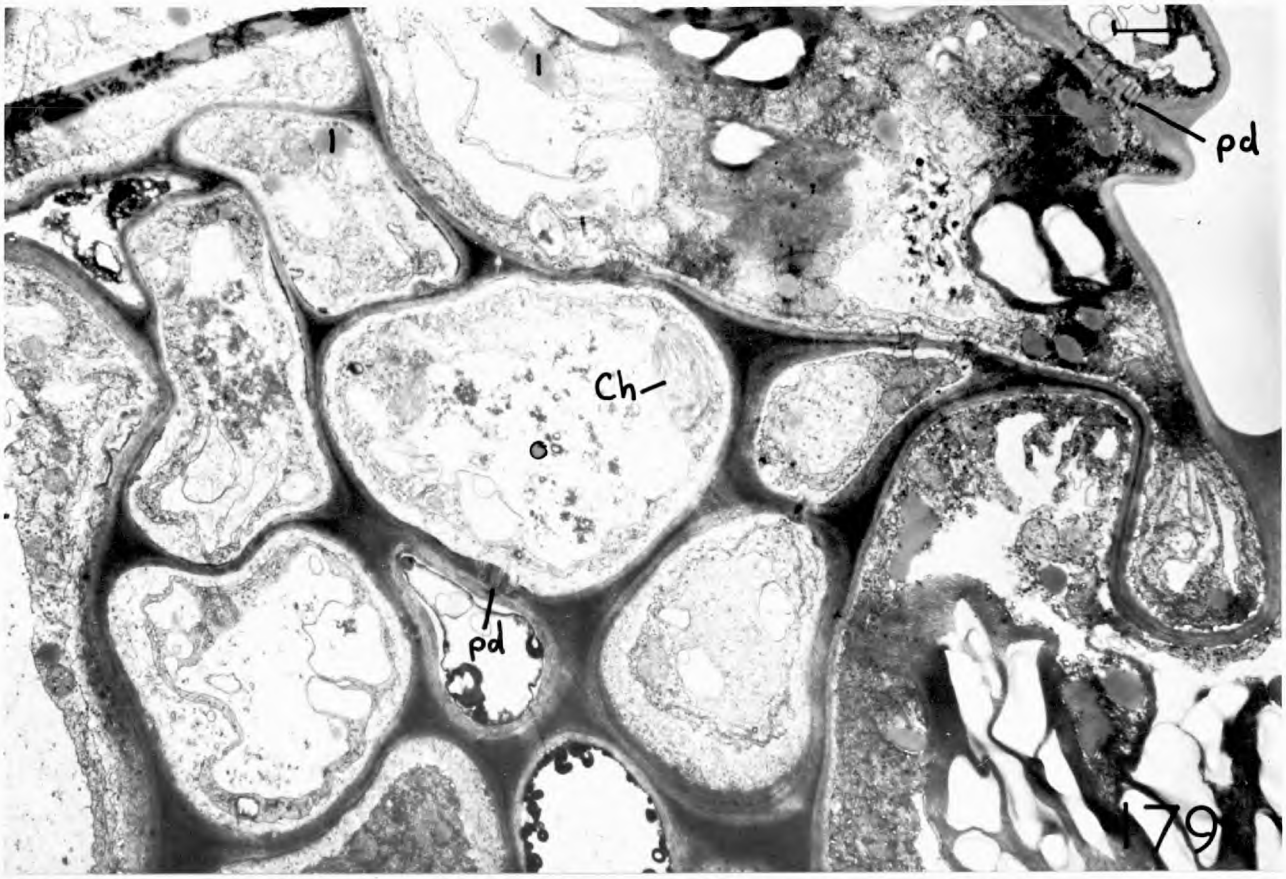
In some host cells near to veins, sometimes containing material between the host plasmalemma and cell wall, single membrane bounded tubular vesicles filled with granular material were present in the cytoplasm in the vicinity of haustoria (Fig. 191). These tubules, which did not appear to be branched, were about 45nm in diameter and up to 800nm in length before passing out of the plane of section. The nature and origin of these tubules and their contents was unknown. They may have originated from the Golgi bodies, as vesicles not greatly different in size from vesicles associated with Golgi and containing granular material were present near to them (Fig. 193). These tubules may also have originated from cisternae of the E.R. which appeared to be associated with the haustoria (Fig. 191, 194), but their lack of branching did not, however, favour an E.R. origin. Tubules were seen in what appeared to be either a state of fragmentation into smaller vesicles or of fusion of vesicles to form tubules (Fig. 192). The granular material contained within the tubular vesicles was similar in appearance to the finely granular component in the outer layer of the body wall of the haustorium (Fig. 192, 194).

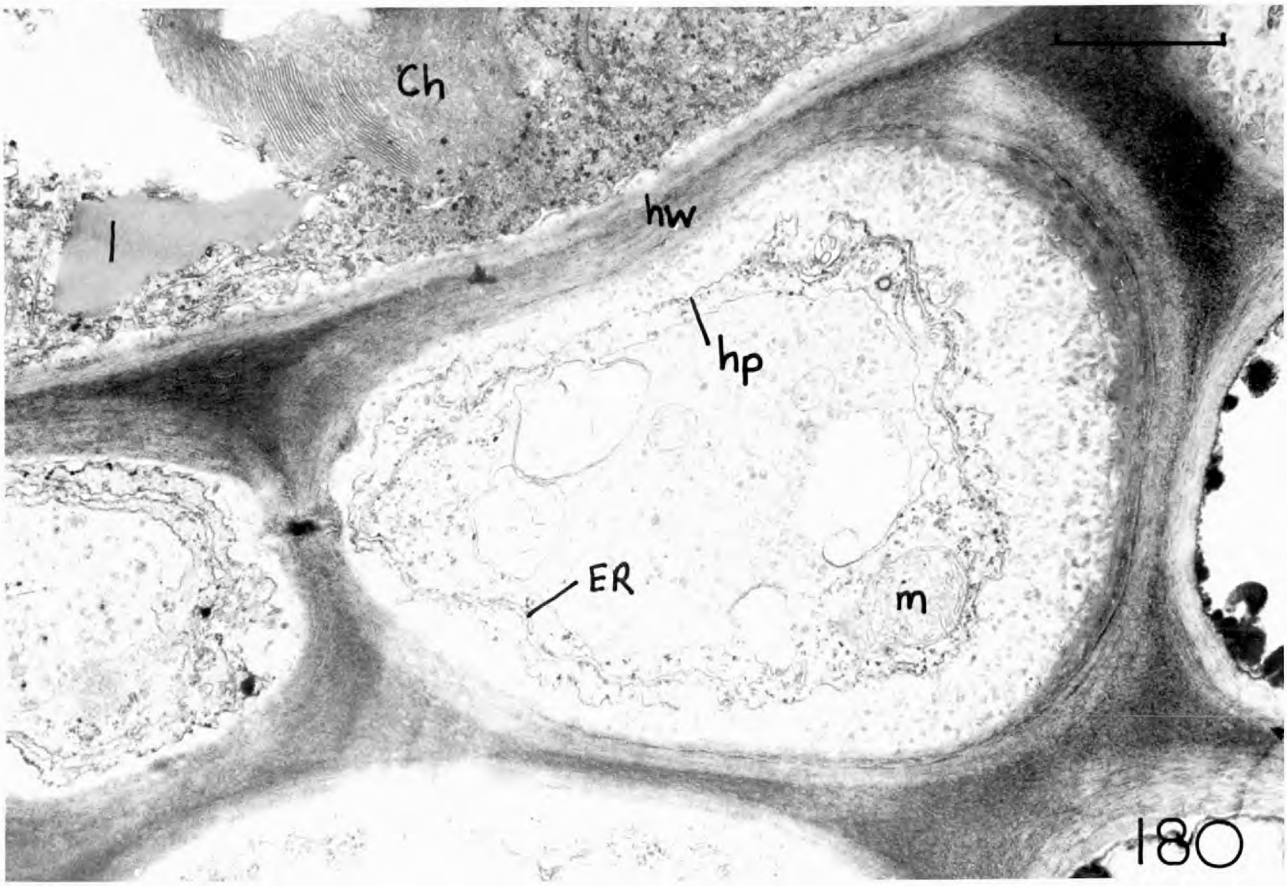
The manner in which individual host cells reacted to the presence of haustoria varied greatly (cf. Fig. 185, 189-191), to a degree which seemed as great among cells from one infection centre as in the whole of a leaf or between leaves of cultivars of different resistance.

Fig. 179-194 are of rust infected Kievit.

179. Low power view of tissue from the vein area showing general vacuolation of cells, plasmodesmata (pd) and numerous lipid droplets (l). 1851A/15. 14142.

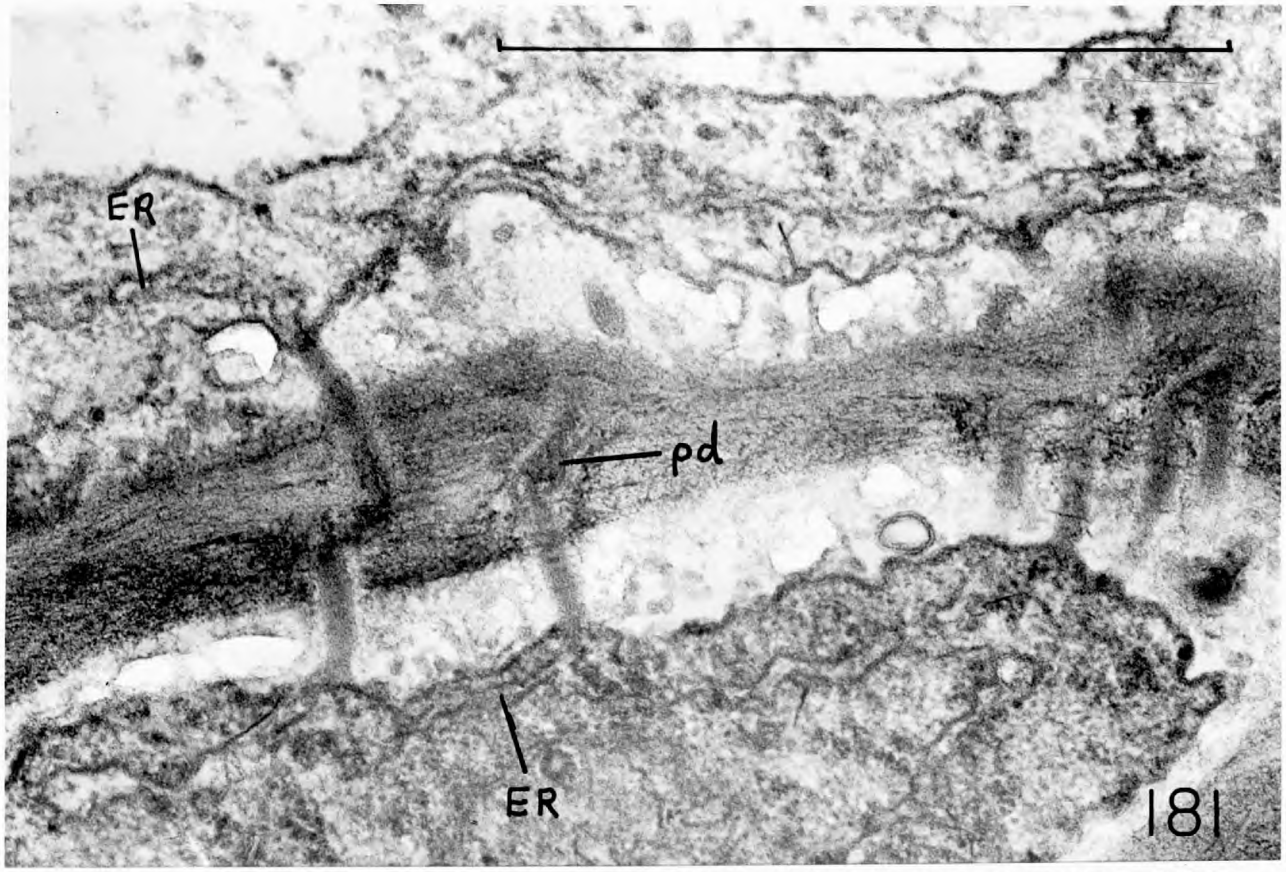
180. Detail from Fig. 179 showing a large deposit of material between the host cell wall (hw) and the host plasmalemma (hp). A cisterna of the E.R. is present around the margin of the cell which has a large central vacuole containing granular material, small empty vesicles are also present within the cytoplasm. 1851A/15. 14144.

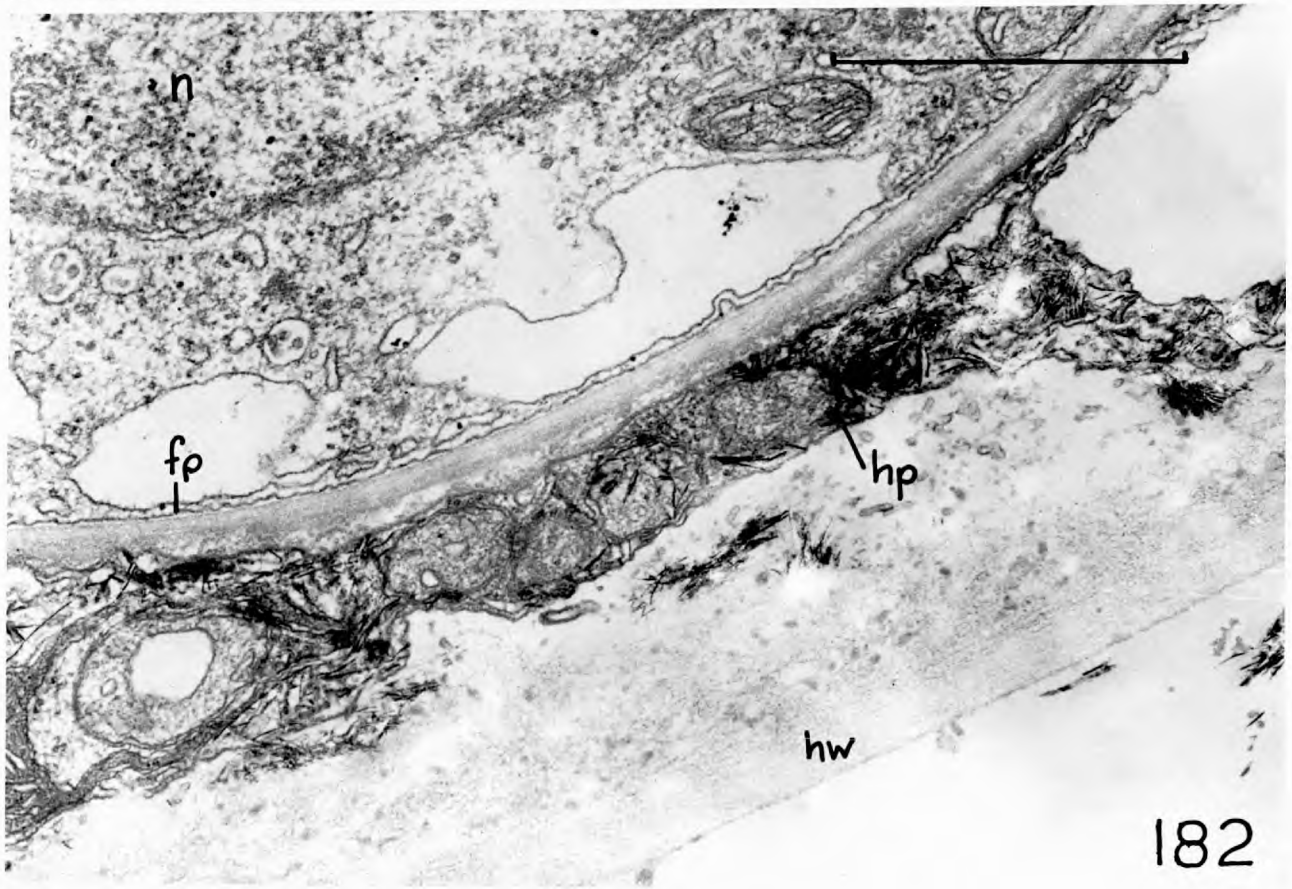

l
l
pd
Ch
o
pd
179


Ch
l
hw
hp
ER
m
180

181. Plasmodesmata (pd) extending across the deposit of material applied to the host wall. Cisternae of the E.R. are present at the ends of these extensions. 1851A/15. 14148.

182. Haustoria from an area of necrotic flecking. Needle-like deposits are present over the host cytoplasm and organelles. Note the fibrillar material between the host plasmalemma (hp) and cell wall (hw). 1851B/10. 14380.


ER
pd
ER
181


n
fp
hp
hw
182

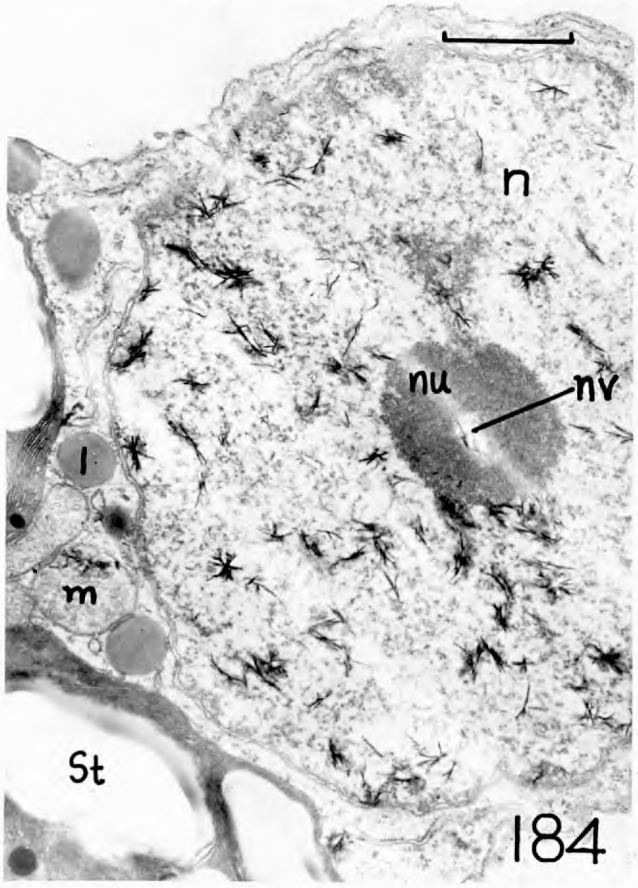
183. Intercellular hypha (I) with a heavy deposit of the needle-like crystals. 1851B/6. 12109.

184. Host nucleus with aggregates of needles in the form of sheaves, also present on the nucleolar vacuole. 1851A/1. 12078.

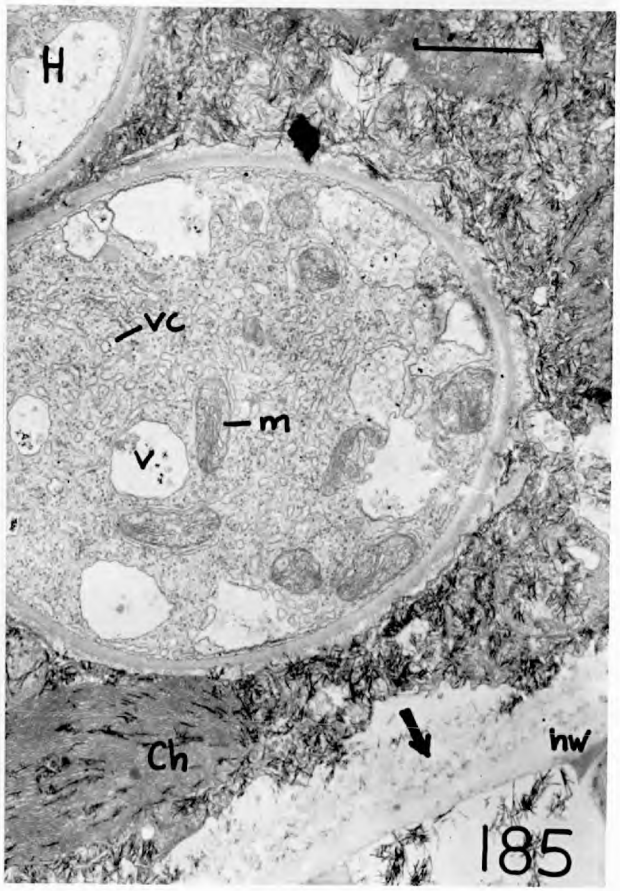
185. Haustorium containing small vesicles (vc) surrounded by host cytoplasm which is obscured by a heavy deposit of crystals. Note crystals on the stroma of the choloroplast (Ch), also the deposit of material adjacent to the host wall (arrow). 1851B/10. 14375.



183



184

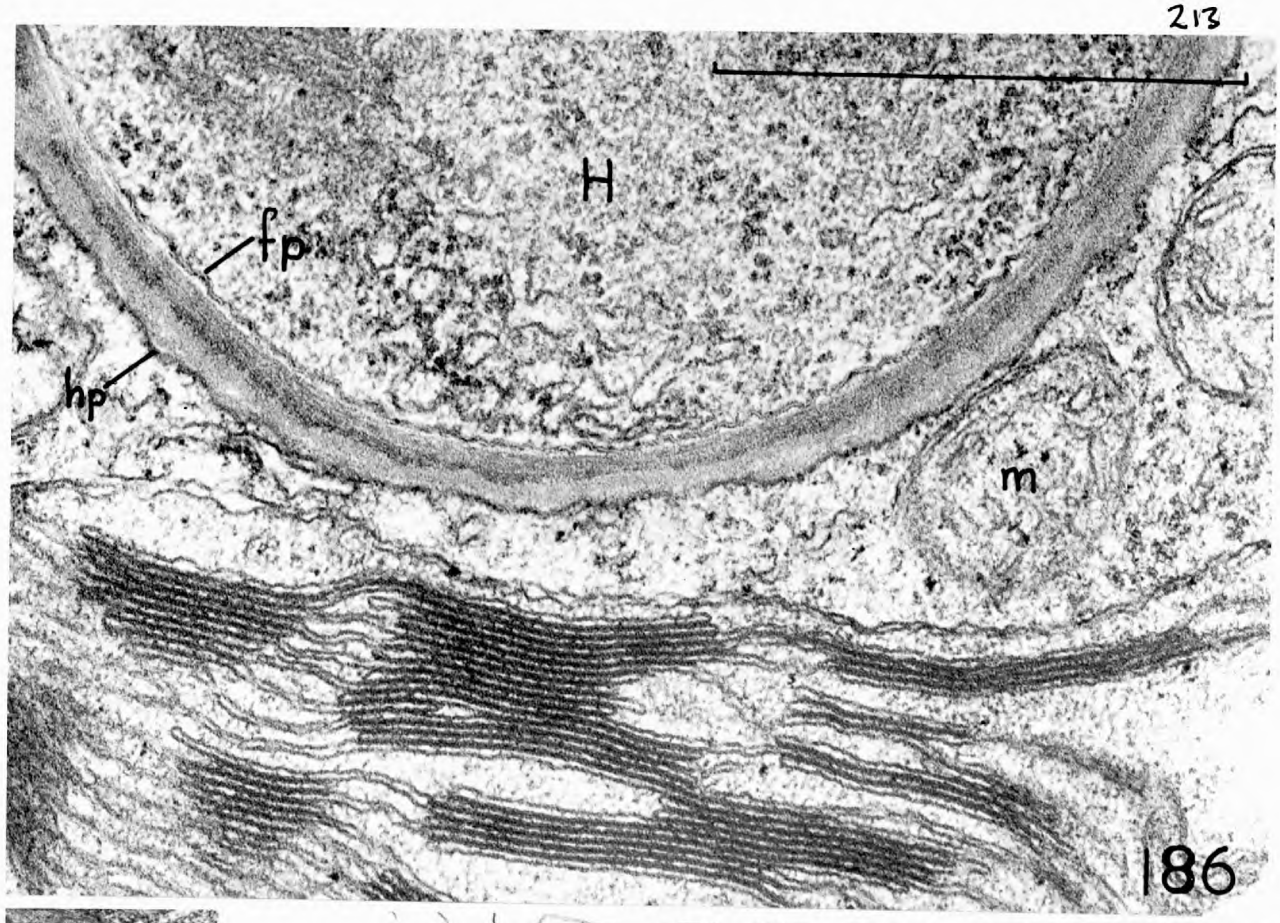


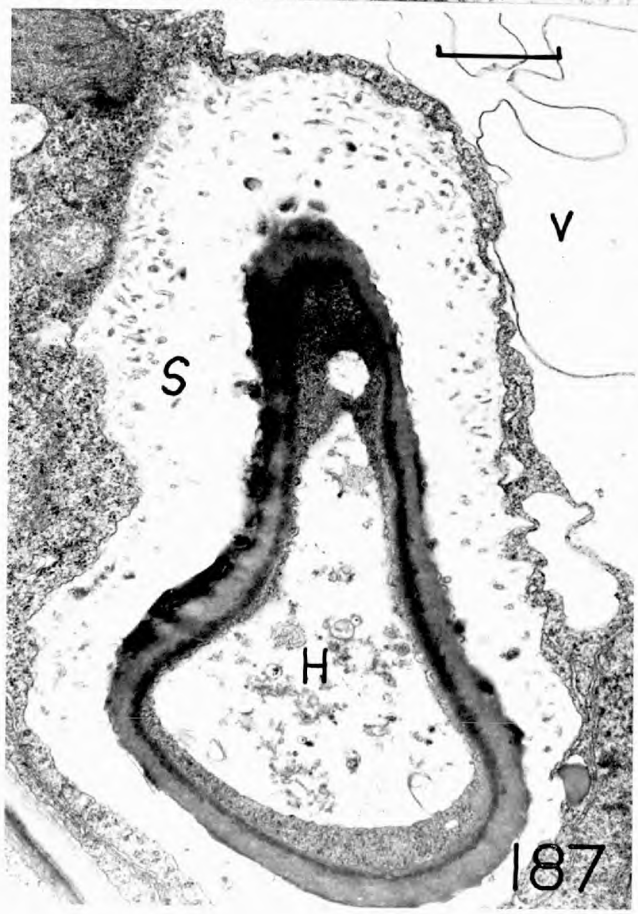
185

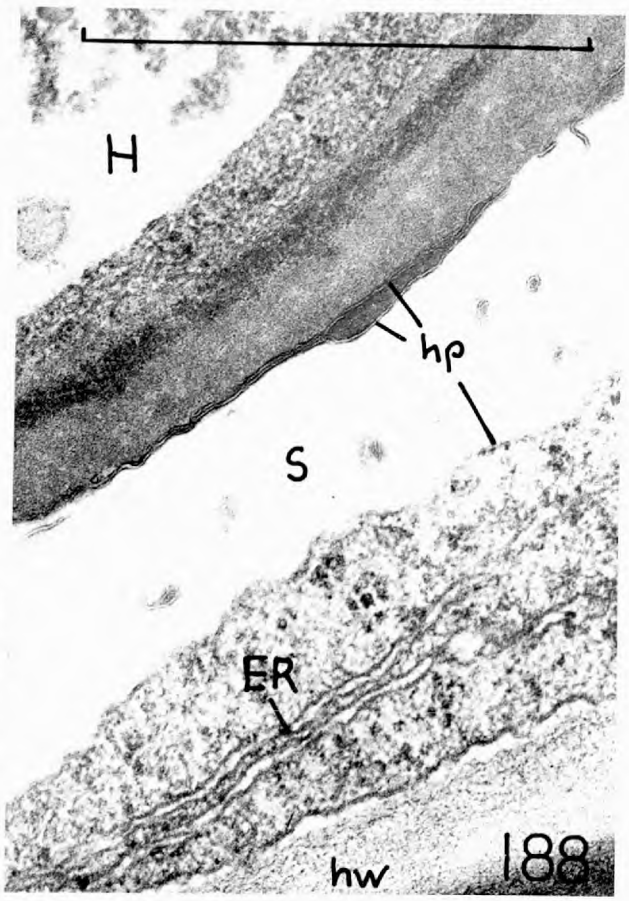
186. Haustorium showing two layered body wall within a cell with no deposit of needles. 1851A/17. 14165.

187. Haustorium with a large sheath (s) with occluded membranes. 1851A/15. 14139.

188. Detail of Fig. 187 to show double host plasmalemma (hp) between the wall of the haustorium and sheath, presumably a re-entrant fold to the neckband. 1851A/15. 14138.

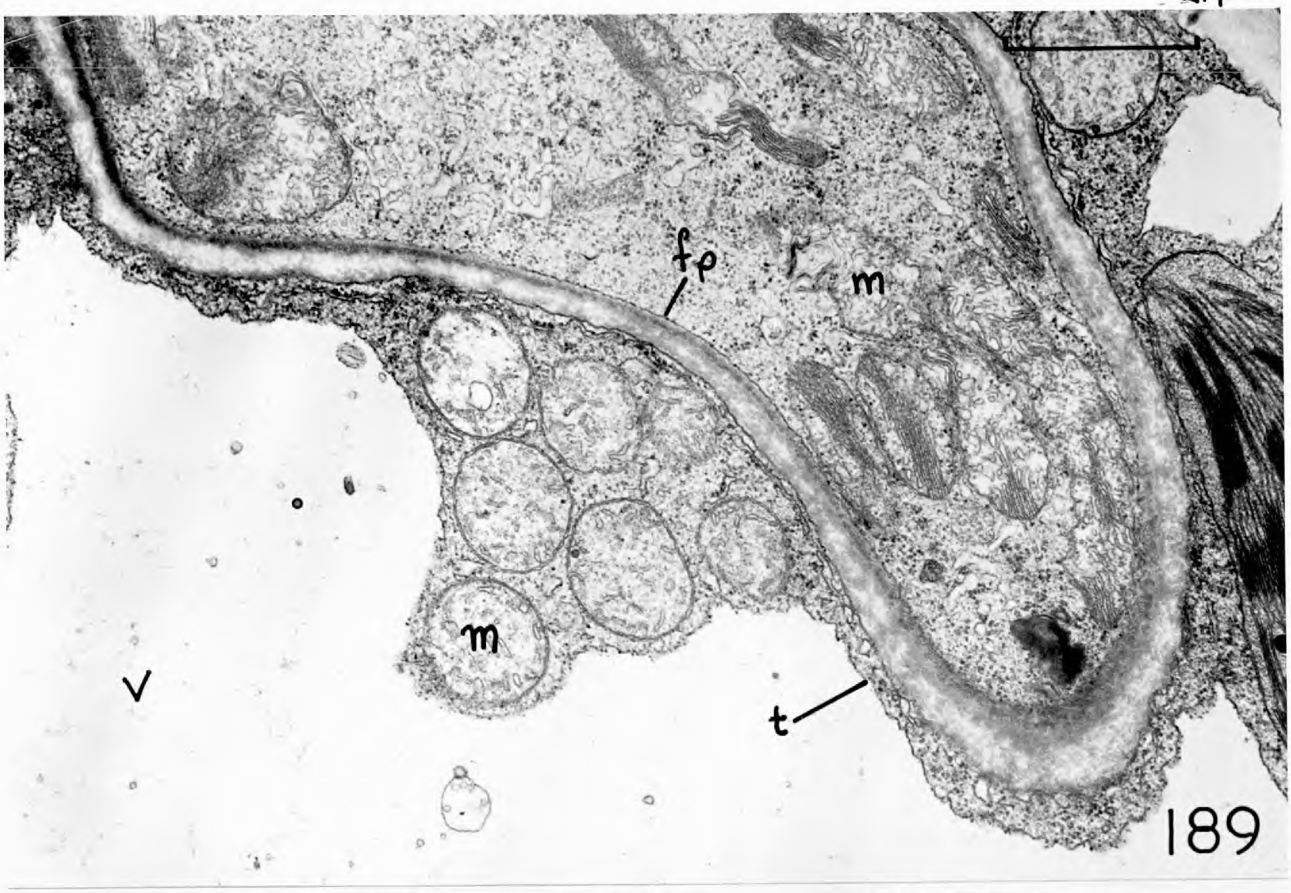

H
fp
hp
m
186


V
S
H
187

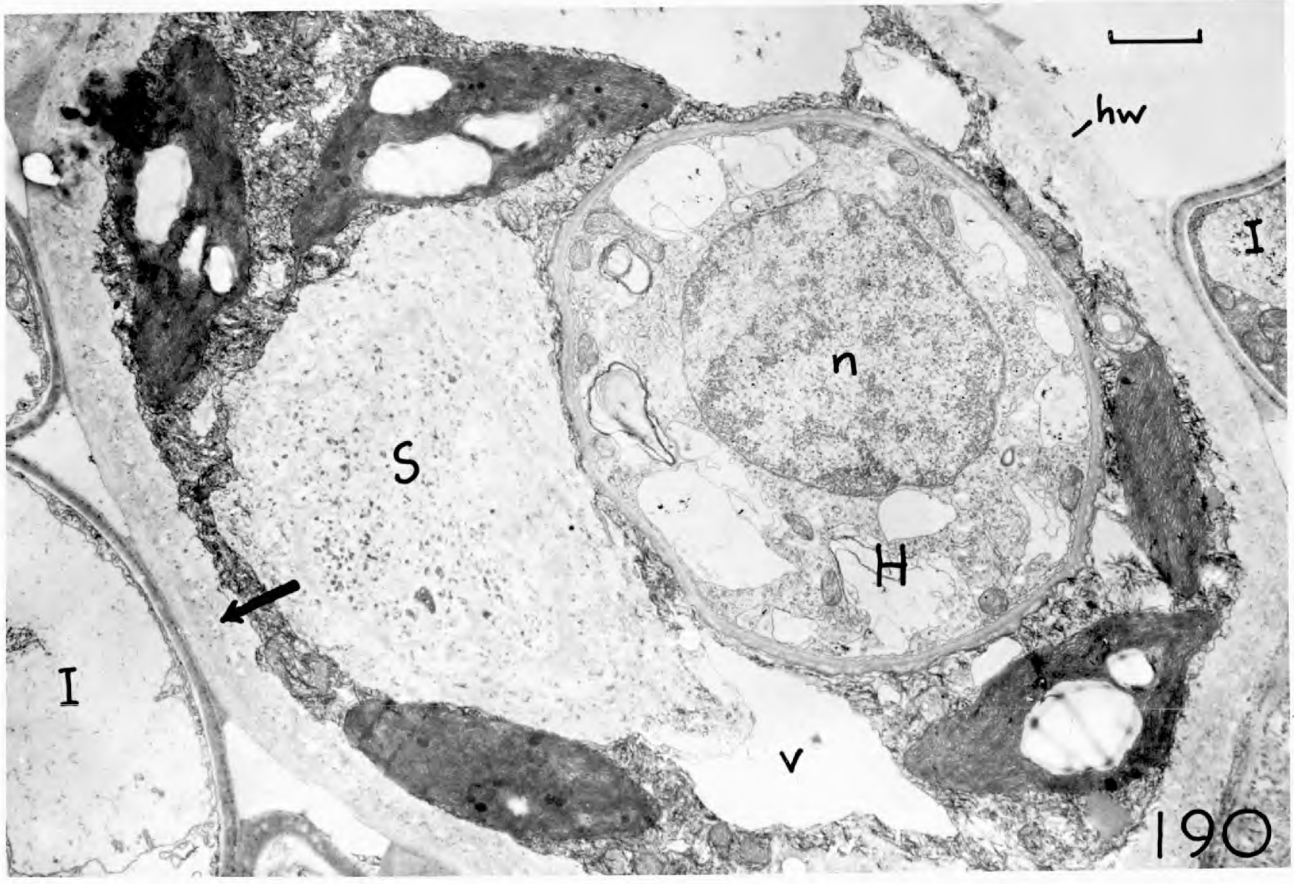

H
hp
S
ER
hw
188

189. Section of a haustorium to show the outer layer of the body wall which is greater in thickness and more open in texture than that of the haustorium in Fig. 186. 1851A/16. 14168.

190. Haustorium with a large sheath (s) of similar material to the new material adjacent to the host wall (arrow). 1851A/10. 14178.



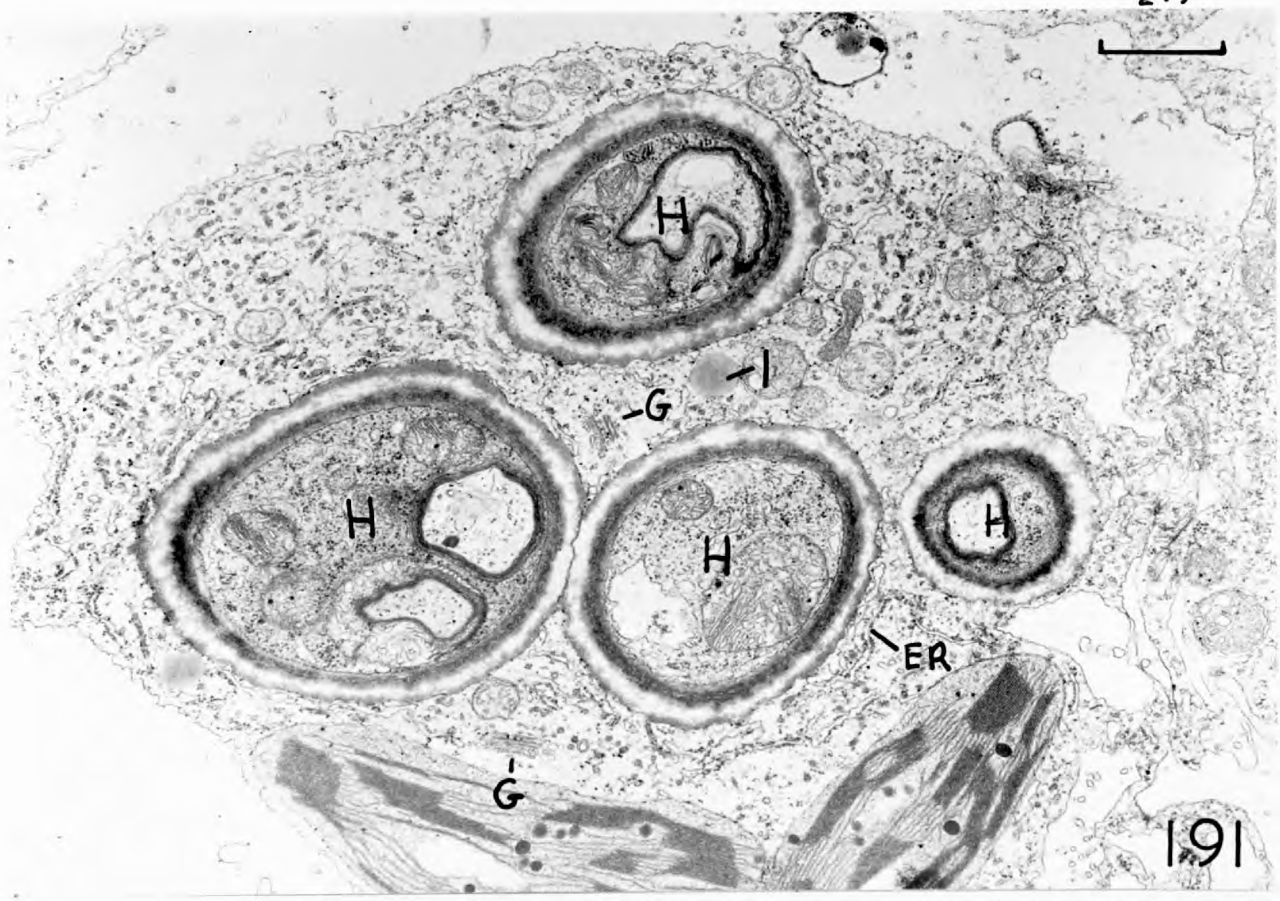
189



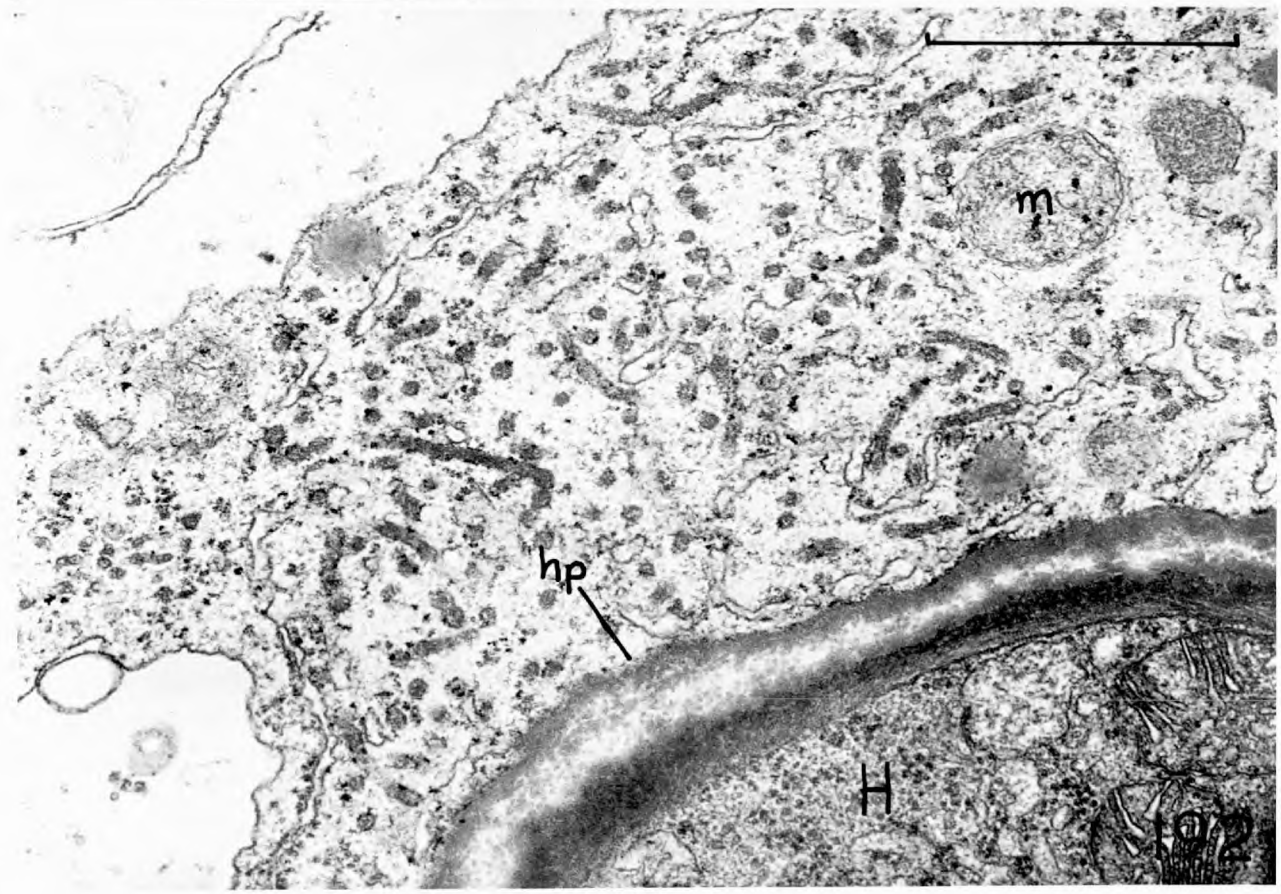
190

191. Low power of a host cell with lobes of a haustorium (H) cut in cross section showing the E.R. in this region. 1851A/16. 14151.

192. Section through the body wall showing single membrane bound tubules containing finely granular material in the host cytoplasm around the haustorium. Some of the tubules (arrows) appear to be formed of a string of smaller vesicles. 1851A/16. 14157.



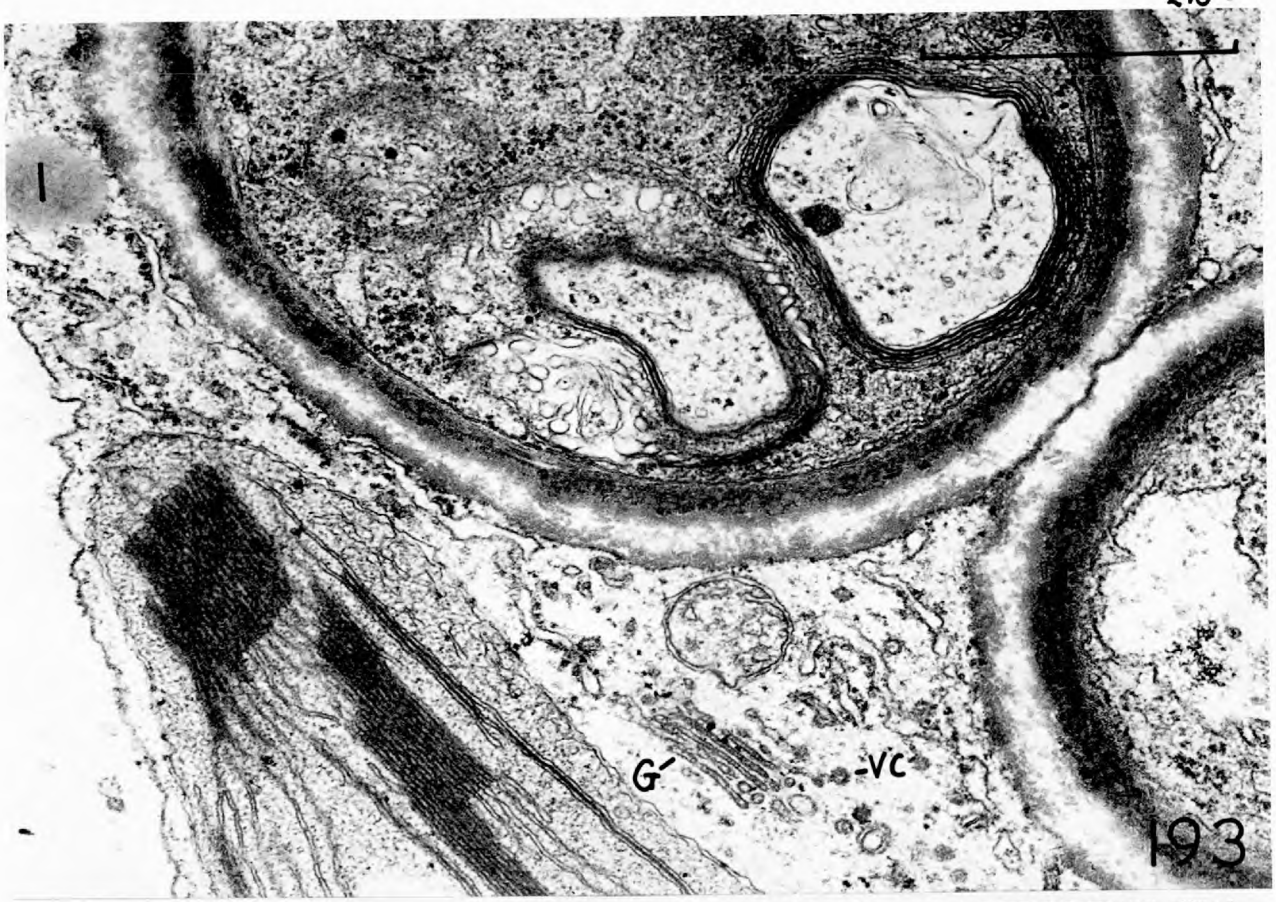
191

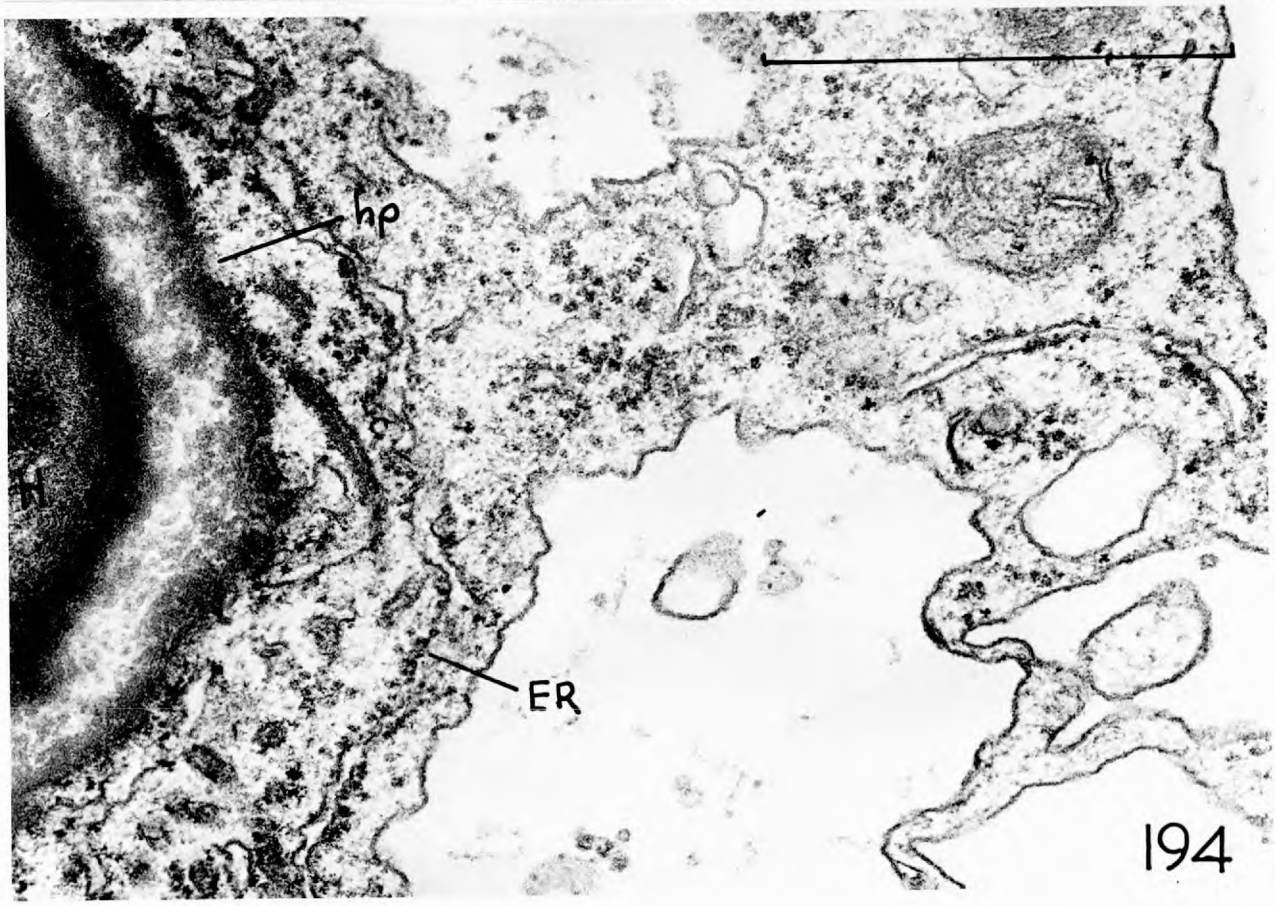


192

193. Detail of Fig. 191 to show a Golgi body (G) with associated vesicles (vc). Note the absence of granular material in the region between the body wall of the haustorium and the host plasmalemma where two areas of host plasmalemma are in contact. 1851A/16. 14153.

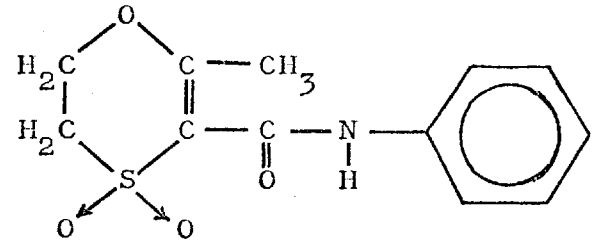
194. Section showing the E.R. around the body of the haustorium (H) and the tubules with material of similar opacity and granularity to that found against the body wall. 1851A/16. 14160.


I
G
VC
193


hp
H
ER
194

10. THE EFFECT OF A SYSTEMIC FUNGICIDE ON FRENCH BEAN RUST

Recently a new chemical has been found to act both as a curative and preventive systemic fungicide against rusts (Schmeling & Kulka, 1966). The compound is an oxathiin (2,3-Dihydro-5-carboxanilido-6-methyl-1,4-oxathiin-4,4-dioxide) which is marketed on a limited scale for experimental use by UniRoyal International under the trade name 'Plantvax' (F 461).



PLANTVAX

Little is known about its mode of action. It is thought that the chemical affects the rust as it enters the plant and is active against mycelium established within the plant. It is effective when applied to soil, seed or as a foliar spray.

A preliminary examination of the fine structure of rust infected Pinto beans treated with Plantvax was made for comparison with that of stages in susceptible and resistant types of infection to assess the likelihood that further investigations on such lines would be informative.

Samples of Plantvax, both of technical grade (t.g.)

(100% active ingredient) and as a wettable powder (w.p.) (75% active ingredient), were supplied by UniRoyal International.

(a) To determine the effect of the compound on the germination of uredospores

New slides, washed in 'Stergene', rinsed in hot water followed by several changes of glass distilled water were soaked overnight in 5% acetic acid, rinsed and soaked overnight in glass distilled water, rinsed in acetone and finally dried at 70°C in covered containers, were placed in sandwich boxes on glass rods over moist paper towels after the method of Deverall (1967). Two 0.01ml drops of a suspension of uredospores, of concentration 10^6 spores/ml in 0.001% Tween 20 (a non-fungitoxic wetting agent), were placed on each of two slides. Each set of four replicates was overspotted with 0.01ml of 0, 50, 100, 150 and 200ppm Plantvax (t.g.) in water respectively. The lids to the boxes were sealed with 'Vaseline' and incubated at 20°C in the dark for 18h, then spotted with cotton blue in lactophenol and scanned under the low power of a microscope for germination and germ tube length (uredospores were deemed to have germinated when the pellicle of the spore had been ruptured by the germ tube and the contents of the tube had stained blue).

The means from 25 spores per drop were recorded and the length of the germ tubes measured to the nearest 5µm.

There follows a table of the results:

Final Concentration of Plantvax in ppm	% Germination after 18h	Germ Tube Length in µm
0	100	400
25	96	60
50	87	40
75	66	50
100	55	35

It was clear, therefore, that the fungicide prevented or retarded the germination of uredospores and, when present in concentrations insufficient to prevent germination, reduced the growth of the germ tubes.

(b) Determination of the concentration of Plantvax which would prevent infection and colonisation by the rust

Plants were prepared as described (p. 44) and 15 day-old greenhouse grown plants were used.

Concentrations of 1, 10 and 100ppm Plantvax were prepared from 75% active w.p. and applied in four different ways, using three replicates for each concentration of Plantvax.

(i) Pots were drained of excess water and 10ml of a suspension of the fungicide ~~was~~ were applied to the vermiculite

from the top.

(ii) One leaf, still attached, of the primary pair of four sets of the replicates was immersed in the fungicide and withdrawn immediately.

(iii) One side of one primary leaf was immersed to the mid-rib.

(iv) One primary leaf was immersed halfway from the base.

One set of replicates were left untreated.

All plants, except three sets of replicates from treatment (ii), were inoculated with rust uredospores on both primary leaves the following day.

One set of replicates from treatment (ii) were inoculated on the second day and one on the third day following the application of Plantvax with the uredospores remaining from the same suspension, stored in the meantime at -20°C, as used on the first day.

The final set of replicates from treatment (ii) were left uninoculated.

All plants were left on the greenhouse bench and the development of symptoms were recorded.

There follows a summary of the results:

The uninoculated but treated control plants remained normal during the course of the experiment.

The inoculated but untreated control plants developed

symptoms as raised chlorotic spots on the leaves five days after inoculation. A density of 10-20 sori/cm^2 was eventually produced.

In the untreated leaf of the primary pair or untreated portion of leaf of the replicates with foliar applied Plantvax symptoms were as described above for the inoculated but untreated controls.

In root applications with 1ppm Plantvax symptoms developed as for the untreated controls. Using 10ppm Plantvax the number of sori was reduced. With 100ppm Plantvax symptom development was delayed for two days and the sori which eventually appeared were situated mainly near the larger veins where higher concentrations of uredospores tend(ed) to accumulate because of the method of inoculation.

In the treated leaves all parts dipped into concentrations of 1ppm Plantvax developed symptoms after five days and eventually a crop of sori as for the untreated controls. Applications of 10ppm Plantvax reduced the number but did not delay the appearance of sori. With 100ppm Plantvax symptom development was delayed by one day compared with the untreated controls and the sori produced were situated next to the larger veins.

With plants inoculated two and three days following treatment symptoms appeared five days after inoculation as in the untreated controls, with no significant decrease in the number of sori produced.

Root appications of the fungicide gave the better overall control of the rust, though the concentrations used proved sufficient only to reduce the severity of the disease and not to prevent the development of symptoms or the production of uredospores.

(c) To determine the effectiveness of Plantvax as a curative agent

To a set of three replicate pots each containing a 22 day-old plant six days after inoculation with rust uredospores, just as raised chlorotic spots were appearing on the leaves, 10ml of 1000ppm Plantvax (t.g.) were added to the vermiculite from the top. Plantvax was added in a similar manner to three replicates of uninfected plants of the same age. The plants were incubated in the growth cabinet and the effect of the treatment noted.

Five days following treatment the inoculated leaves were completely necrotic, whereas the uninoculated trifoliate leaves of the same plants and all the leaves of the uninoculated controls were unaffected.

In this experiment the fungicide had killed a well established rust infection. The results raised the possibility that the death of the fungus may have upset a dependant relationship which had become established between the host and its parasite and lead also to the consequent early death of the infected leaves.

(d) Fine structure of rust infected French bean plants with a post inoculative application of Plantvax

To two sets of three replicates of 23 day-old French bean plants cv. Pinto, 20ml of 100ppm Plantvax (t.g.) were added at the top of the pot to the vermiculite which had been drained of excess water. One set of plants had been previously inoculated with rust 17 days after soaking the seed. Four days later material was taken from each set of replicates and processed for electron microscopy as described (p. 48).

(i) Control Material

Uninfected, treated material differed little from uninfected, untreated material (see p. 70) except that the tonoplast was frequently damaged (Fig. 203). Damage to other membranes was infrequent and such as was seen was probably due to fixation. The cell organelles appeared generally normal (Fig. 203, 204).

(ii) Infected Material treated with Plantvax

The host cytoplasm frequently showed damage and disruption of internal membranes, especially the tonoplast (Fig. 195) and the cytoplasm and organelles were dispersed throughout the cell lumen. Mitochondria were swollen and rounded-off and their cristae were reduced in length and

number (Fig. 196). Rough and smooth E.R., Golgi bodies and microbodies were still visible in the disrupted cells (Fig. 195). Lipid droplets (Fig. 196), not previously seen in cells of Pinto beans, were observed and also resembled those found in disorganised cells of Kievit (e.g. Fig. 191).

The chloroplasts still possessed intact envelope membranes but were swollen and contained large starch grains (Fig. 195). The osmiophilic droplets appeared to be unaffected in size and number and there was no disruption of the lamellae (Fig. 196).

The bodies of the haustoria were frequently flattened and their contents were dense, lacking vacuoles, as though shrunken and were probably necrotic (Fig. 195, 197, 198), resembling those found occasionally in healthy cells of untreated plants (e.g. Fig. 56, 57). Other haustoria did not have the dense appearance described above but certain abnormalities were apparent. Mitochondria observed in the haustorial body were unusually swollen, some being almost totally devoid of cristae with a more dispersed matrix of correspondingly less density (Fig. 196, 199); some large vacuoles were present with distinctive granular contents (Fig. 196).

The sheaths were large and extended as collars for various distances along the stem, similar to more voluminous sheaths generally (e.g. Fig. 55, 56). The contents comprised a relatively wide inner electron-lucent layer,

often including fragments of sequestered membrane which were, however, most numerous at its boundary with the surrounding zone of fibrillar material. The latter had a deeply indented interface with the host protoplast and also contained small pockets of membrane.

The body wall of the haustorium did not differ significantly from that of the untreated cells (e.g. Fig. 60) and consisted of an inner, electron dense layer and an outer pale layer of equal thickness (Fig. 196). In some cases, typically in host cells with disordered membranes, the outer layer was more uneven in thickness and electron-opacity.

The intercellular hyphae observed were well filled with cytoplasm (Fig. 200). The nuclei and lipid droplets appeared normal but the ribosomes were distributed evenly and not grouped in irregular clusters like those observed in untreated cells (e.g. Fig. 26, 68, 79). The mitochondria (Fig. 200, 201) were greatly swollen with dispersion of the matrix and were frequently bereft of cristae and thus converted to a condition in which they resembled vacuoles or cytoplasmic vesicles from which they could be distinguished only by close examination at high magnification, where the presence of two containing membranes rather than a single one could be detected (Fig. 202).

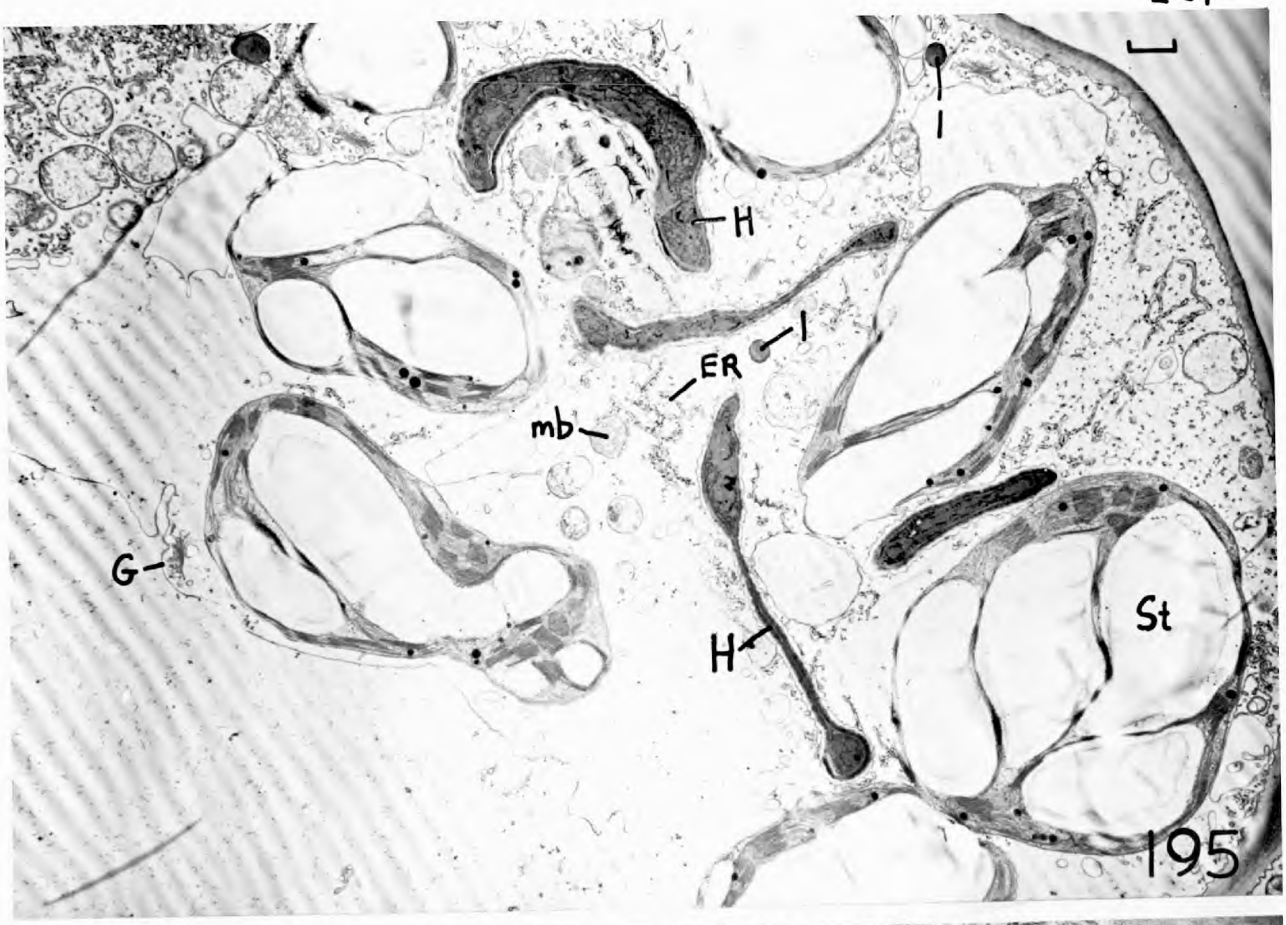
Uredospores had not been produced nine days after inoculation, but a stroma was apparent beneath the epidermis and beginning to raise the latter from the underlying

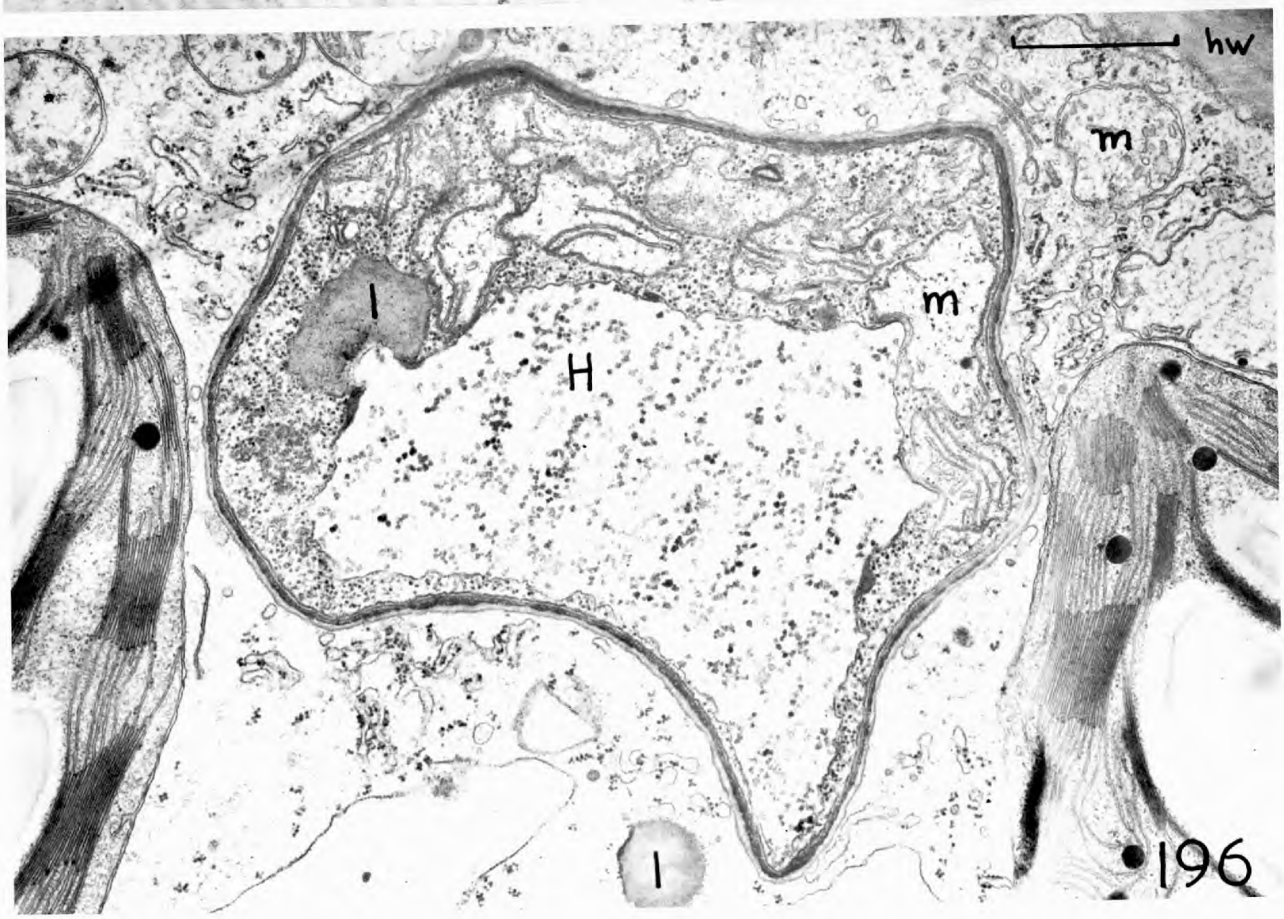
palisade cells. It was at this stage in the development of the rust that the fungicide had originally been applied and, therefore, no further development in the colonisation of the host had taken place during the four days between treatment and removing material for electron microscopy.

195. General view of an invaded cell, haustoria (H) are collapsed and apparently necrotic. Lipid droplets (l) are present within the cell, the chloroplasts are swollen and contain large starch grains (St). 2213A/1. 16137.

196. Body region of a haustorium showing the typical two layered structure of the body wall. Host mitochondria (m) appear swollen with loss of the cristae. 2213A/1. 16136.

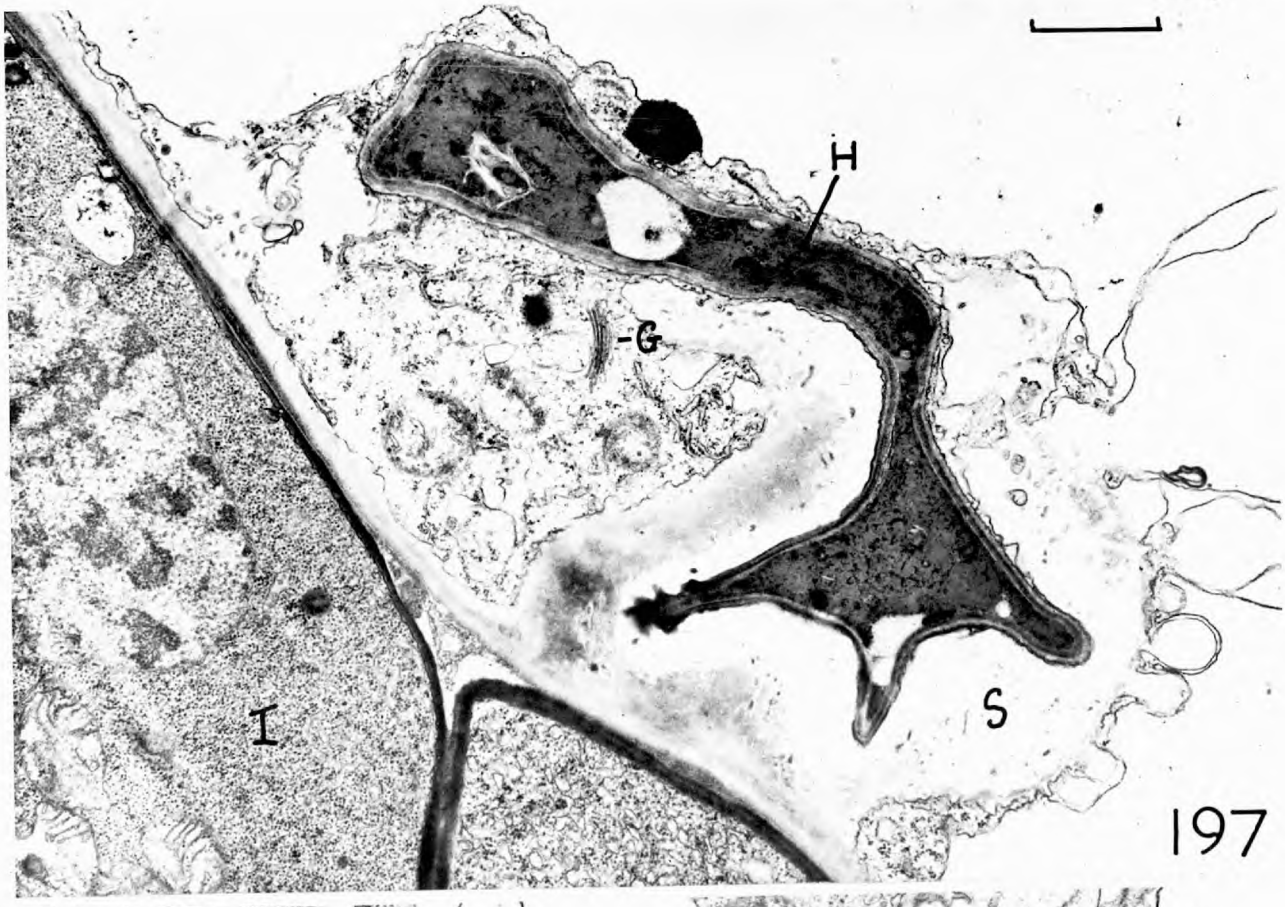
Fig. 195 - 204 are of material treated with Plantvax.


I
H
I
ER
mb
G
H
St
195

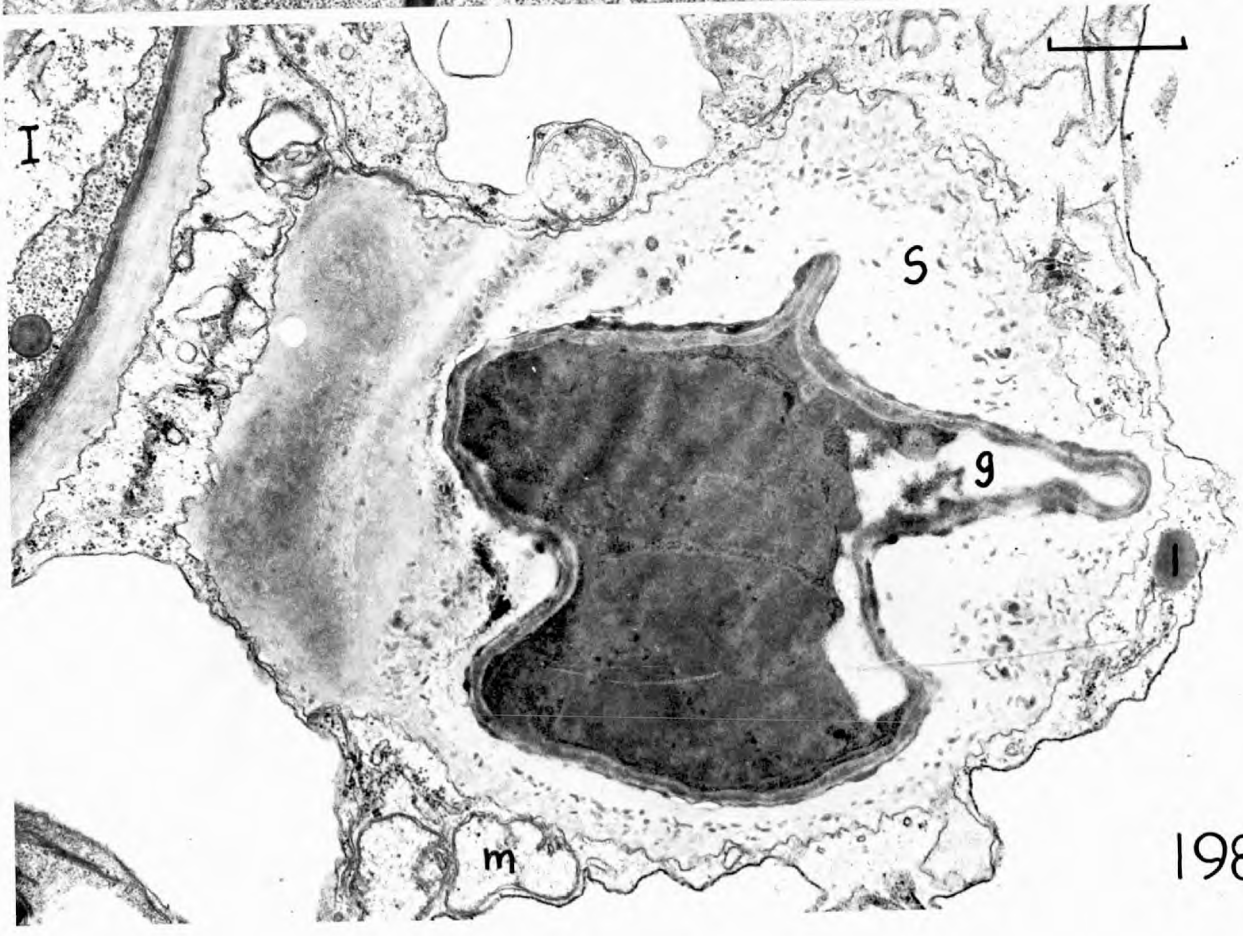

hw
m
I
m
H
I
196

197. Haustorium (H) with a large sheath (s) of two zones, an inner electron-lucent and an outer fibrillar zone. The haustorium has dense contents with no vacuoles. 2213A/9. 16147.

198. Haustorium with a large sheath, occluded membranes are present, concentrated at the interface between the inner electron-lucent and the outer fibrillar zone. 2213A/9. 16150.



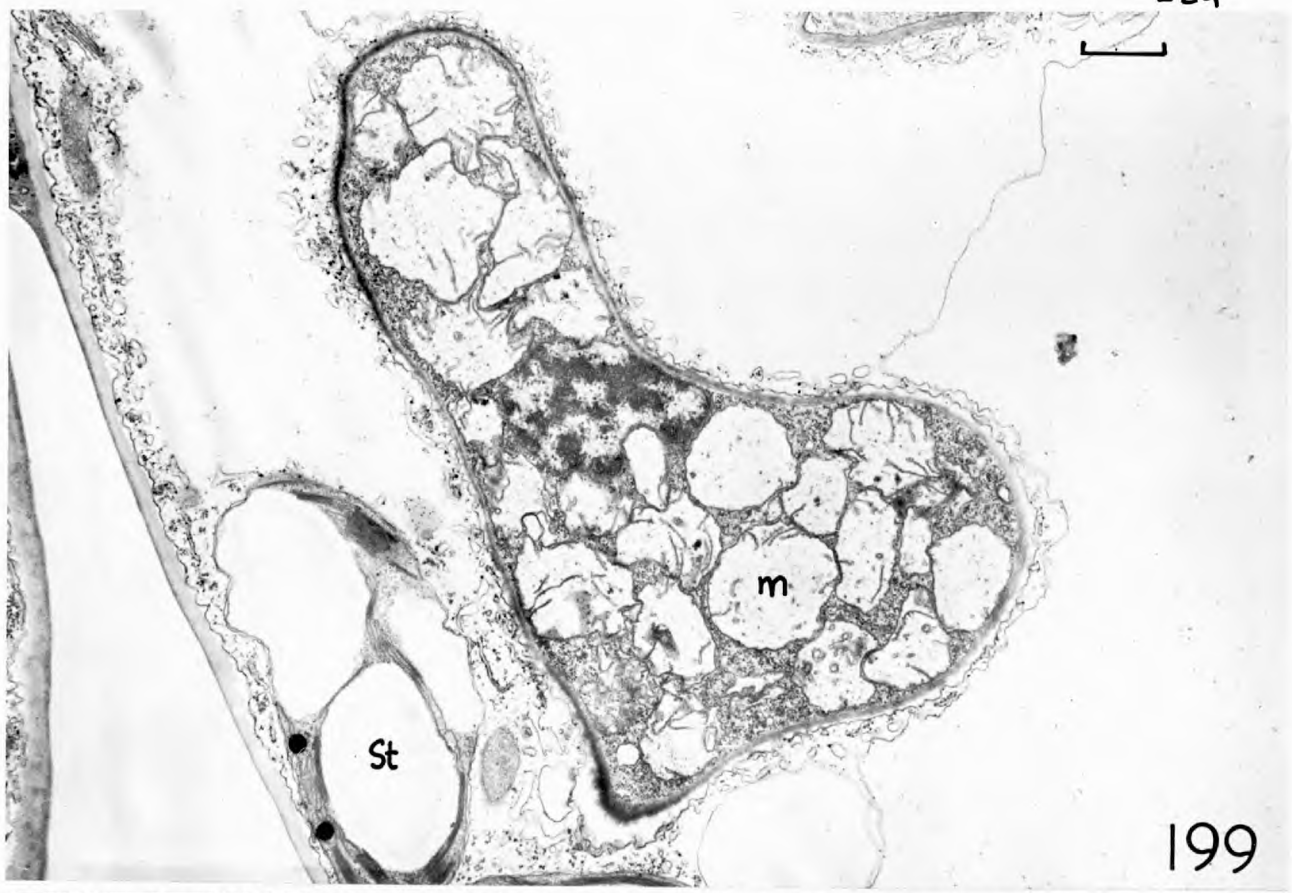
197



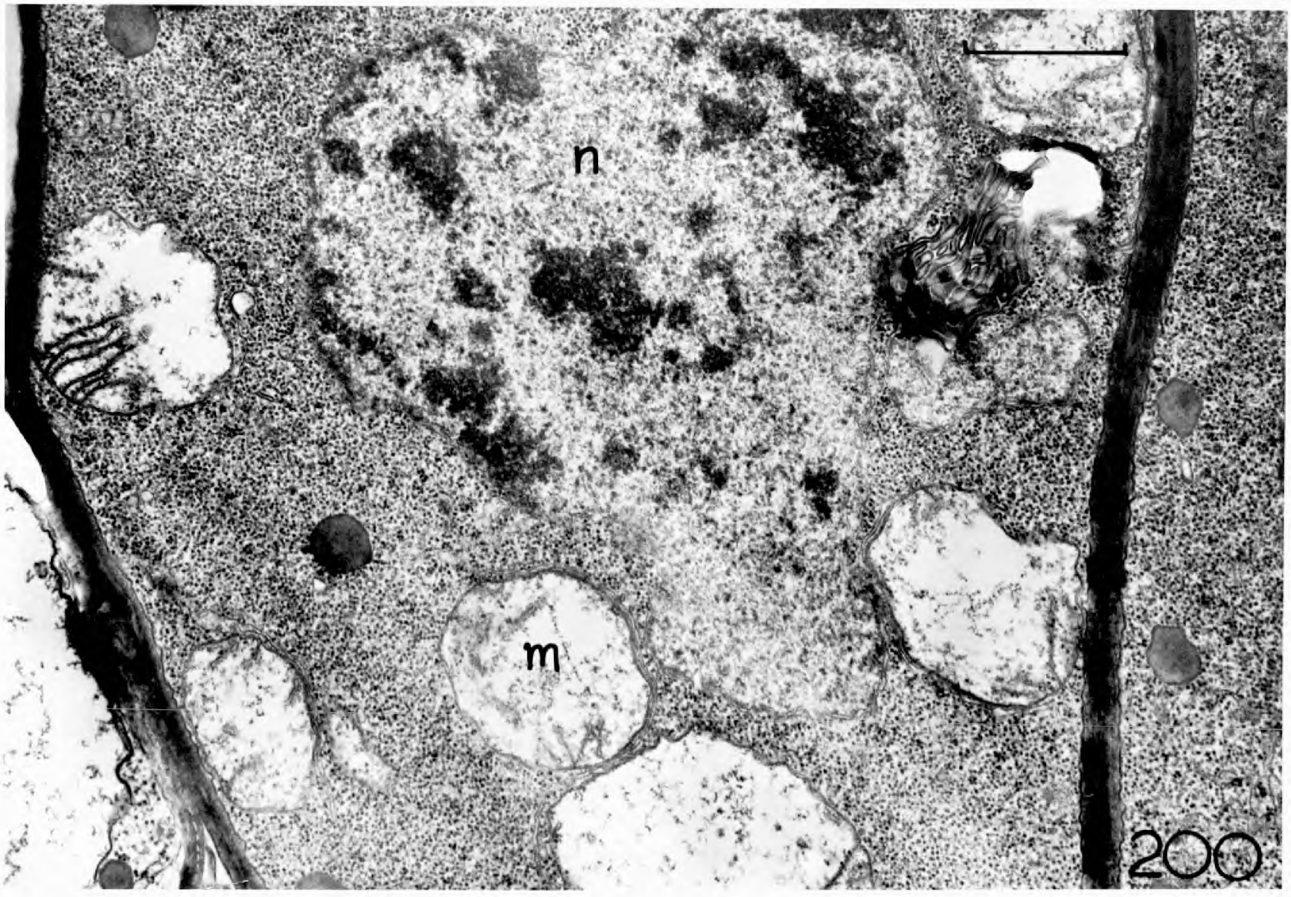
198

199. Haustorium containing swollen mitochondria (m) with a reduction in the number of cristae. 2213A/3. 16139.

200. Intercellular hypha packed with ribosomes, not formed into aggregates or chains but dispersed evenly throughout the cytoplasm. 2213A/3. 16140.



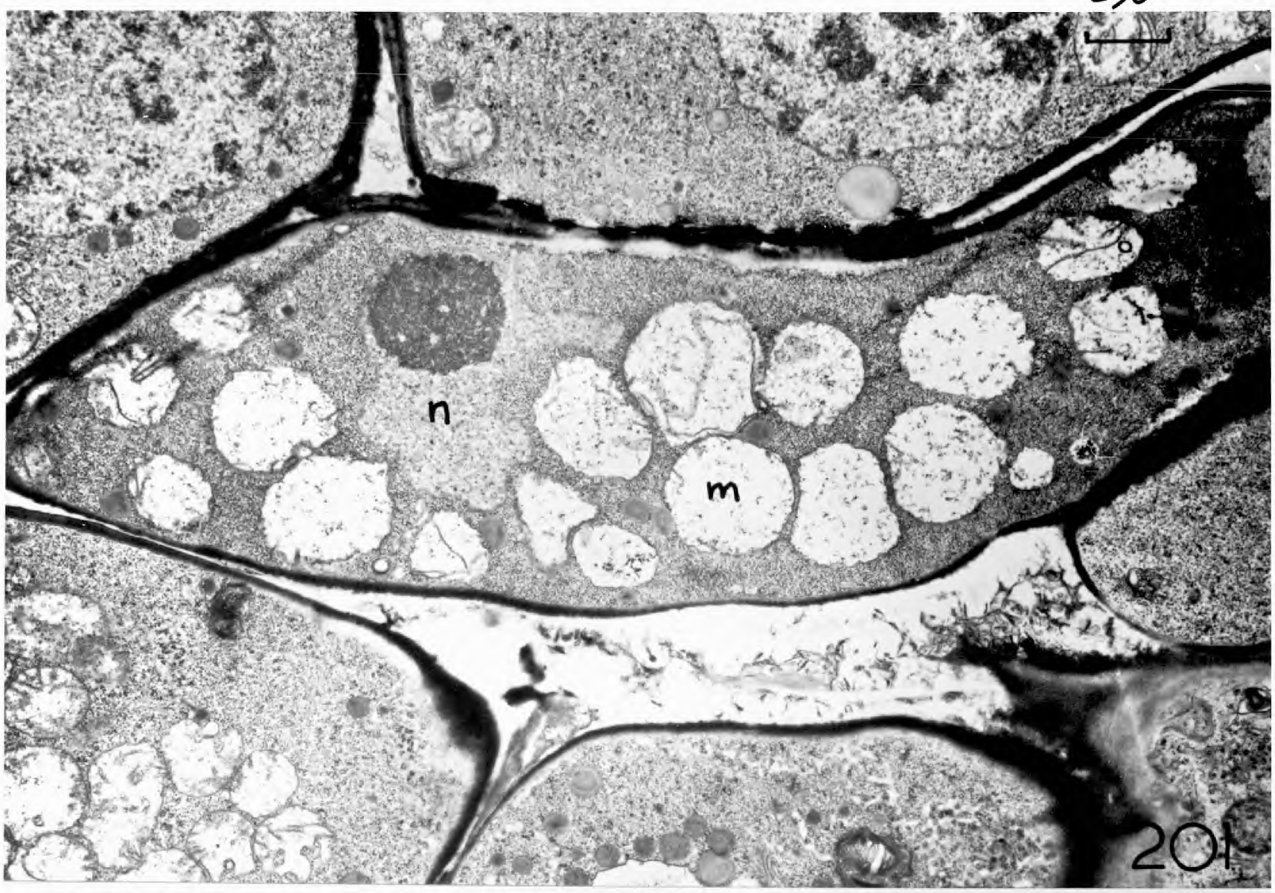
199

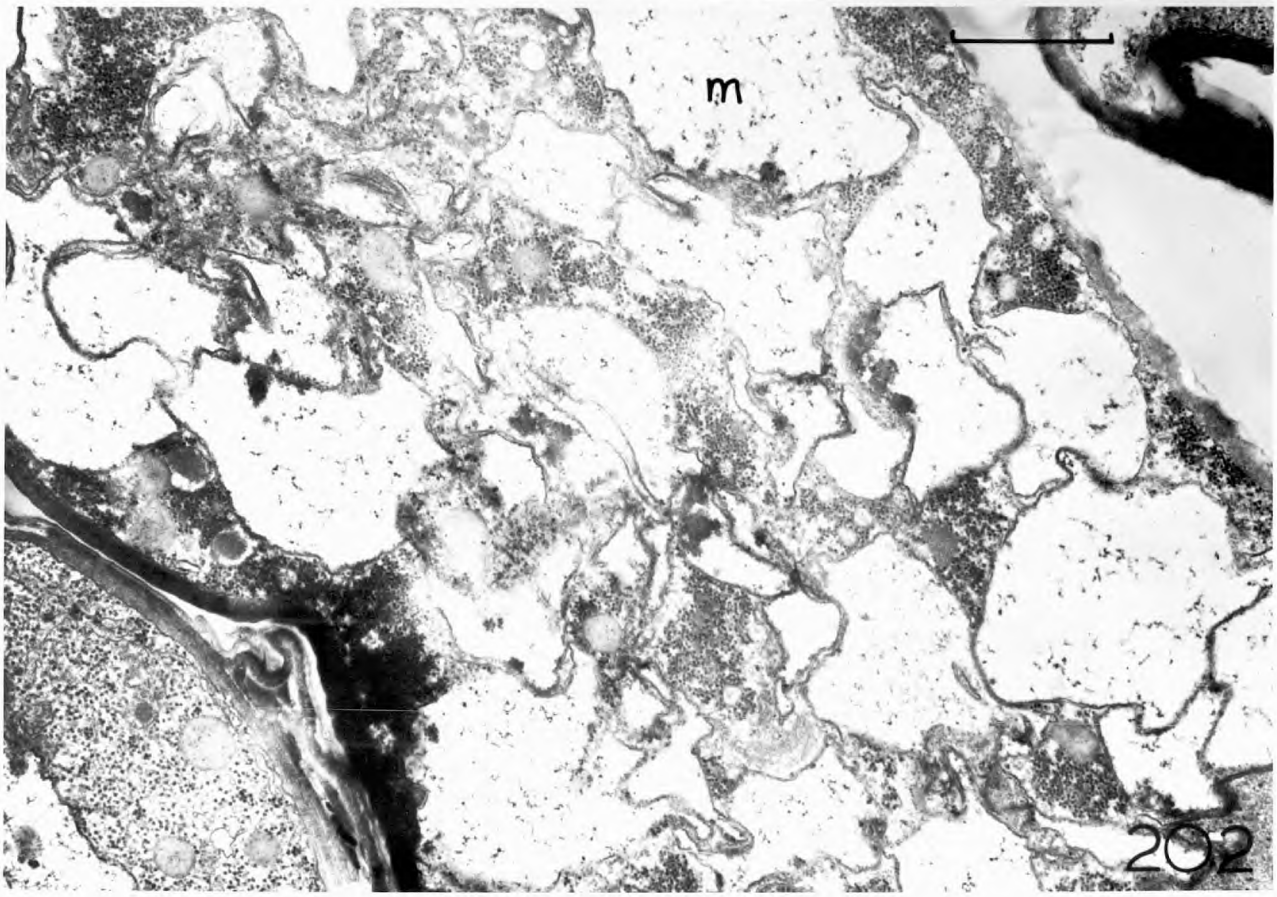


200

201. Intercellular hypha with greatly swollen mitochondria (m) almost totally devoid of cristae. 2213A/4. 16145

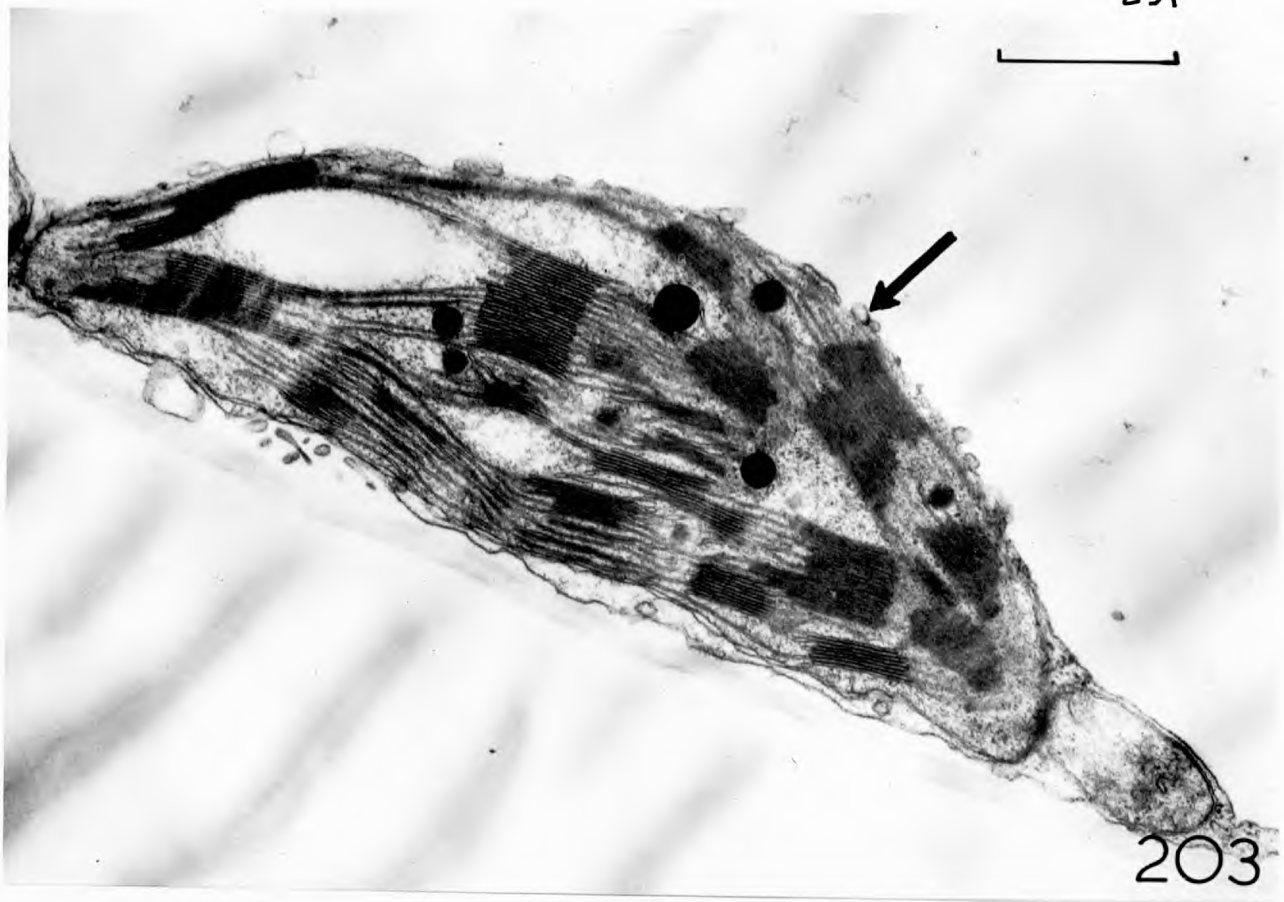
202. Intercellular hypha in a state of disorganisation, the mitochondria are only recognisable by their surrounding double membrane. 2213A/3. 16142.


n
m
201


m
202

203. Chloroplast from an uninfected but treated plant. Some breakage of the tonoplast (arrow) has occurred. 2216A/1. 16156.

204. Mitochondrion within the cytoplasm lining the cell of an uninfected, treated plant. The tonoplast is unbroken. 2216A/2. 16158.



203



204

11. FINE STRUCTURE OF THE HAUSTORIAL APPARATUS OF UROMYCES VICIAE-FABAE

Uromyces viciae-fabae (Pers.) Schroet., a species closely related to U. appendiculatus, parasitic on broad beans (Vicia faba L.), was investigated primarily to see if a neck-band was present in the stem of its haustorium.

Nine days after inoculation sori were cut from the fourth pair of leaflets of 40 day-old broad bean plants, which had been grown in the growth cabinet and inoculated with uredospores of U. viciae-fabae as for French beans (p. 44) and fixed and embedded as described (p. 48).

Results are presented from an examination of six haustoria.

(a) Host and Fungal Cytoplasm

The presence of mature haustoria within cells of broad bean caused little disruption of the cytoplasm, chloroplasts and mitochondria (Fig. 205, 208, 209). A coating of host E.R. was not observed on the sheath or stem of the haustorium. The cytoplasm of the intercellular hyphae was not investigated in detail but it closely resembled that of U. appendiculatus.

(b) The Haustorium

The haustorium was similar in general shape and size to that of U. appendiculatus. However, the stem was characteristically constricted at a point in the neck region at varying distances from the base (Fig, 205, 208, 209).

The haustoria were nucleate (Fig. 205) and in one haustorium two nuclei were observed. A group of coiled or concentric membranes were visible in the stem of the haustorium (Fig. 205, 208).

(c) The Haustorial Mother Cell

The wall of the h.m.c. consisted of four distinct layers, the relationships of which are best appreciated in its zone of contact with the host cell wall where it was frequently expanded as in U. appendiculatus.

A thin superficial layer, comparable with that of U. appendiculatus, was not clearly evident over the free surface of the h.m.c. but a thin, less electron-opaque layer was often present in the region of its contact with the pad of intercellular material in the area of adhesion with the host cell (Fig. 206).

The layers distinguished were, from the outside inwards:

(i) An outer layer (Fig. 206) terminating within the area of cell contact, usually somewhat swollen at the margin of this area and often slightly separated from the inner layers of the wall.

(ii) A thin electron dense layer, terminating a short distance from the penetration pore (Fig. 206, 208, 209). This layer was characteristically uniform in thickness, without marked expansion in the contact zone, but frequently appearing partially degraded near its termination.

(iii) A paler layer, forming about a third of the thickness of the h.m.c. wall, which was considerably swollen and more electron dense in the contact area before it tapered to an abrupt termination at the margin of the penetration pore (Fig. 206, 208, 209). No sub-layers of greater electron density, comparable with those seen in the corresponding layer of the h.m.c. wall of U. appendiculatus, were observed in this layer.

(iv) An innermost layer (Fig. 206) which, with little or no change in thickness, was continued through the penetration pore, around and along the neck where it terminated at a conspicuous dark ring identical in appearance to the boundary of the neckband of U. appendiculatus (Fig. 207).

(d) The Sheath

The sheath was composed of an inner layer, formed by the fungal wall of the neck, and an outer zone around the neck of of the haustorium which was in its turn surrounded by material of a different nature (Fig. 205).

The outer zone which invested the neck itself was seated

on the original host wall immediately surrounding the penetration pore. This part of the sheath was generally more electron-opaque than the host wall and was also quite distinct in opacity and texture from a lateral extension of the base of the sheath which was spread over the host wall in the form of an annular disk. The material of this basal extension of the sheath closely resembled that of the host wall, particularly at the boundary between the two layers where its presence was, however, made clearly evident by changes in textural pattern, including the presence of less opaque areas and by the position of infolds of the host plasmalemma over its surface and the presence within it of occluded portions of membrane (Fig. 206).

(e) The Neckband

The sheath thinned to a width only slightly greater than the fungal wall at the constricted region of the neck (Fig. 205) and the two layers continued without change in thickness to the dark ring which was situated at varying distances from the base (Fig. 207). In U. viciae-fabae there was no region corresponding to the short structurally distinct part of the neckband basal to the dark ring characteristic of U. appendiculatus (e.g. Fig. 45, 66). The electron dense ring was no greater in thickness than the neck wall adjacent to it and the stem wall continued beyond it without change in thickness.

(f) The Body Wall

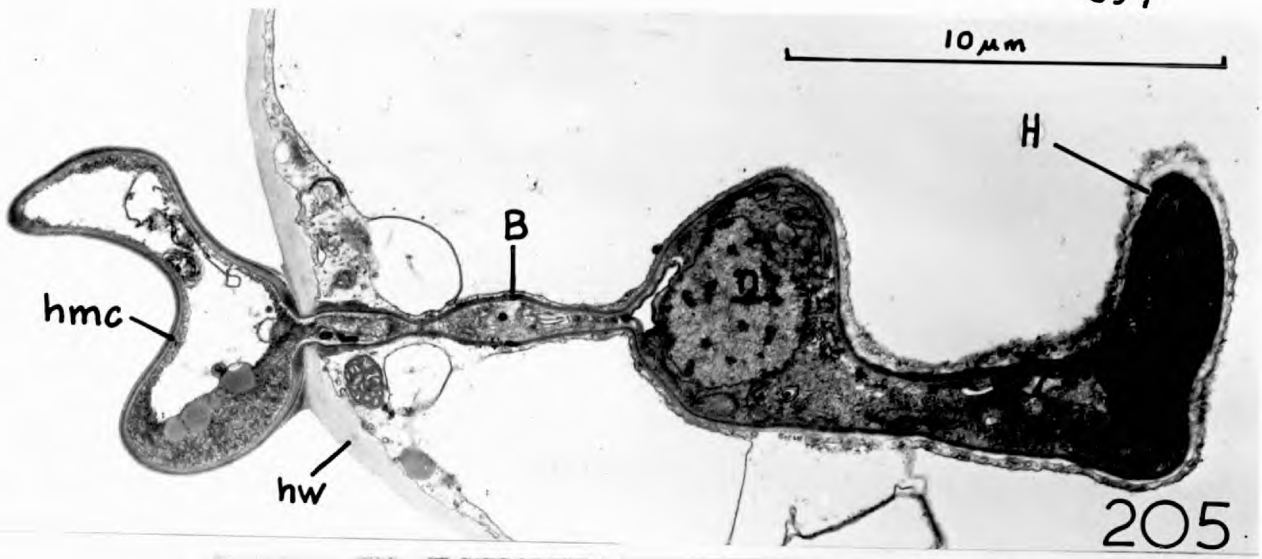
Beyond the neckband the stem wall consisted of two distinct layers, equal in thickness but differing slightly in electron-opacity, the inner layer being more electron dense (Fig. 205, 208). The stem wall continued unchanged and eventually enclosed the expanded body of the haustorium. The structure of the wall in this region closely resembled that seen in U. appendiculatus but a detailed examination was not made.

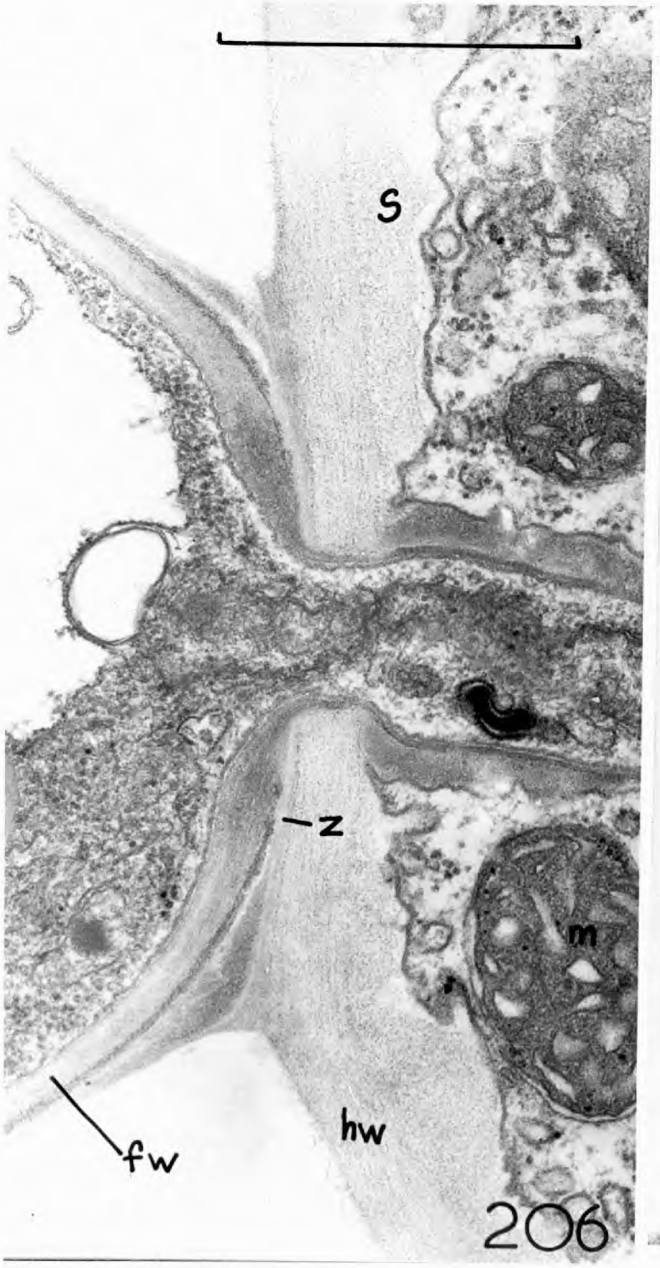
Fig. 205-209 are of *V. faba* infected by *U. viciae-fabae*

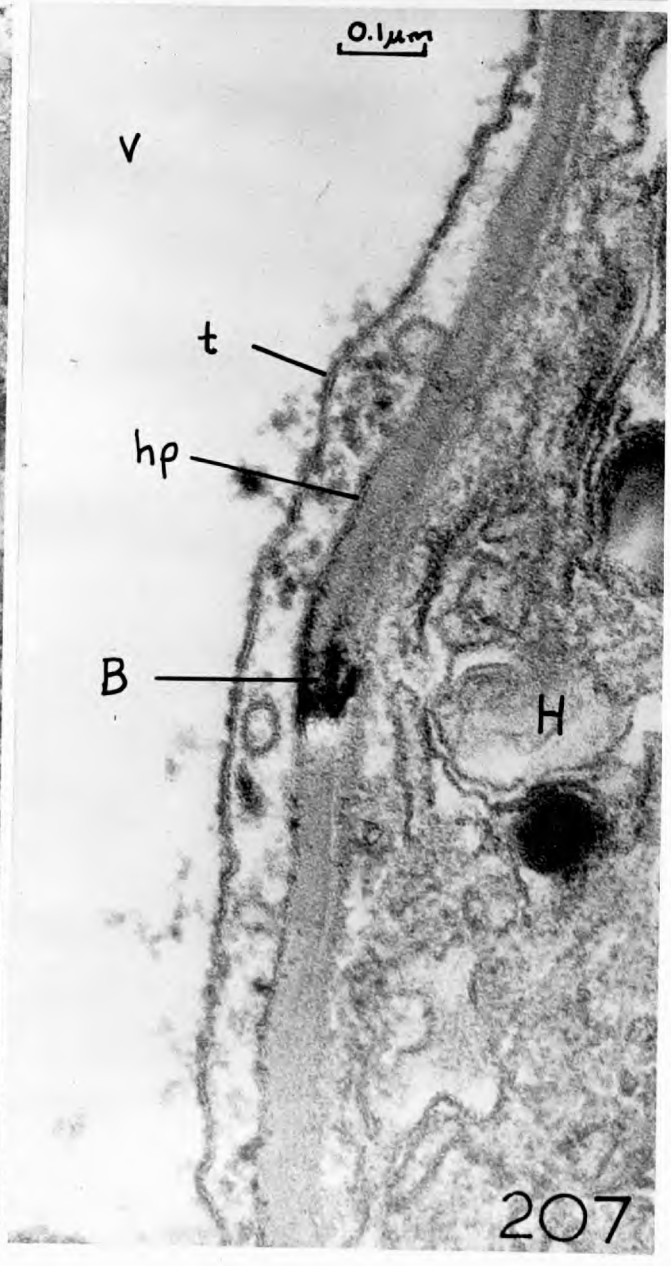
205. General view of a haustorium showing the neckband (B) and two layered body wall. Note the constriction in the neck region. 1717/3. 7400.

206. Section median to the penetration pore showing the four main layers to the h.m.c. wall (fw). Note the continuity of the innerlayer through the penetration pore, the opaque zone of the sheath immediately surrounding the neck wall and the annular disk of randomly orientated fibrillar material (s) against the host wall (hw). 1717/3. 7406.

207. Detail of the neckband region showing the ring of dense material (B) terminating the neck (haustorium entering from the bottom). 1717/3. 7407.

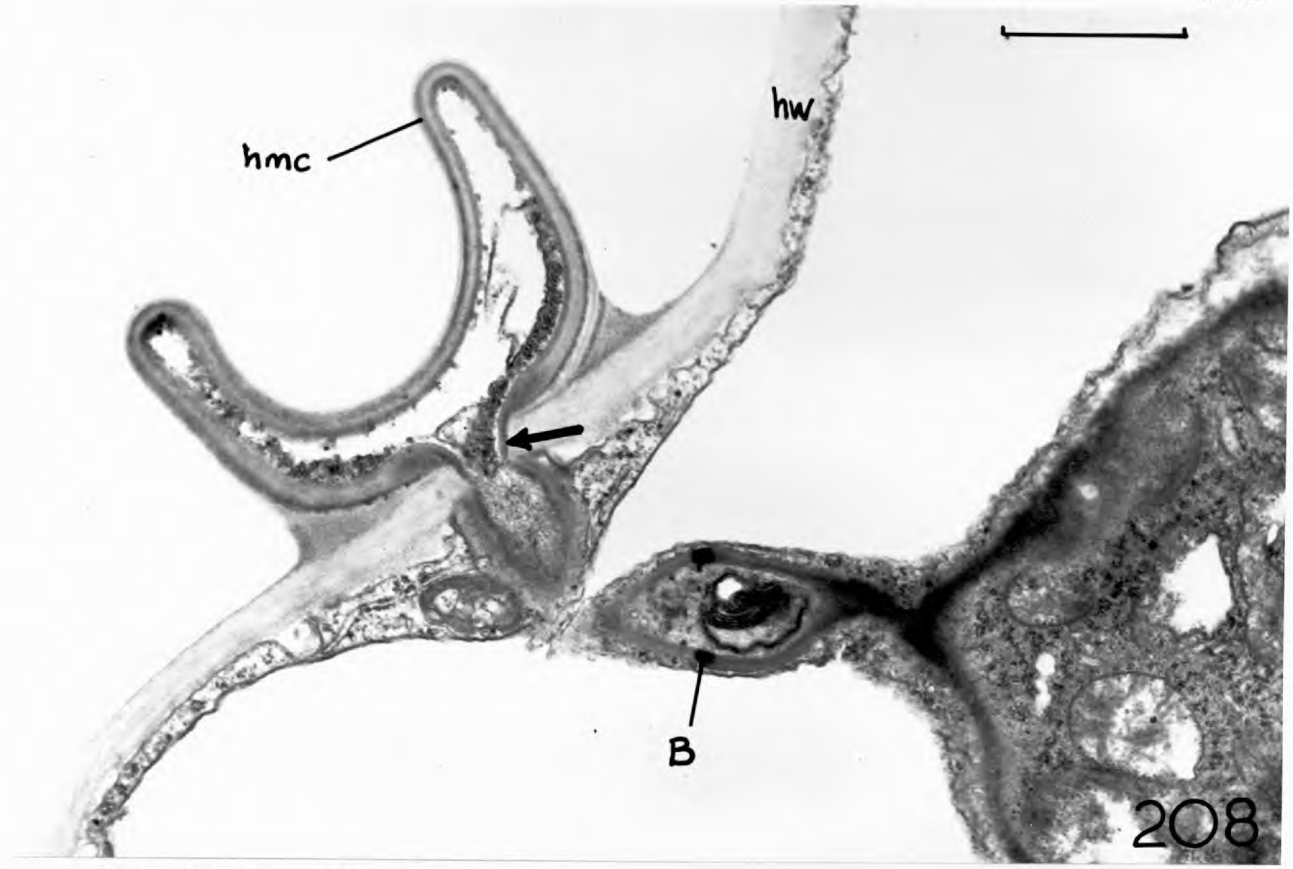

10 µm
H
B
hmc
hw
205

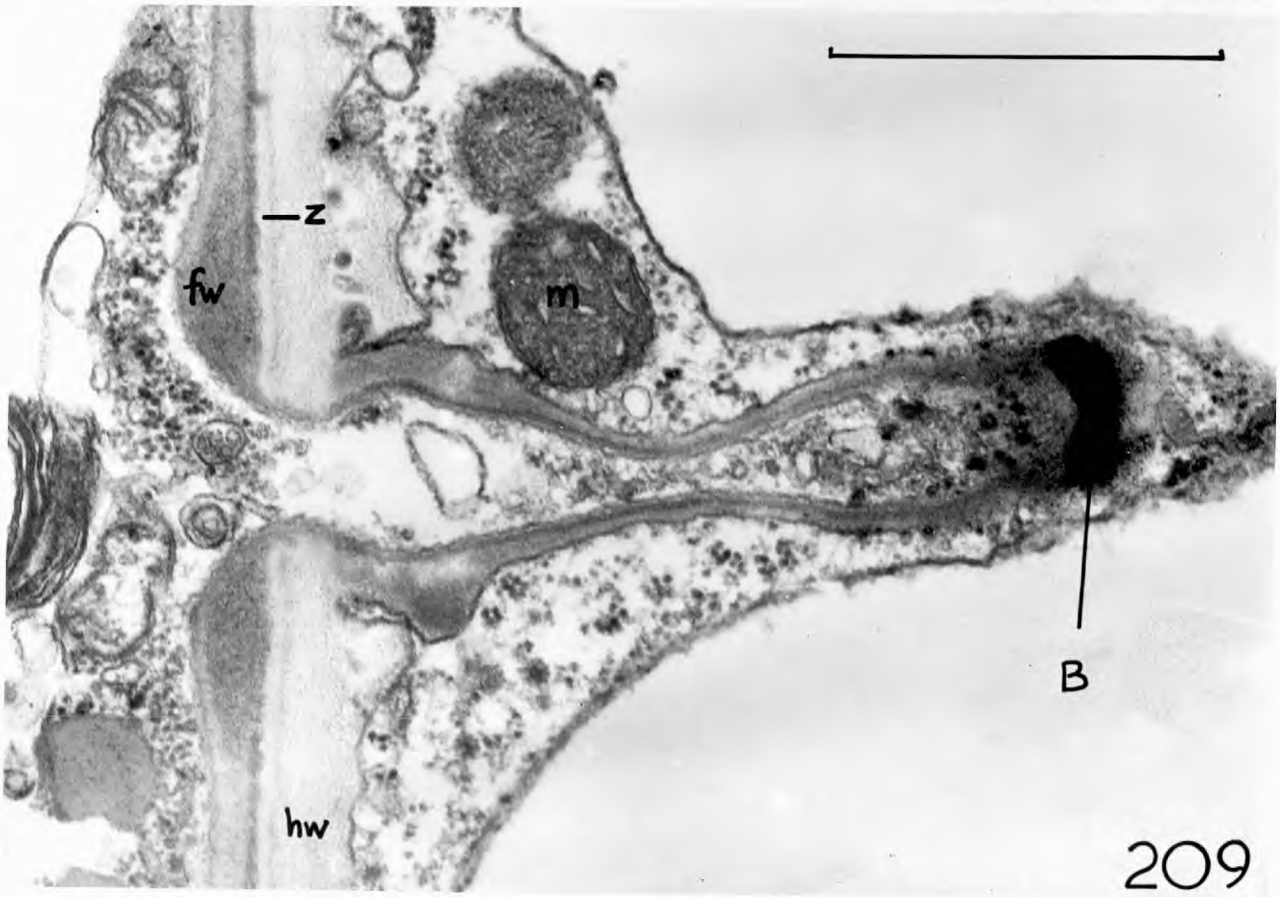

S
z
m
hw
fw
206


0.1µm
V
t
hp
B
H
207

208. Haustorium showing the continuity of the inner layer of the h.m.c. through the penetration pore (arrow). 1717/1. 7392.

209. Section median to the penetration pore and neck region of a haustorium showing sequestered membrane in the pale annular disk layer of the sheath and the inner electron-opaque layer extending to the neckband which has been cut at grazing incidence. 1717/5. 7411.


hmc
hw
B
208


z
fw
m
B
hw
209

D I S C U S S I O N

1. SPECIMEN PREPARATION

(a) Fixing and Embedding

The variations in quality and the reproducibility of results was no greater than is common in the 'art' of electron microscopy. The fixing and embedding of spores presented difficulties which could not be completely overcome. Uredospores when fixed and embedded at all stages of development before detachment from their stalk cells or when germinating usually gave good results, but detached uredospores with concave sides, even when still within the sorus, fixed and embedded badly, probably because of the dehydration of the protoplast and the impermeability of the pellicle or spore wall to the fixative and to the resin. Teleutospores, which have even thicker walls than uredospores, were only cursorily examined but with results which indicate that they present similar difficulties.

(b) Embedding and Sectioning

Success in embedding depended on a choice of resin mixtures of appropriate hardness and cutting properties. Different batches of resin prepared quantitatively from

identical mixtures of constituents and different parts of individual blocks did not always behave identically.

To obtain good sections of the host cells and uredospore walls required a resin of comparably great resistance to the knife. The routine use of diamond knives was a great advantage because glass knives were blunted after cutting only a few sections. However, even with the use of a diamond knife all spores, except those at stages in development when the wall was relatively thin and easy to infiltrate with resin, tended to be pulled out of the resin matrix. The hard resin mix (Luft's, 1961, Epon 812 mixture B) was the most effective in reducing this tendency. This mixture also gave least variation in hardness from place to place within the blocks, but it was difficult to trim prior to ultramicrotomy. For routine purposes a useful compomise was found in the use of mixtures 3A:1B or 4A:1B, which were equally suitable for the examination of mycelium and host cells within the same block.

(c) Staining of Sections

Although carefully standardised procedures were adopted when uranyl acetate followed by lead citrate were used for section staining, it was not uncommon for crystals of stain to occur on certain components of cells in some sections, though neighbouring sections, even on the same grid, would be free from such deposits.

A recurring artefact of this nature took the form of unusually large rosette-like crystals of stain deposited on one or more of the following components: starch grains, fungal or host cell walls and uredospore spines. Necrotic cells were often obscured by sheaves of needle-shaped crystals distributed more generally rather than on one or several particular structures or organelles.

These artefacts have in common that they are superficial accretions on preferred areas of the section which appear to have developed at randomly distributed centres of crystalisation. In no cases were any distinctive structural details identified which could serve as such centres. It seems likely, therefore, that the initial sites of deposition are determined either by physical characteristics of the surface, e.g. features of small scale textural roughness, which favour the initiation or adhesion of a crystal, or by the interaction of the stains with localised chemical residues peculiar to the organelles concerned, e.g. phosphorylated derivatives of carbohydrate in the walls or starch grains, or the products of structural breakdown in necrotic cells, which, though labile, may not always be completely removed or washed out during processing.

2. GENERAL FINE STRUCTURE

(a) Host Cytoplasm

Fragmentation of the host vacuole was found only in the resistant variety Kievit, but has been reported as a normal condition for cells invaded by Cronartium ribicola (Boyer & Isaac, 1964) and Puccinia graminis (Shaw & Manocha, 1965b). In the range of examples studied the cells of the susceptible variety (Pinto) are affected remarkably little by invasion of the haustorium. It is possible that fragmentation of the vacuole may be a factor in the resistance of the variety Kievit to U. appendiculatus.

In necrotic cells the host plasmalemma is one of the last structures to disappear. This has also been reported in wheat infected by P. graminis (Shaw & Manocha, 1965b). The retention of form and contact of the protoplast with the cell walls in late stages of necrosis suggests that turgidity of the cells is maintained until rupture of the plasmalemma eventually occurs.

Nuclei seen in the invaded cells were often situated close to, though rarely in contact with, the body of haustoria and were usually grossly distorted in shape. Nuclei were not only simply invaginated to accommodate the haustoria, often including some adjacent organelles and cytoplasm, but possessed projections, apparently wrapping round the haustoria,

and deep indentations and furrows on sides remote from the latter. The frequent close proximity of the host nuclei to haustoria is unlikely, therefore, to be merely a consequence of the passive transport of the nucleus carried forward by the body of the haustorium as it expands within the cell; the appearance of the nuclei would be consistant with intrinsically active changes of shape and position. Many associations of this type have been reported for rusts (Rice, 1927), but difficulties occur in the further interpretation of apparent associations from fixed material, the plane of section may not intersect a nucleus adjacent to a haustorium when it is present, or the cells may have been fixed at a stage either when an earlier association has been lost or before an association was formed.

The internal contents of nuclei and nucleoli seen in the invaded cells were not markedly different from those in uninfected tissue, in contrast to the observations on wheat infected by P. graminis by Manocha and Shaw (1966) which showed a correlation between the presence of larger interchromatin areas in sections of nuclei of invaded cells and an increase in nuclear RNA.

Whilst, therefore, there is evidence in U. appendiculatus of an active association between the host nuclei and the haustoria there is no structural evidence that this is accompanied by given changes in the nuclear protein content of the nuclei.

The presence of deeply constricted chloroplasts has also been observed in healthy cells of P. vulgaris and also in Spinacea oleraceae and Vicia faba (Greenwood, 1968: illustrated in Clowes & Juniper, 1968 and personal communications). Hohl (1960) reported that chloroplasts in Datura stramonium increased in number after wounding and Frey-Wyssling and Mühlethaler (1965) have reported the division of mature chloroplasts in uninjured plants. It is likely, therefore, that the division of chloroplasts at late stages in their development occurs frequently in higher plants and that the examples seen in invaded cells were not a specific consequence of invasion.

The pattern of break-down of chloroplasts in invaded cells of P. vulgaris appears to be similar to that reported for the break-down of chloroplasts in the cotyledons of Brassica juncea infected by Albugo candida (Harding et al, 1968).

The frequent occurrence of large numbers of mitochondria around haustoria is an indication that the metabolism of the host cells may have become modified, and in particular that the pathways of energy production and utilisation have become adjusted in directions towards the invading haustorium.

Cytoplasmic microtubules (Ledbetter & Porter, 1963) were only rarely seen in non-invaded or invaded cells, even after fixation at room temperature which has been reported to be necessary for the retention of these structures (Tilney &

Porter, 1967); the observations were not, however, sufficiently numerous to relate them to infection.

Membranous intravacuolar bodies resembling those in rust infected wheat (Thomas & Isaac, 1967b) were only occasionally seen in both healthy and infected cells of P. vulgaris and these facts suggest that, like those described by Thomas and Isaac (1967b), they were not connected with the infected condition and they may have been artefact.

The structures around the invading haustoria of P. graminis described as lomasomes by Ehrlich et al (1968b) possessed the characteristics of the sequestered membranes in the sheaths around haustoria of U. appendiculatus and may be equivalent structures. Lomasome-like bodies (Moore & McAlear, 1961a) in other parts of the cells were observed no more often in invaded than in non-invaded cells. They were not associated with any special feature of the cell and since they have been reported in numerous other uninfected higher plant cells (e.g. Echlin et al, 1966; Esau et al, 1966; Walker & Bisalputra, 1967) there is no reason to believe they are implicated in the reaction of the cell to invasion.

Plasmodesmata, apparently with cisternae of the E.R. entering and possibly continuous through them, are an uncommon feature of mature cells and as such are a subject of controversy (O'Brien & McCully, 1969). Considerable interest, therefore, attaches to the example of a plasmodesma containing a strand of E.R. from an infected host, not found

in resistant material. This raises the possibility that in susceptible host varieties the establishment or re-opening of such potential channels of transport between cells in the vicinity of infection centres may be involved in the development of an effective host-parasite relationship in which the movement of host products is channelled towards the sites of infection. It has already been suggested that the E.R. may have such a rôle in cells of wheat infected by P. graminis by Ehrlich et al. (1966)

Lipid droplets are conspicuous in invaded cells of the resistant variety Kievit and the invaded cells of Plantvax treated Pinto, but were not observed in similar cells of untreated Pinto. This similarity in response to invasion indicates that there may be a common factor underlining the natural resistance of Kievit and the resistance induced in Pinto by Plantvax. In the case of Kievit the fragmentation of the tonoplast and the presense of cytoplasmic lipid suggests that the cells are more labile to structural damage and to derangement of the normal metabolism in the presence of the haustoria of U. appendiculatus than those of the non-resistant variety Pinto. The cells of Kievit may, therefore, be less capable of entering into a balanced relationship with U. appendiculatus essential for the normal and complete development of the latter, which is so clearly evident in Pinto and so obviously lacking in the hypersensitive reaction of Kievit and other resistant varieties of beans (Wingard, 1935) and of other disease, including P. graminis on wheat (Stakman,

1915), where the early senescence or death of an invaded cell arrests or temporarily halts the progress of the disease. On this interpretation the action of Plantvax may therefore be to slightly impare the normal physiological tolerance of the host cells and their capacity to adjust to new metabolic patterns imposed by the entry of a normally compatible parasite. One direction of this common response would seem to be towards allowing the cells to react to attack by the sequestration of cell products and potentially nutritive metabolites in the insoluble and less accessible form of lipid droplets. These considerations raise the possibility that the diversion of metabolism towards lipid accumulation may be an essentially non-specific mechanism of general importance in cell defence under abnormal conditions.

(b) Fungus Cytoplasm

Generally cells of U. appendiculatus were dikaryotic. Uredospore and stalk cells consistently possessed two nuclei but in the haustorium the number varied from one to three. The unusual number of three nuclei found in haustoria of U. appendiculatus has also been observed in haustoria of other rusts (Puccinia violae, Rice, 1927; P. triticina, Allen, 1926b), but haustoria of P. podophylli (Moore, 1963a) and Melampsora lini (Manocha & Shaw, 1967) were reported to be regularly dikaryotic. Allen (1926b) found that one nucleus

was the most common condition in haustoria of P. triticina but, on the evidence of two small dense bodies lying close to the recognisable nucleus of an apparently uninucleate haustorium, she suggested that two nuclei had become degenerate as the haustorium matured. No obviously degenerate nuclei or possible residual bodies were found in the haustoria of U. appendiculatus but the presence of three nuclei in a young haustorium and the frequent occurrence of apparently uninucleate, mature haustoria would be consistent with the ultimate total loss of two nuclei from an original complement of three, though it leaves quite open the question whether such loss may involve the degeneration of the nuclei or their migration into the supporting hyphae during the course of maturation.

Nucleoli were present in nuclei of uredospores, intercellular hyphae and haustoria of U. appendiculatus at all stages of development observed. Conspicuous differences were found only in mature spores where they had changed both in size and content, if infact the two pale bodies observed were nucleoli and not some other type of nuclear inclusion. The absence of nucleoli has been reported in the mature hydrated uredospores and germ tubes of M. lini (Manocha & Shaw, 1967). In U. appendiculatus there is no doubt that nucleoli of normal appearance are present in nuclei of germ tubes at early stages of development prior to the formation of appressoria, but it is unknown whether they appear prior

to division of the nuclei of the spores.

The mitochondria of U. appendiculatus possessed plate-like cristae and were in appearance similar to those described for other rusts (Williams & Ledingham, 1964; Manocha & Shaw, 1967). Plate-like cristae are present in the mitochondria of the majority, if not all, of other fungi, apart from the Oomycetes which uniformly, whether parasites or saprophytes, possess tubular cristae (Ehrlich & Ehrlich, 1966; Gay & Greenwood, 1966; Bracker, 1967). The basic type of cristae is not, therefore, related to the nutritional habit of the fungus. No distinctive or systematic changes in the size, number or arrangement of the cristae were noticed in U. appendiculatus which could be correlated with its life cycle or with the course of the disease.

Lomasomes have been implicated in cell wall synthesis in fungi (Marchant & Robards, 1968), but the scattered distribution and frequent absence of these bodies in regions where wall growth may be expected to be rapidly taking place does not support this notion; a similar conclusion has been drawn by Heath (1969) in his work on Saprolegnia ferax. Lomasomes in U. appendiculatus were not of constant form and some of these variations may ultimately be found to be artefacts of fixation as appeared to be the case in S. ferax where the shape and composition varied markedly following different fixation conditions (Heath, 1969). These bodies appear to be in essence pockets of excess membrane, probably often of the plasmalemma accumulated where the processes of

wall formation and membrane synthesis are locally not synchronous.

Some confusion has arisen as to the definition of lomasomes. Marchant and Robards (1968) have proposed that a general term 'paramural body' (from a mixture of Greek and Latin meaning 'against the wall') be applied to all groups of membranes lying between the cell wall and the plasmalemma. They propose to limit lomasome to mean a paramural body the membranes of which have been derived from cytoplasmic membranes rather than from the plasmalemma and to use the existing term 'plasmalemmasome' (earlier used in reference to morphologically comparable structures in prokaryotes by Becking et al., 1964) for paramural bodies originating from the plasmalemma. The acceptance of these proposals meets with the difficulty that the distinction between 'lomasomes' and 'plasmalemmasomes' in the sense of Marchant and Robards (1968) is still a matter of controversy and that the criteria required to distinguish them (e.g. detailed serial sections, developmental sequences or distinctive features of the membrane sub-structure), cannot in any case be applied to the data of Moore and McAlear (1961a) who devised the term to designate structures of this general type occurring in fungi and who in so doing first drew wide attention to their existence as a distinct structural component of cells. It is, therefore, perhaps better for the time being to retain the original usage of the term lomasome (from the Greek meaning 'border-body') for fungal

structures only, as defined by Moore and McAlear (1961a). The non-commital phrase 'boundary formations' (Esau et al., 1966), which has precedence over paramural body, is available as a general term in application to cells in all types of plants where more precise designation is impossible.

Golgi-like bodies were not observed in any part of the cytoplasm of U. appendiculatus examined, although their presence has been reported for other members of the Heterobasidiomycetidae: P. podophylli (Moore, 1963b) and M. lini (Manocha & Shaw, 1967). Lamella systems similar to those described for P. podophylli have also been observed in basidiospores of Exidia nucleata by Wells (1964) who was more cautious about equating those features with the classical Golgi apparatus as observed in animal cells (e.g. Dalton, 1961) and in plant cells (e.g. Whaley et al., 1960). Golgi or Golgi-like bodies have not been reported in other members of the Heterobasidiomycetidae examined (e.g. Ehrlich & Ehrlich, 1963a; Ehrlich et al., 1968a,b). True Golgi bodies of the dictyosome type occur in both saprophytic and parasitic Oomycetes (Peyton & Bowen, 1963; Heath, 1969).

Aggregations of granular material with particles of generally similar, though more variable size to ribosomes were present in the cytoplasm of all stages of U. appendiculatus examined, apart from appressoria and sub-stomatal vesicles. Material of granular form, similar to that observed in U. appendiculatus, has been identified as glycogen in animal cells (Reve et al., 1960). Glycogen, though not

reported in rusts, is stored by many fungi (Zaloka, 1965). Amylase digestion has been successfully used in combination with electron microscopy to identify glycogen in epon embedded thin section of rat tissue (Monneron, 1966), but it was unfortunately not possible in the time available to apply this technique in attempts to chemically characterise the granular material in U. appendiculatus. The marked reduction in the density of staining of the granular material within the germinating spore, the reduced size of the aggregates subsequently present in the germ tubes and their absence within the appressoria and sub-stomatal vesicles are good evidence to suggest that hydrolysis and utilisation of this material takes place during the initial development of the hyphae.

A feature of the cell walls of U. appendiculatus is their affinity for lead stain. The walls are strikingly electron dense compared with those of the host. However, this useful distinction, which has made possible the detailed structural analysis of the walls, appears to depend on some minor factors, as yet uncertain, in the preparative treatment. Dennington (1969: personal communication) also working in this laboratory with the same organism and following a similar fixing and staining schedule to that described here, was unable to stain the walls of the fungus.

Moore (1965b), on little evidence, suggested that thallus formation in P. podophylli was arthrogenous and distinct in this respect from other Basidiomycetes which have an incomplete type of cell division (Moore, 1965). The mycelium of

U. appendiculatus, whilst divided into a series of short cells by septa and pseudosepta, resembled more closely the Basidiomycete pattern. Perforated septa of a similar type to U. appendiculatus have also been described for P. graminis and P. recondita (Ehrlich et al., 1968a). After treatment with Schweitzer's reagent it can be observed in U. appendiculatus that the lateral wall of the hypha in contact with the edge of the septum breaks down completely, in contrast to the remainder of the wall of the septum itself which is only partially affected and the pellicle which remains intact. Following standard methods of preparation and staining there are no differences of texture or structural detail by which this localised ring of wall material, which is evidently less resistant than the remainder of the wall, can be detected.

The primary wall of the spore initial appears to be continuous with and in no way different in appearance from the wall of the stalk cell, but in the process of maturation it becomes completely removed as far as the level of the septum between the two cells, indicating either that it is chemically distinctive or that the process of removal depends upon a mechanism strictly localised to its area of contact with the underlying secondary wall of the spore itself. The pellicle, which is unaffected, remains the only continuous part of the wall surrounding the two cells and apart from this the mature spore is held on its stalk only by the adhesion of the two halves of the septum.

The septa of hyphae of U. appendiculatus typically taper radially to the edge of a central pore from which cell organelles appear to be excluded. The margin of the pore is not thickened as reported for Basidiomycetes in general (Moore & McAlear, 1962) and in this respect the septum is similar to those of the Ascomycetes (Bracker, 1967). The dolipore septum present in the Basidiomycetes is thought to preserve the dikaryotic state of the cells, though this idea has not received universal acceptance (Bracker, 1967). The absence of the dolipore from intercellular hyphae in the Uredinales has been considered to facilitate the free movement of nuclei (Bracker, 1967). This is of interest as a possible mode of origin of the several nuclei reported in rust cells by Allen (1926a); but in this study of U. appendiculatus no examples of nuclei or any other organelle situated within true septal pores have been seen. Organelles are frequently present where pseudosepta partially occlude the hyphae and these structures, therefore, appear capable of permitting the movement of organelles along the hyphae. However, Ehrlich et al. (1968a) have suggested for similar pores in P. graminis and P. recondita that pseudoseptae may be no more than compensatory ingrowths or infoldings of the wall occurring in the short axis of a hyphal bend and not partitions between segments functionally analogous to separate cells.

The septum which separates the sub-stomatal vesicle of U. appendiculatus from the appressorium is thicker rather than

thinned towards its centre, which may finally be imperforate and thus provide for the effective isolation of the sub-stomatal vesicle and its products from the external environment in the event of damage to the germ tube on the leaf surface. A feature of the haustorium of Erysiphe graminis, which unlike that of U. appendiculatus is formed directly from the appressorium on the epidermal cell, is the presence of a septum in its stem (Bracker, 1968). This structure of analogous function to the septum in the sub-stomatal vesicle may provide a comparable defence mechanism in the event of damage to the external appressorium on the leaf surface and perhaps also against the effects of adverse reaction by the host on the haustorium being transmitted to the surface mycelium.

3. FINE STRUCTURE OF HAUSTORIA

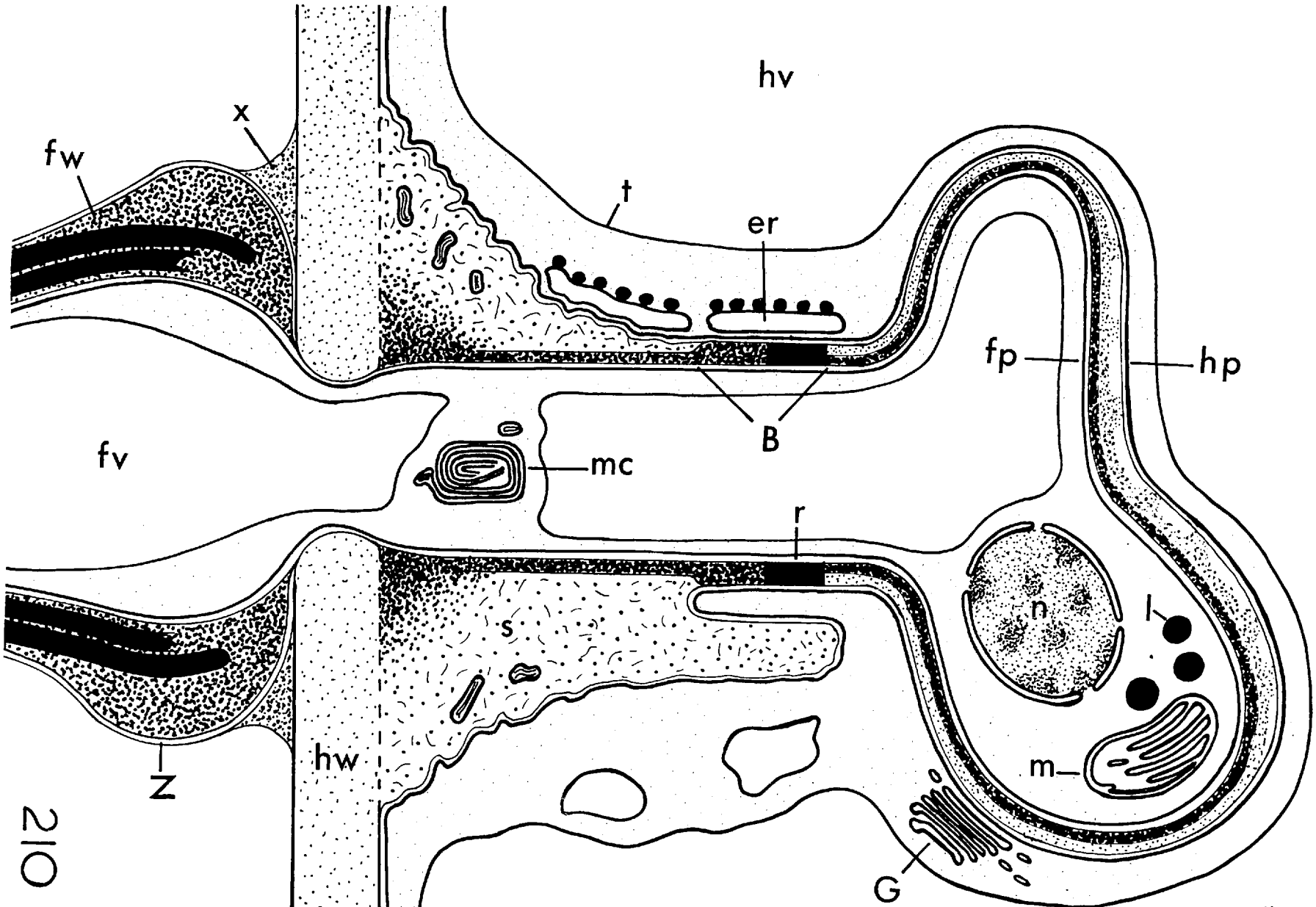
A summary of the results is presented diagrammatically in Figure 210. Further discussion will be limited to major points of interest or of difficulty of interpretation.

There is considerable controversy regarding the terminology of the various intercellular materials investing the haustoria of obligate parasites generally. The detailed examination of the penetration region of U. appendiculatus clarifies several points of difficulty, notably relating to regions which have been termed 'sheath' and 'encapsulation',

210. Diagram summarising the structure of the haustorium of Uromyces appendiculatus in a susceptible host.

The wall of the h.m.c. (fw) at its point of contact with the host wall (hw) is frequently expanded to reveal electron dense sub-layers which terminate a short distance from the penetration pore. Around the h.m.c. wall is a pale layer (z) which continues to the margin of the pore. Between the expanded wall of the h.m.c. and the host wall is an accretion of intercellular material (x) presenting a smooth surface at its free face. The host wall is pierced to form a small pore which does not appear to be lined by a structural fungal wall, although the fungal plasmalemma (fp) is continuous through this region. Immediately within the host cell there appears to be a mingling of wall material from both host and fungus before a defined stem wall is apparent lining the sheath (S). The host plasmalemma (hp) is lifted away from its original position against the host wall by the penetrating haustorium. Beneath this new area of plasmalemma electron-lucent material surrounded by a fibrillar zone, or only fibrillar material may be deposited, usually these outer layers of the sheath contain sequestered membranes. The host plasmalemma first contacts the electron dense layer lining the sheath at an expanded region of the former which has been termed the neckband (B). The neckband terminates at a ring of electron dense material of a diffuse nature (r). The outer layer of the sheath may continue to be deposited over the neckband in the form of a collar, but where this occurs the plasmalemma returns to the neckband as a re-entrant fold. Beyond the ring the stem wall continues as a two layered wall, the inner electron dense, the outer less opaque and eventually surrounds the body which expands into the host vacuole (hv) covered by a thin layer of host cytoplasm. The outer layer of the body in the distal parts of the haustorium may appear more eroded and the host plasmalemma less closely applied than in its more proximal parts to the stem.

Haustoria generally contain one nucleus (n), several mitochondria (m) and lipid droplets (l). A complex of coiled or concentric membranes (mc) is usually present in the stem region, the haustorium is vacuolated to varying extents (fv). Host E.R. (er), rough on the cytoplasmic face, is usually associated with the sheath and stem of the haustorium. Golgi bodies (G) though present near to haustoria do not appear either numerous or active judging by the small number of associated vesicles.


hv
x
fw
t
er
fp
hp
B
fv
mc
r
n
l
s
m
hw
Z
G
N.V.H.

and in so doing provides a clearer understanding of the processes of penetration by haustoria.

(a) Use of the term 'Sheath'

Bracker (1968) has recently suggested that 'sheath' should be used for non-host-wall material between the haustorial wall and the host protoplasm surrounding the body of the haustorium and as such should be substituted for the terms 'encapsulation' (Ehrlich & Ehrlich, 1963a), 'sack' (Hirata & Kojima, 1962; McKeen et al., 1966) and 'zone of apposition' (Peyton & Bowen, 1963). The term 'collar' is proposed for the host-derived layer in the region around the stem of the haustorium. Bracker (1968) claimed that his usage of 'sheath' was in line with the original proposals of G. Smith (1900) in his early account of the detailed structure of the haustorium of Erysiphe communis. G. Smith (1900) described an apparent ingrowth of host wall surrounding the penetrating haustorium through which the latter eventually broke, leaving remnants of the original ingrowth around the stem of the haustorium and to this he applied the term 'collar'. He noted that beyond the collar the newly-formed distal parts of the haustorium were often coated with a deposit and he believed this deposit was a product of the further disintegration of the collar, therefore, though changed in nature the collar was regarded as derived from the

cellulose of the host wall and G. Smith described the haustorium as being sheathed by this material. It is clear at this early date that G. Smith (1900) realised that both plasmalemmas were probably intact and thought that the intercellular material between them was mainly, if not entirely, a breakdown product of the original ingrowth of host wall, though he also observed that it sometimes appeared eventually to lack any solid material, being then a space between host plasmalemma and haustorial wall which was merely fluid filled.

It is not possible to transfer G. Smith's (1900) terminology to the present knowledge of the fine structure of the haustorium without altering the meaning of the terms. Subsequent light microscopists (Rice, 1927; Blackwell, 1953; Fraymouth, 1956) used the word sheath restrictively to denote only the first formed investments of the haustorium, especially in cases where this was prominent because of its thickness and chemical characteristics or where it could be seen to originate as an initial infection peg which was pierced later by the haustorium in its more extensively invasive phase of expansion; by implication not all haustoria necessarily possess a sheath. This apt and easily understood usage was retained in the early electron microscopic studies. To alter its meaning now to designate the investments of the body part of the haustorium on grounds of prior use (for which, from the reasons given above, the evidence is of doubtful validity) would add still further to the present confusion of terminology.

On balance the term 'collar' is perhaps best retained, not as a general term as proposed by Bracker (1968), but as an appropriate description for sheaths which, as in E. graminis, do have the form of a collar or, as sometimes is the case for U. appendiculatus, for the terminal part of the sheath when this has a collar-like form. Terms such as 'callosity' (Young, 1926), 'ligni-tuber' (Fellows, 1928) and 'papilla' (Corner, 1935) only further describe special types of sheaths.

In U. appendiculatus the use of the term sheath adopted in this thesis, while similar to that advocated by Berlin and Bowen (1964), differs in important respects. Those authors suggested that the term be reserved for material homologous with the original host wall whatever form it may take. It is used here in a more restricted morphological sense as a structure which ends at the neckband. In U. appendiculatus it cannot be regarded as homologous with either host or fungal wall because at its base products from both partners appear to be mingled (Fig. 210). Sometimes (probably arrested haustoria) where a neckband is absent the sheath may form a complete covering to the haustorium.

(b) Use of the term 'Encapsulation'

Bracker (1968) used 'sheath' in place of the term 'encapsulation' applied to E. graminis by Ehrlich and Ehrlich (1963b)

for the material external to the inner layer of the body of the haustorium, which is generally assumed in this case to be fungal wall.

Earlier, in reference to P. graminis, Ehrlich and Ehrlich (1963a) defined 'encapsulation' in terms which are difficult to apply generally to other pathogens because they suggested that the material of the encapsulation was essentially of fungal origin, even possibly containing fungal cytoplasm. This was contested by Shaw and Manocha (1965b) who thought that the encapsulation of P. graminis was deposited by the host. In an investigation of Albugo candida Berlin and Bowen (1964) took the view that the so-called 'zone of apposition', a term first used by Peyton and Bowen (1963) for Peronospora manshurica*, should not be used in future because it was equivalent to the 'encapsulation' used earlier by Ehrlich and Ehrlich (1963a). McKeen et al. (1966) used encapsulation for the investments of the body region of Erysiphe cichoracearum and in the same year its general use for all parasites was suggested by Ehrlich and Ehrlich (1966).

In U. appendiculatus the neckband marks quite precisely a point of change in the nature of the intercellular deposits

---

* It should be noted that in the work of Peyton and Bowen (1963) on P. manshurica and Berlin and Bowen (1964) on A. candida both the 'zone of apposition' and the 'encapsulation' are described and illustrated as being continuous through the penetration pore and surrounding the intercellular hyphae. If the encapsulation is a deposit of the host cell then these observations clearly require further investigation.

from those of the sheath to those characteristic of the body of the haustorium where distinct inner and outer layers are present. Ehrlich and Ehrlich (1966) have already suggested that the outer layer of the two layered body wall of the haustorium of Uromyces caladii (Moore & McAlear, 1961b) should be called an encapsulation. This term may also be applied to U. appendiculatus (and U. viciae-fabae) where the limits of this outer layer of the body wall and the host plasmalemma can also be readily and precisely defined.

It is therefore proposed that 'encapsulation' be used for the outer of the two layers of the body wall in the rust fungi, where it is descriptively useful and need not be defined in terms that pre-suppose its origin. In the present state of knowledge it cannot be said that the corresponding outer layer of the body wall of the other groups of parasite are its exact equivalent. There are wide differences in the detailed morphology of these zones. They may be equally diverse in composition, origin and mode of development and it is too early to generalise by assuming that one term alone will be adequate or appropriate for all types of haustoria. For the time being the term 'body wall', which can be applied to all haustoria, will suffice for general purposes.

(c) Fine Structure of the Haustorial Mother Cell and Penetration

The wall of the h.m.c is swollen at the point of

penetration by U. appendiculatus and U. viciae-fabae, as was also reported for M. lini (Manocha & Shaw, 1967), but the darker sub-layers of the wall do not take part in the swelling. The degree of swelling in the rest of the wall appears to be in inverse relation to the area of contact between the host and parasite cells.

The termination of the dark sub-layers of the h.m.c. some distance from the edge of the penetration pore is significant in considering the method of penetration. The almost total absence of fungal wall material at the point of penetration in the base of the haustorium of U. appendiculatus could result if an existing wall was removed by local action (probably chemical), or alternatively if the wall at this point was incompletely formed at the time of penetration and, after modification rather than removal, failed subsequently to develop completely. The second alternative appears the most likely if, as in M. lini (Manocha & Shaw, 1967), the point of contact between the h.m.c. and the host wall originates at the growing apex of a hypha.

The h.m.c. wall of U. viciae-fabae was generally similar to that of U. appendiculatus but differed in respect of the number and form of apparently distinct layers which it contained. A pale outer layer like that of U. appendiculatus could not be detected around the h.m.c. of U. viciae-fabae; the dark sub-layer of the former terminated within the h.m.c. wall a short distance from the penetration pore,

whereas, in U. viciae-fabae the equivalent layer, whilst also terminating before the penetration pore, was, unlike the former, in final contact with the pale zone between the walls of the h.m.c. and host cell. An inner layer, present in U. viciae-fabae, could only be detected in U. appendiculatus after permanganate fixation, but it did not continue through the penetration pore unaltered as in the former example.

The absence of displaced fragments of host wall near the point of penetration or of disturbance to the structure of the wall surrounding the base of the haustorium and the smooth sides and slightly rounded edges of the pore itself show that a remarkably localised dissolution of the wall, limited strictly to the track of the haustorium, has taken place. The results of Schweitzer's reagent show that some degree of chemical alteration has also occurred throughout the wall for a radial distance of about 0.8μm from the margin of the pore. These observations on two species of Uromyces are in agreement with the earlier conclusions of Allen (1923a) and Ehrlich and Ehrlich (1963a) for other rusts that the removal of the host wall by entering haustoria is mainly a chemical (possibly enzymic) process rather than a mechanical one. In so far as fine structural data may be expected to assist further in resolving this question special importance attaches to observations made on the early stages of penetration.

In the present investigation little information has been

obtained about the early stages of invasion by haustoria. The difficulties of identifying unequivocal sections of these stages lie not only in finding early penetration sites, which are necessarily few and inconspicuous, but also in distinguishing a casual fungal-host contact from a potential point of penetration. It is often not possible to decide in any one section if a profile of a seemingly small haustorium is of an early stage of penetration or merely of an oblique glancing section through the base of a more mature haustorium. Ehrlich and Ehrlich (1963a, Fig. 4) figure a supposedly early penetration of a wheat cell by P. graminis which, from its general shape, could equally well have been a glancing section over the basal region of a fully formed haustorium. However, Figure 2 of the same paper is more convincing and suggests that in P. graminis the plasmalemma of the entering haustorium may come into contact, at least initially, with that of the host cell. This would imply, at that stage, an absence of sheath material in the area of contact between the two membranes, but would not exclude the presence of an early deposit on the host wall surrounding the point of penetration. In general the apparent infrequency of early compared with later stages in the sections examined suggests that the phases of penetration and the main period of growth are quickly accomplished.

It has been suggested that haustoria are able to enter the host because of differences of turgor between the host

and parasite (Allen, 1923a; Thatcher, 1939). For this to occur a good seal and mechanically rigid attachment between the h.m.c. and host walls at the point of penetration would be essential. The swelling of the h.m.c. wall in its area of contact with the host wall surrounding the entry pore is possibly related to such requirements because the swelling is least where the area of contact is largest. The intercellular material in the angle of contact possibly also contributes to the effectiveness of the seal. Although turgor differences may assist in the entry of the haustorium the uniformly cylindrical shape of the stem suggests that the growth of the latter was under some specific morphogenetic control and not subject merely to forces of inflation immediately following entry into the host cell.

A definite layer of fungal wall is present at the base of the haustorium of U. viciae-fabae; this layer is continuous with the inner layer of the h.m.c. wall and appears to represent only a part of the latter. The fungal wall at the base of U. appendiculatus is still further reduced, being at most a very thin layer (apparently enhanced by treatment with Schweitzer's reagent) derived from the inner layer of the h.m.c. wall, probably usually fenestrated and often present only as a superficial but dense staining of the surface of the host wall forming the sides of the penetration pore.

The lack of a definite fungal wall in the base of

U. appendiculatus makes the haustorium potentially weak at this point and the manner in which the h.m.c. had broken away from the host wall in one of the examples described here gives support to this view.

The two species of Uromyces investigated appear to differ from those of other parasites so far examined by electron microscopy in the absence of a fully organised fungal wall at the base of the haustorium. In both downy (Peyton & Bowen, 1963; Berlin & Bowen, 1964) and powdery mildews (Bracker, 1968) the wall of the h.m.c. continues without much change in thickness or composition through the penetration pore and for a varying distance along the stem. In the powdery mildews this wall stands in a normal relation to the septum which is typically present at a point between the base and body of the haustorium. It can, therefore, be concluded that in these cases a more or less normal fungal wall is at the base and along most of the stem of the haustorium.

(d) The Sheath

In U. appendiculatus there appears to be an intermingling of extracellular products from both host and fungus in the base of the sheath (not observed for U. viciae-fabae); a distinct layer adjacent to the fungal plasmalemma only becomes evident in its more distal parts. This condition

may be causally related to the mode of penetration. It could be accounted for on the assumption that following contact between the naked protoplasts of host and fungus, in the manner proposed by Ehrlich and Ehrlich (1963a) for P. graminis, there is at first a simultaneous depostion and a consequent mingling of the intercellular materials from both organisms during the initial and possibly rapid stages of inward expansion of the haustorium. However, in U. viciae-fabae the structural pattern shows that inner and outer layers of the sheath are from the first laid down as separate deposits. In both species of Uromyces examined the close relationship of the host plasmalemmas to the sheath are consistent with the supposition that the bulk of this material, apart from the dark inner layer, is produced by the host cell. The structure of the sheath is not identical in the two cases. This is particularly evident at the base of the sheath where in addition to the differences in the inner layer, already commented upon, the fibrous material in the outer zone of U. viciae-fabae is limited to a disk on the host wall surrounding the pore and that surrounding the neck is of a different texture. It is not known if these differences in appearance reflect differences in chemical composition or are merely different patterns in the distribution and quantity of identical constituents present, and the extent to which these differences are specific to the species of host and parasite involved also remains to be investigated.

The fibrillar material which is usually particularly conspicuous in the outer, later formed layer of the sheath, and in some cases in U. appendiculatus comprises the whole of it, resembles that of the original host wall, though tracts of parallel fibres orientated in any one direction tend to be small and are often curved, in contrast to the extensive layers of parallel fibres characteristic of the host wall.

(i) Composition of the Sheath

Mangin (1892), who named callose because it was the main component of the calli which occlude sieve tubes, also found that this material accumulated in irregular positions in epidermal and parenchyma cells, often in association with haustoria of the Peronosporales and Uredinales but, because it also occurred in healthy cells, he was unable to link its presence with any particular pathological state.

The sheath of U. appendiculatus varies considerably in its degree of development and is heterogeneous in appearance. Some, though not all sheaths, gave a staining reaction (toluidine blue O and resorcinol blue) and fluorescence (aniline blue) characteristic of callose. Attempts to remove this reactivity by prior treatment with a callase extracted from papain (B.D.H.) by the method of Eschrich (1961) were unsuccessful. However, callose is characteristically electron-lucent and lacks a fibrillar element (Heslop-Harrison, 1966) and areas of material with these characteristics were present in varying quantities in the sheaths of

U. appendiculatus. So far as can be judged by the frequency of the electron-lucent regions in electron micrographs of the sheaths and from the number of sheaths giving strong staining reactions for callose in the light microscope callose may be present in varying amounts in the haustorial sheaths of U. appendiculatus.

In Peronospora parasitica the presence of callose sheaths has been frequently demonstrated (Fraymouth, 1956). Callose could not be demonstrated in the collar of E. graminis by Bracker (1968), but in this laboratory some but not all cells penetrated by this parasite gave a fluorescent reaction at the point of penetration when treated with aniline blue (Stanbridge, 1969: personal communication). It is interesting to note that when cells of P. vulgaris hypocotyls are injured by pricking with a fine needle localised areas of U.V. fluorescent material, characteristic of callose, are found at sites of injury, but completely negative results were obtained in cells of the some host species penetrated by Colletotrichum lindemuthianum (Mercer, 1969: personal communication).

Although it cannot be altogether excluded that the negative results are due to a masking of the reactivity of callose in sheaths it seems likely that the deposition of callose cannot be considered a universal or inevitable local response of the host to penetration of the wall by a haustorium, even in species where it can readily be elicited by

mechanical pricking.

The metachromatic reaction of toluidine blue O, which is absent from the fungal walls, is usually strongest in the outer layers of the sheath where it is often more intense than the original host wall. It is possible, therefore, that the fibrillar material in both cases consists largely of polyuranide hemicelluloses, to which the reaction of toluidine blue O in host walls is thought to be due (O'Brien et al., 1964). This would be in agreement with the evidence for the origin of the sheath, particularly its outer layers, mainly or entirely from the host.

(ii) Formation of the Sheath

Manocha and Shaw (1967) stated that the host wall thickens to form a collar around the stem of the haustorium of M. lini, but they did not determine whether the collar was merely a swelling of the host cell wall or a fresh deposit of material. In U. appendiculatus this material is clearly a secondary deposit.

The cisternae of E.R. which lie against the sheath may be implicated in the deposition of the latter. However, E.R. cisternae may be completely absent from the sheath surface and in other cases may extend often in contact with the plasmalemma distal to the neckband where a sheath had not been deposited at the time of fixation. These facts admit two different interpretations: either the E.R. is concerned

with the deposition of the sheath and perhaps disperses when this is completed, or alternatively the coating of E.R. develops only when sheath formation is nearing completion; it cannot be excluded that it may also have a function at a late stage in preventing the further extension of the sheath.

Esau et al. (1966) reported that E.R. applied to the site of sieve plate pores was associated with callose deposition until the pore was formed and Northcote (1969) has pointed out that callose accumulates in areas beneath cisternae. E.R. has been found in various types of cells where a normal cellulose wall is not laid down (Clowes & Juniper, 1968) and Picket-Heaps and Northcote (1966) have suggested that the E.R. has more than one rôle in wall formation; it may direct and channel certain materials, such as callose, to sites of deposition and exclude others. It is possible, therefore, that the E.R. applied to the sheath, which like that of the developing sieve plate, is 'rough' only on the cytoplasmic side, is involved in the deposition of callose. Whatever the rôle of the host E.R. maybe in relation to the sheath and stem of the haustorium the decoration of membrane-bound ribosomes suggest that here, as in other cases (e.g. Caro & Palade, 1964), the E.R. cisternae are likely to be involved in the production of proteins (which could be enzymes).

The deposition of callose as a constituent of the

sheath may be no more than a non-specific response to wounding and which varies considerably in different cells and between different plant species (Currier, 1957; Currier & Shih, 1968).

Callose deposition is characteristically rapid (Heslop-Harrison, 1966), as probably is also the remainder of the sheath. This may explain the sequestration of portions of the covering plasmalemma.

(e) The Body Wall of the Haustorium

Reasons have been given for supposing that the electron dense inner layer of the body wall of the haustorium belongs to the fungus. This layer is more or less uniform in thickness over its whole extent and is greater in mature haustoria than in younger ones. The body differs in this respect from the stem where the thickness and character of the dense inner layer change in a systematic manner from the base to the neckband. This could be readily accounted for if the body is formed, perhaps rapidly, after the haustorium has burst through the sheath by expansion as a consequence of its greater turgor into the host cell. It then becomes coated with wall material evenly over the whole of the body rather than sequentially in relation to an apical or other special region of growth. Such a mode of development would be quite different from the growth of hyphae which appear generally in fungi to be by apical extension (Aronson, 1965; Robertson,

1965). The more irregular shape of the body compared with that of the stem would support the view that turgor differences between host and parasite may play a greater part in determining the form of the body than that of the stem.

The encapsulation of the body wall continues to develop after the dark inner layer has formed, as was also observed in P. graminis (Shaw & Manocha, 1965b). In the resistant variety examined the encapsulation was looser in texture and usually more extensively developed than in the susceptible one and it resembled more closely that described for P. graminis by Ehrlich and Ehrlich (1963a). In some examples of haustoria from the resistant variety small vesicles close to Golgi bodies associated with the haustoria contained material apparently similar to the encapsulation. Small vesicles derived from Golgi bodies in certain higher plant cells have been shown, by use of tracer precursors in combination with electron microscopic studies, to contain non-cellulosic polysaccharides or hemicelluloses involved in wall synthesis (Northcote, 1969). Similar experiments with infected leaves might give information about the chemical nature of the contents and the functional rôle of the Golgi-associated vesicles in cells containing haustoria, but the combination of two organisms and the probability that the growth of the encapsulation occupies only a brief phase in the life of the haustorium are two of a number of special factors adding to the difficulty of such a line of investigation.

The absence of E.R. at the body interface of U. appendiculatus in the susceptible reaction does not indicate that it is involved in the deposition of the encapsulation or concerned in movement of products to or from the haustorium as suggested by Ehrlich et al. (1966) for P. graminis.

(f) The Neck and Neckband

The two regions of the haustorium, base and body, are separated by the stem which includes the neckband region where there is a striking change in the intercellular deposits lying between the plasmalemmas of host and parasite.

The termination of the neckband is marked by the ring, a band of diffuse electron dense material (frequently with clear areas as if material has been lost during processing). After permanganate fixation there is a break in the wall at the ring, indicating that the material at this point is less chemically resistant than at any other part of the haustorial wall.

The neckband marks an area in which a pronounced change takes place in the interaction between host and parasite, perhaps a change in response of the host when the haustorium passes from a phase of penetrative activity to one of a more invasive nature when the body expands into the cell, during which it forms a distinct wall of its own.

Rice (1927) reported a thickening in the stem of the

haustorium of Puccinia sorghi which she suggested may mark a change in host parasite relations. It is possible that this feature corresponds to the neckband of the two species of Uromyces examined. A re-examination of the fine structure of Puccinia spp. and other rusts may now reveal an equivalent structure.

Although the sheath may project beyond the neckband, sometimes as a short collar, these extensions are covered by host plasmalemma re-entering to the beginning of the neckband. This structural arrangement is essentially similar to that envisaged by Bracker (1968) for E. graminis, where the sheath rises as a collar from around the penetration pore and extends along the whole length of the cylindrical stem. On the interpretation put forward here for U. appendiculatus the haustorium of E. graminis does not possess a neck (that part of the stem between the base of the haustorium and the neckband). There is infact no evidence for a neckband-like structure in E. graminis, but if such were present it should be located close to the base of the collar.

In considering the development of the neck in U. appendiculatus two obvious possibilities present themselves. The neck may develop by apical or intercalary growth of the stem before the body of the haustorium has formed, or it may arise by intercalary growth of the stem during or subsequent to the formation of the body of the haustorium.

The host membrane at the neckband is a small area of

plasmalemma situated between two larger areas, one associated with the sheath and the other with the body of the haustorium. Observations on the structure of the plasmalemma in these different regions do not show any distinctive features which would assist in deciding whether the membrane of the neck-band is newly formed or whether it is perhaps a remnant of the host plasmalemma originally situated at the penetration pore and later carried inwards at the tip of the advancing haustorium.

(g) Host and Fungal Plasmalemmas

Once fully expanded the plasmalemma of P. vulgaris can be traced unbroken around the haustorium of U, appendiculatus This has not been demonstrated previously for a rust and confirms the suppositions of Rice (1927) and Thatcher (1939). Also, the plasmalemma of the fungus continues unbroken from the h.m.c. through the penetration pore and stem into the body of the haustorium.

Four distinct regions can be identified in the host plasmalemma of an invaded cell.

(i) The part of the plasmalemma against the original host wall where it is not associated with any new deposit of intercellular material.

(ii) The part that is lifted away from the host wall in both P. vulgaris and V. faba by lateral extensions of the

sheath of the invading pathogen for variable distances from the edge of the penetration pore and extends over the whole of the sheath. This area of the plasmalemma is new membrane and may be involved in the production of the sheath material with which it is associated.

(iii) The small area of host membrane associated with the neckband and further characterised by the occlusion of the small space between it and the neckband by material apparently diffused from the wall in this region as if it were incompletely formed and contained labile components. This area of the plasmalemma appears to lack an associated deposit of host material.

(iv) The newly proliferated and deeply invaginated membrane around the body of the haustorium associated with its encapsulation, which is presumably also the area of host membrane through which the transport of products for the nutrition of the fungus must mainly, if not entirely, take place.

The sub-structure of the membranes over the different areas of the haustorium has not been examined in any parasite. High resolution micrographs of thin sections from these regions around the haustorium of U. appendiculatus have not revealed any visible differences in the composition of these areas. However, the freeze-etch method (Branton, 1966) might reveal any changes of associated membrane granularity that may be present.

Bracker (1968) has noted that the membrane around the 'sheath' of the body of the haustorium of E. graminis shows slight differences in the thickness of the constituent layers of the triplet compared with the plasmalemma against the host wall. It was reported by Hirata and Kojima (1962) that the membrane covering the 'sack' of the powdery mildews was more resistant to damage than other areas of the plasmalemma, due possibly to the action of calcium. The extraction of the sheath membrane with the haustorium of Sphaerotheca fuliginea suggested to Dekhuijzen and Scheer (1967, 1969) that the membrane was fungal in origin; also proposed by Ehrlich and Ehrlich (1963a,b) for the membranes around the haustoria of P. graminis and E. graminis. There is no evidence to support this view for the membrane around the body of the haustorium of U. appendiculatus as its continuity with the host plasmalemma is not in doubt.

It cannot thus be assumed that the plasmalemmas of host and fungus investing the haustorium are identical in properties with the areas of plasmalemma adjacent to their respective vegetative cell walls. The membrane of the fungus is less complex in several respects; it follows closely the simple shape of the haustorial protoplast and there is less distinction between the materials deposited adjacent to it in the different regions of the haustorium.

4. FINE STRUCTURE OF UREDOSPORES

(a) Spore Wall and Spine Formation

In most respects the development of the spines follows a pattern similar to that observed for the uredospores of P. graminis described by Thomas and Isaac (1967a), though it has been possible in the present study to carry the analysis and interpretation of the wall dissolution further. The work by Angold (1969: verbal communication to the Federation of British Plant Pathologists) on Puccinia striiformis also supports the main conclusions on the processes of spine development in uredospores of U. appendiculatus.

The wall of the uredospore initial consists of the original hyphal wall (primary wall) covered by the pellicle. During development a secondary wall is deposited against the primary wall but in the process of maturation the latter is lost and finally the spore is enclosed by a thick uniform wall of secondary origin, still surrounded by the pellicle. The simple structure of this wall differs from that of M. lini (Manocha & Shaw, 1967), which is multilayered and of P. graminis (Williams & Ledingham, 1964; Thomas & Isaac, 1967a), which has three layers, of which the outer electron dense, hydrophobic one is probably equivalent to the pellicle of U. appendiculatus.

The disintegration of the primary wall appears to begin

around the tips of the spines before they are extruded to the surface and eventually spreads around the spore.

The disintegration of the primary wall in this way involves the removal of one wall layer lying external to another. In considering the mechanism by which this is achieved three possibilities emerge: (i) that enzymes for the dissolution of the primary wall move through the secondary wall leaving the latter unaltered; (ii) that enzymes were produced from the tips of the spines and spread there throughout the primary wall upto its junction with the stalk cell. It was not possible to obtain glancing sections over the primary wall at this stage in development because on contact with the knife the spores usually became pulled out of the sections. It could not, therefore, be determined whether the dissolution of the primary wall, which undoubtedly first occurs at the tips of the spines, then spreads by extension of the areas first dissolved, or whether new areas of dissolution are initiated independently and perhaps simultaneously; or (iii) that the enzymes for the removal of the primary wall originate from within the wall itself.

It is possible that the spines, open at their bases to the cytoplasm of the spore until fully developed (which is after the dissolution of the primary wall has started), may provide a pathway for enzymes or activators of this process. The presence of linear flecks running vertically through the spine is suggestive that the spine may act as a mediator

between the cytoplasm of the spore and the primary wall.

Hutchins and Lutman (1938) suggested on the basis of an examination of drawings made by Sappin-Trouffy (1896) that the development of spines in the smuts was analagous to echinulation of uredospores. Gelatinisation of the outer layer of the wall described for rusts (Savile, 1954) and smuts (Hutchins & Lutman, 1938) was not observed in U. appendiculatus and the possible relationship of such processes to fine structural changes during development remains to be investigated. Savile (1954) was concerned with the rôle of cytoplasmic movements in spine formation and its relationship to meteorological changes in the environment and was not aware of the part played by cytoplasm, organelles and the plasmalemma in the synthesis and movement of materials within cells. It is not possible to determine in detail how spine formation in smuts is analogous to that in the rusts until fine structural observations have been made.

(b) Dissemination and Germination

During the deposition of the secondary wall cytoplasmic continuity between the spore and its stalk cell is maintained via the pore in the hilum region and presumably when the spore is fully formed and dehydration occurs water is lost via the pore after detachment. It is possible that

rehydration also occurs through the pore, the observation that the uptake of ruthenium red into the spore begins at the hilum region supports this view. The intensity of ruthenium red initially at the hilum region is more likely to be due to uptake into the spore cytoplasm than to the presence of pectin which has not been demonstrated in rust spores (Shaw, 1964).

The mechanism by which the spores become freed from their stalks is unknown but may be connected with the sudden expansion of the spore accompanied by the elevation of the spines to its surface and the simultaneous dissolution of the primary wall, which appears to be the major component binding the spore to its stalk. Final detachment and rupture of the pellicle is probably brought about by the pressures of one spore upon another within the uredosorus.

After detachment from the stalk the uredospores collect in the sorus and are eventually dispersed by wind. The dehydration and consequent loss in weight will be a contributing factor to effective wind dispersal. The spines on the uredospores are thought to increase their buoyancy in the air (Duff, 1918; Savile, 1954).

Perhaps a more important aerodynamic feature in U. appendiculatus is the 'dough-nut' shape of the detached uredospores which must aid dispersal because this shape creates greater tubulence than a sphere thus aiding transport to greater distances. Uredospores of U. viciae-fabae,

which have three to four germ pores, are collapsed on as many faces as there are pores and the uredospores of other rusts which possess various numbers of germ pores, e.g. P. graminis, 4 and P. striiformis, 8-10 (Wilson & Henderson, 1966), are probably also of different shapes.

Spines may aid in attachment to leaf surfaces, especially as in the dehydrated condition in U. appendiculatus they are arranged around the rim of the dough-nut and thus provide a large annular area of contact with these projections than would be available on the contact area of a sphere. In the dehydrated condition a germ pore is usually situated on a side in contact with the leaf surface.

Fromme (1915) reported that in the rusts generally germination occurred from a germ pore facing away from the source of light and suggested that the presence of a number of germ pores in uredospores increased the chances that one pore would always be favourably situated for germination on the leaf. However, experiments with uredospores of U. appendiculatus showed that the emergence of the germ tube from one or other of the two germ pores was not influenced by the direction of illumination. The frequent occurrence of a depression at the site of the ineffective germ pore in germinating spores suggests that the germ tubes usually commence to emerge from both pores more or less simultaneously and that the one which is first successful in rupturing the pellicle is the one that continues to grow, whereas the other then ceases to develop, probably rarely rupturing

the pellicle, and eventually collapsing. The orientation of the germ tubes of U.appendiculatus was also independent of the direction of light. Dickenson (1949a) has suggested that the orietation of germ tubes of P. graminis was due to a contact or humidity response and this would appear equally likely to be the case in U. appendiculatus.

5. HISTOCHEMISTRY

(a) General Considerations

The precise nature of the components of the cell walls of host and fungus cannot be determined by the standard histochemical methods as their specificity has been frequently doubted. However, the reagents used have given useful information about the sub-structure and composition of the walls and have helped in the elucidation of the structural relationships between host and parasite.

In thin sections polysaccharides are characteristically electron-lucent and without visible structure (Frey-Wyssling & Mühlethaler, 1965). However, the untreated cell walls of fungus and host react to the 'electron' stains. The host cell wall contains a distinct fibrillar element, thought to be revealed by lead citrate bonding to noncellulosic polysaccharides surrounding the cellulose microfibrils following Glut/Os fixation (Clowes & Juniper, 1968). The

presence of protein within plant cell walls could possibly contribute to their capacity to become stained with electron scattering metals. Hydroxyproline, found in structural proteins, is thought to provide disulphide linkages between the cellulose microfibrils of higher plant cell walls and to be possibly involved in cell extension; this hydroxyproline-rich primary cell wall protein has been called 'extensin' (Lamport, 1965). Proteins often stain with osmium (Clowes & Juniper, 1968) and it is possible, therefore, that the fibrillar component showing in the plant cell wall after Glut/Os, U/Pb treatment may be at least a protein network. Thus the fibrillar appearance of the cell wall is a 'negative' pattern imposed by the staining of protein or other material lying between closely aggregated non-staining fibrils.

The dense staining of the fungal wall may indicate the presence of a high proportion of lipid. Protein as an integral part of the cell walls of fungi has not been conclusively established (Aronson, 1965). The osmium or osmium-bound material was removed from the fungal cell wall by the ammonia treatment, which does not appear to affect the staining characteristics of the host wall. The nature of this reaction is unknown. Germ tube walls do not stain under normal fixation conditions and it is possible that the incorporation of stainable materials does not occur until the fungus is established within the host.

The ready removal of the encapsulation of the haustorium by the various treatments described below seems to indicate that it is of a much less cohesive nature than other wall materials. That this layer is of host origin is further illustrated by the fact that treatments which remove host materials also remove the encapsulation.

(b) Treatment with Schweitzer's Reagent

Schweitzer's reagent as well as removing cellulose also removes mannan, xylans and glucans which have β 1:4 linked residues (Roelofsen, 1959). After treatment with Schweitzer's reagent the remaining fibrillar material of the cell wall of P. vulgaris is probably protein and polyuranide hemicelluloses with α 1:4 linkages which are not removed by the reagent (Siegel, 1962).

The clearer areas revealed around the penetration pore contained fewer fibrils than the remainder of the wall and it is concluded that the fibrillar material removed had been altered, probably chemically, before treatment, but not so as to affect its reaction to fixation and staining for routine electron microscopy.

Allen (1923a) has noted a localised change in the staining reactions of the area immediately around the penetration pore made by P. graminis, which she regarded as a chemical alteration related to the penetration of the wall

by an enzymic process. Recently in plants infected by members of the Erysiphaceae McKeen et al. (1969) have observed alterations in the fine structure of the wall around the penetration pore which they believe are also formed by enzymic means.

Material lining the penetration pore appears to originate from the fungus; it is more prominent in the extracted material than in most examples observed without treatment and it may represent, at least in part, a thin deposit of material extracted from the diffusely stained zone of the host wall surrounding the pore and consolidated on its surface. Within the host cell a definite fungal component can be seen throughout the neck, although apparently bonded to the outer layer of the sheath, which is in agreement with the association or mingling of these two zones during the initial stages of invasion.

The more open texture of the fungal wall following treatment with Schweitzer's reagent suggests that some constituent has been removed, possibly a β 1:4 linked polysaccharide such as mannan which is known to be present in rusts (Shu et al., 1954). However, Schweitzer's reagent did not appear to remove any material from the walls of the mature uredospore and was without affect on the spines.

(c) Treated with KOH/Acetic Acid

Hot concentrated alkali is known to dissolve most

higher plant wall substances (Siegel, 1962), and after treatment with KOH/acetic acid for the removal of chitin no fibrillar structure was seen in unfixed host cell walls, which appeared to be almost completely removed. Granular material which remained in the host cell walls treated in the same way but following Glut/Os fixation may possibly be protein that has been rendered more resistant by fixation and coagulated by the subsequent treatment. Evidence to support this idea is found in the fact that more cytoplasm remained in the cells of fixed than in unfixed material.

Treatment of hyphal and haustorial walls did not remove the bulk of the material comprising the wall, though other tests confirmed the removal of chitin previously present in the walls. It is possible, therefore, that chitin is present only in small amounts. Thatcher (1943) obtained a positive result for chitin in hyphal and haustorial walls of P. graminis.

Shu et al. (1954) concluded from results of chemical analyses that only small amounts of chitin were present in uredospores of P. graminis. No positive tests for chitin were obtained from fresh uredospore walls of U. appendiculatus but they completely dissolved when treated with KOH/acetic acid and its presence cannot be completely excluded. The spines, not stainable in any reagent tried, were removed, as were the germ pore plugs following KOH/acetic acid treatment. The germ pores of uredospores of M. lini are

thought to be occluded by a form of polysaccharide (Manocha & Shaw, 1967)

The material left in the uredospore wall after treatment is possibly fixed protein as the uredospores in unfixed sori disappeared after similar treatment. There is an indication that lipid, possibly in large amounts, is present in the wall, as treatment with concentrated H_2SO_4 does not completely dissolve it but converts it to a translucent shell. Lipid is thought to play an important part in the architecture of the yeast cell wall (Nickerson, 1963) and it is not inconceivable that 'bound lipids' (resisting extraction with neutral solvents) are also present in uredospore walls.

6. EFFECT OF A SYSTEMIC FUNGICIDE ON FRENCH BEAN RUST

The preliminary experiments with 'Plantvax' indicated several features concerning its mode of action. It works directly on the spore, reducing germination and the subsequent growth of the germ tubes. At concentrations that would not totally prevent symptom development Plantvax reduces the number of lesions eventually produced. Haustoria appear to be affected directly after the fungicide is applied to already successfully established rust lesions and results in the death of the parasite and also of the invaded host cells. However, if the lesions are numerous the leaf as a whole dies and falls off as would have occurred later

in the natural course of the disease, but at least in the former instance the fungus is also killed and dissemination prevented.

These effects of treatment with Plantvax further demonstrate the intimate balance between rusts and their hosts. It is well known that factors which affect the growth of the host plant also affect the development of the rust (Mains, 1917; Hart, 1926; Harter et al., 1935), it is of interest that in this case the reverse also appears to be true.

The swelling of the mitochondria in the parasite cells and the dispersion of the polysomes after treatment with Plantvax suggests that this fungicide acts through a direct effect on respiration and possibly on RNA synthesis. It also appears that since haustoria are killed before the intercellular mycelium the movement of the fungicide is via the haustoria and not directly through the walls between adjacent fungal and host cells.

7. CONCLUSIONS

(a) The Importance of Fine Structure in Relation to Disease Resistance

Many factors, physiological, biochemical and mechanical, all influenced by the genetical make up of the host and fungus (Person, 1959; Hooker, 1967) are involved in resistance to haustoria-forming, obligate parasites.

In the present investigation the observations have particular relevance to two rather different phenomena of host-parasite interactions that may be important in the mechanisms of resistance in the rust disease of French beans and indicates possible approaches to means of control. On the one hand there is the occasional presence of haustoria apparently arrested at an early stage of growth in some cells of the susceptible variety and on the other there is the evidence of greater structural damage in cells of resistant (hypersensitive) host plants and in susceptible plants treated with Plantvax. In the first case damage to host cells is prevented or reduced to a minimum and the parasite, denied access to nutriment from the host, develops only so far as the food reserves of the spore allow. The second case involves essentially the well known phenomenon of hypersensitivity where an incompatibility between the two organisms prevents the establishment of an effective nutritive link between the parasite and the living cells of the host, resulting in the early death of the former; damage and death of the host cells occurs to a varying degree but the lesions are self limiting.

Initially no differences between the infection processes of U. appendiculatus were demonstrated between resistant and susceptible varieties, which is in agreement with many previous reports for other rusts (Marryat, 1907; Ehrlich & Ehrlich, 1962; Hilu, 1965; Zimmer, 1965).

(i) Isolation of Haustoria within the Sheath

Examples of varieties of host plant in which resistance to obligate parasites is regarded as due to the capacity of the former to arrest the haustoria by forming an impenetrable sheath appear rarely, if ever, to have been reported. However, variations in sheath development and instances of individual haustoria surrounded by a sheath have often been noted and commented upon in relation to resistance. The resistance of a plant as a whole to fungal invasion may conceivably be, at least in part, a function of the proportion of its cells capable in this way of arresting haustoria at early stages of penetration. In their study of the fine structure of A. candida Berlin and Bowen (1964) drew special attention to a few examples of necrosed haustoria surrounded by unusually large sheath deposits and inferred that the death of the haustoria could have resulted from their isolation in this way. In U. appendiculatus the variable amount of sheathing material formed in different cells, even among those from the same infection centre, indicates that each cell reacts independently and varies in its response to invasion. Ito (1949) has suggested that the variability of the amount of sheath produced may merely result from variations in the cells response to wounding. Instances of incompletely developed haustoria which lack a stem or a neckband and completely enclosed in a sheath, though not often encountered in the material examined, indicates that in this organism haustoria can sometimes be arrested

within the sheath, although in the examples seen, without the latter being grossly hypertrophied. Specific search may reveal cultivars of P. vulgaris exhibiting a significantly great capacity for sheath production and consequent resistance to the rust disease. Blackwell (1953) noted that large sheaths were typically present around the haustoria of Phytophthora infestans in tubers of potato but were small or absent around haustoria in the leaves. It may be possible to define conditions of cultivation within the range of agricultural practice which will encourage greater sheath production and so increase the resistance of plants to attack.

Heslop-Harrison (1966) put forward the idea that the temporary callose walls surrounding pollen meiocytes function as barriers to the passage of physiologically active, large molecules between the sporophyte tissue and the developing gametophytes. Tupy (1959) has noted that greater amounts of callose are formed in pollen germ tubes of incompatible reactions than in compatible ones. It is, therefore, conceivable that callose when present in the sheaths of haustoria may be effective in the functioning of the latter as a chemical in addition to a mechanical barrier between host and parasite protoplasts. Davison (1968) has made the somewhat teleological suggestion that the deposition of callose in P. parasitica represents an attempt by the host to form an impermeable barrier to products diffusing from

the parasite.

(ii) Hypersensitive Reaction

Hypersensitivity is a well known and common mechanism of resistance against rust diseases generally.

Several different effects on the fine structure of the host can be distinguished: fragmentation of the host vacuole, increase in lipid content of host cells and the deposition of new materials. The hypersensitive response of the host has negative and positive aspects. Irreversible damage to the host cells by framentation of the vacuoles and damage to cell membranes, resulting ultimately in their death, are examples of the former, and the stimulation of the host to produce new products an example of the latter. Allen (1923a) noted that in the hypersensitive cells of wheat the host wall was thickened at the points of contact with healthy cells and suggested that these thickening were impervious to substances from the diseased cells affecting the neighbouring healthy ones. In the hypersensitive cells of Kievit infected by U. appendiculatus both membrane breakage and the production of new materials occurs as a response to invasion. The whole of the host plasmalemma is reactivated in some cells to produce a deposit of material over the cell walls. Also large deposits of encapsulating material are produced around some haustoria in the resistant reaction, this may have the

effect of reducing the influence of the parasite on the host, or it may indicate an extra-stimulatory response to substances present in the fungus, evidenced by the hypersensitive response. Because of the sensitivity and early death of the host cells the hypersensitive plant is, as well as plants with a large capacity for sheath production, also effective in localising and arresting the development of the disease.

The action of Plantvax, already discussed in some detail earlier (pp. 246 & 290), may be regarded as inducing hypersensitivity in varieties not naturally giving such reactions. However, the production of new wall material was not found in this instance, or an increased amount of encapsulation.

Further work on the fine structure of resistant varieties and the effect of systemic fungicides on susceptible varieties in combination with physiological studies may provide valuable information on the mechanism of resistance in the rusts.

(b) Movement of Materials between the Host and Parasite

There was no evidence at any stage of development that continuity existed between the fungal and host cytoplasm, in particular no structures in the least resembling plasmodesmata as described for P. graminis by Ehrlich and Ehrlich

(1963a) could be demonstrated. However, the distribution of stainable material within the encapsulation would be consistent with transport of materials from host to pathogen via this structure in the manner proposed by Shaw and Manocha (1965b) from their investigations on the fine structure of P. graminis.

There appears to be little basic difference between the structure of the body wall of the haustorium and intercellular hyphae, except in thickness. In effect the only barrier between the two protoplasts completely removed in the formation of haustoria is that of the host wall. That the host wall is not in itself a barrier to the movement of metabolites is demonstrated by the number of pathogens that can grow without initially killing or penetrating the host cells (e.g. Taphrina deformans, Venturia inaequalis).

It is of course possible that the penetration by haustoria stimulates the host to produce products necessary for the parasites growth that are not normally present within the cell and also that the influence of the haustorium on the host is to affect the leakage of metabolic products, in a more controlled way than proposed by Thatcher (1943) who suggested that the entrance of a haustorium into a resistant (hypersensitive) cell caused a fatal alteration in its permeability. It has been observed in lichens that the fungal component influences the leakage rate of metabolites from the algal cells (Smith & Drew, 1965). However, it is unlikely

that these are the only factors concerned in the host dependant nutrition of the parasite in view of the inability to produce an artificial medium which will support the growth of rusts generally and enable them to synthesis proteins, RNA and DNA (Shaw, 1963, 1964).

(c) In vitro Culture

It has been demonstrated that the germ tubes of rusts can be stimulated to produce infection structures on artificial membranes (Dickenson, 1949a-d, 1955; Maheshwari et al., 1967a,b). Mains (1917) found that peptone increased the branching of germ tubes. Only recently have particular strains of rust been successfully grown in axenic culture (Williams et al., 1966, 1967; Coffey et al., 1969; Turel, 1969) following the disputed work of Cutter (1959, 1960).

Attempts to grow U. appendiculatus and U. viciae-fabae in vitro, using the medium of Williams et al. (1967) and numerous variations including leaf extracts, by the author and Bau (1969: personal communication) have been unsuccessful. The problems of obligate parasitism are thus still unresolved because although some strains of rusts will grow successfully on media containing commonly used constituents and be found to reinfect host material (Williams et al., 1967) others will not.

The work presented in this thesis has tried to present a general picture of the fine structure of one rust in relation to its host in an attempt to elucidate problems of host-parasite relations. It is clearly inadequate in many respects, but the author hopes that some light has been shed on areas of research which will prove profitable for further work, not only in answering fundamental questions about the fine structure of this group of fungi, but of wider areas concerned with plant diseases and their control.

A P P E N D I X

1. SCANNING REFLECTION ELECTRON MICROSCOPY

Results and Discussion

A description of the material used is presented in the plate legends for figures 211 - 220.

(i) Freeze-Drying

For all the material except that shown in Fig. 216 the first method of freeze-drying (p. 56) proved to be inadequate. Presumably the brass block failed to prevent the thawing out of the specimens before sublimation was completed, so that surface tension effects distorted the cells on drying (Fig. 211, 213). However, the material illustrated in Fig. 216 did not distort markedly, possibly because it was sectioned before freeze-drying and thus had a large surface to volume ratio, so the ice sublimed completely before it thawed. Cells of the mid-rib were noticeably more rigid structures than the cells of the mesophyll and were able to give more support to the sections in this region.

Method (ii) (p. 56) also had its limitations, though generally better preservation of cell structures was obtained

compared with method (i). The tissue was probably not frozen quickly enough and damage may have occurred to the cells through the formation of ice crystals within the cells. This damage was not so critical as for high resolution microscopy of freeze-etched cells, as exemplified by the work of Branton (1966) and Moor and Mühlethaler (1963). The minimum temperature achieved (-20°C) may not have been sufficient to completely freeze the contents of the plant cells and may account for the variability in the results using this method (Fig. 215).

The problems of freeze-drying may be overcome by combining the various techniques developed for fixing material as used in the freeze-etch method (Moor, 1965). Echlin (1968) discussed the advantages and disadvantages of various methods employed in specimen preparation for scanning reflection electron microscopy and he described the use of the Pease Tissue Drier as a method for freeze-drying.

Results recently obtained using a combination of methods (i) and (ii), that is rapid freezing of the specimens in liquid nitrogen, transferring to a vacuum desiccator at -20°C and evacuating via a rotary pump, has given more reproducible results and with little collapse of the cells.

(ii) Specimens

Sectioning prior to freeze-drying removed the cell

contents from ruptured cells and frequently affected neighbouring cells. The use of freeze-dried squares of leaf tissue and then sectioning ensured that cytoplasmic contents remained within the cell and also freshly cut or broken edges to cells were exposed (Fig. 214). Some collapse of the cell wall around the chloroplasts was visible, a feature not found in embedded material (Fig. 9).

Figures 218 and 219 show wax crystals preserved on the epidermis of two different plant species. The wax crystals on Pisum sativum (Fig. 218) were similar in appearance to replicas of the leaf surface obtained by Juniper (1968: illustrated in Clowes & Juniper, 1968).

The ability of the microscope to accommodate large specimens enabled sizable areas of leaf surface and sections to be investigated (Fig. 211, 216). The great depth of focus obtainable with the microscope enabled details from within the cavities of stomata (Fig, 212), intercellular spaces (Fig. 214) and vessels (Fig. 217) to be seen. Another useful feature was the ability to be able to move the specimen within the electron beam, enabling details of structure to be accurately documented (Fig. 144, 145).

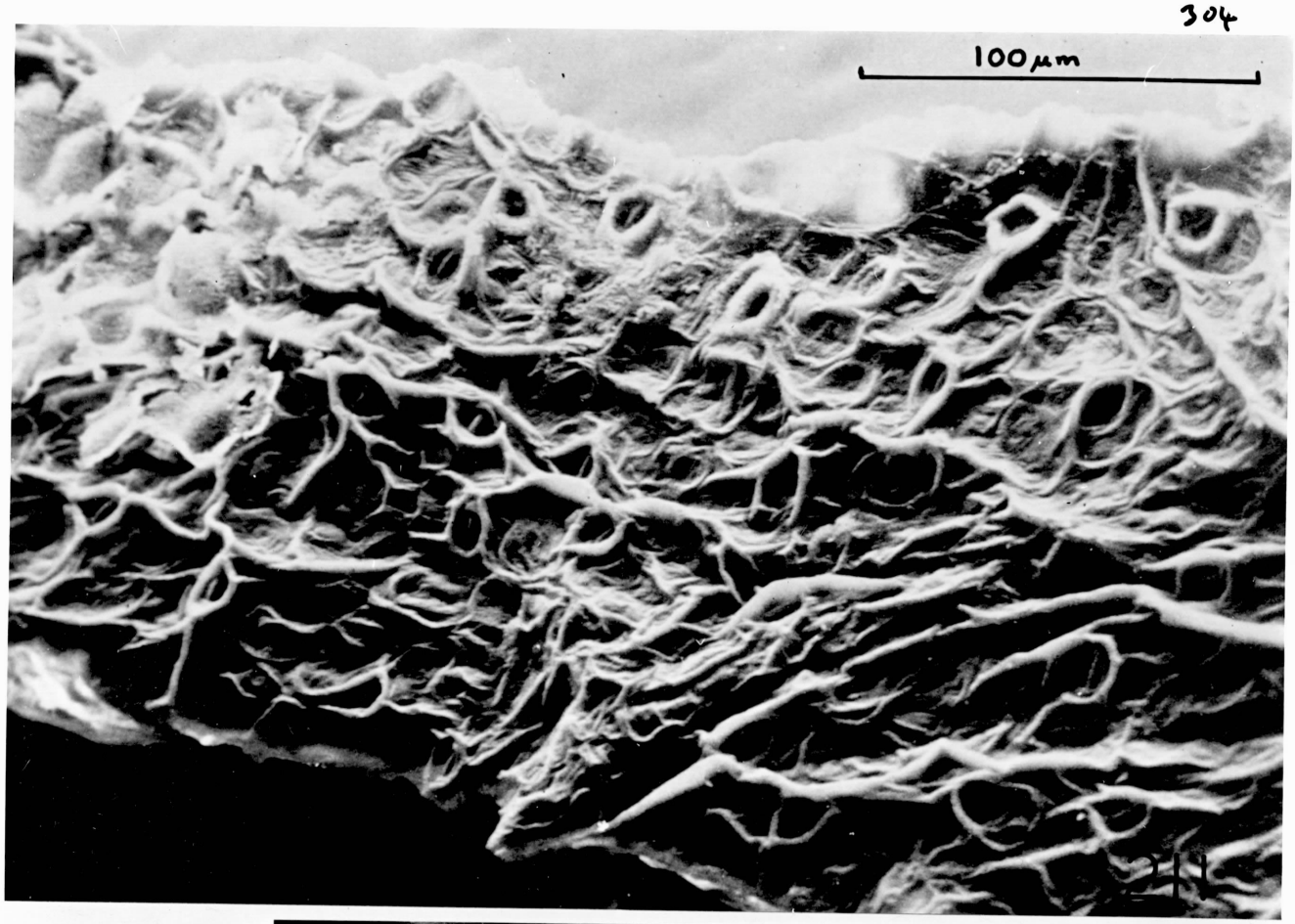
Figure 220 demonstrated the potential of the microscope in the investigation of diseases such as the powdery mildews, which grow on the surface of plants, and following the effect

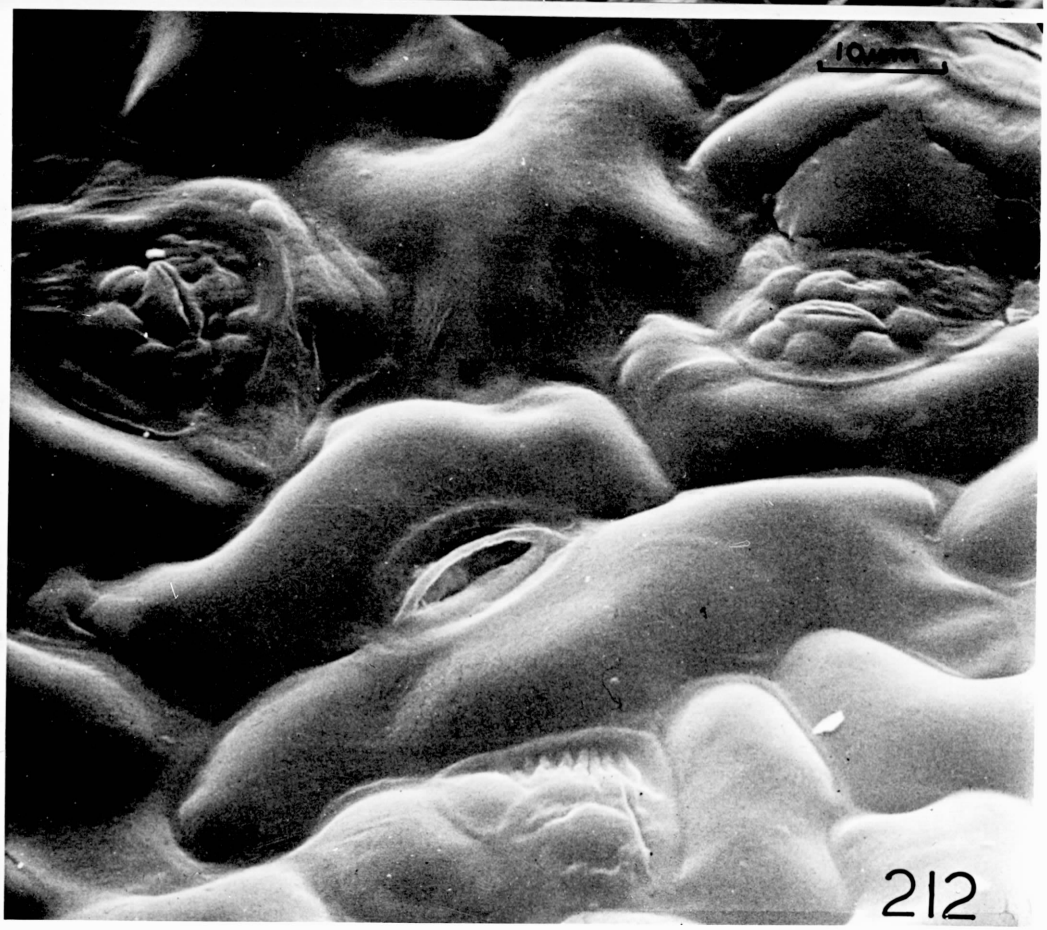
of applications of chemotheraputants on the development of the mycelium and of sporulation.

The scanning reflection electron microscope has been found to serve as a useful bridge between the often inadequate resolution and depth of focus of the light microscope (cf. Fig. 74, 76) and the restricted application of the replica technique in transmission electron microscopy (e.g. Stanbridge & Gay, 1969) in elucidating several features of the structure and development of the rust disease of French beans.

211. A piece of Pisum sativum leaf freeze-dried by method (i) (p. 56). Severe collapse of the epidermal cells has occurred, large amounts of wax crystals are present around the guard cells. 1364/5b. E14.

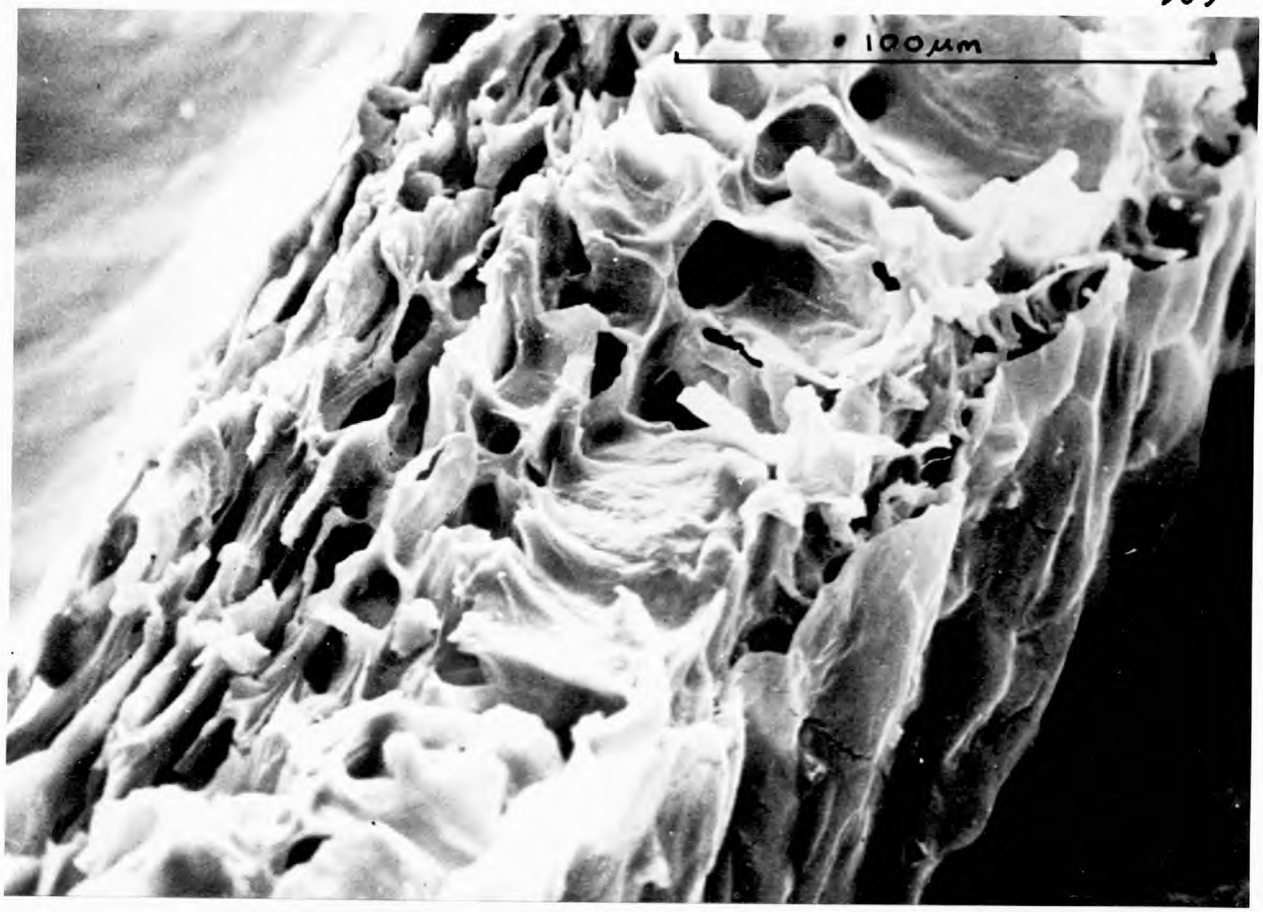
212. A piece of leaf of Phaseolus vulgaris freeze-dried by method (ii) (p. 56). Note that one stoma is open. Probably some collapse of the guard cells of the closed stomata has occurred and has revealed the ring of chloroplasts beneath. 1790/1. B25A.

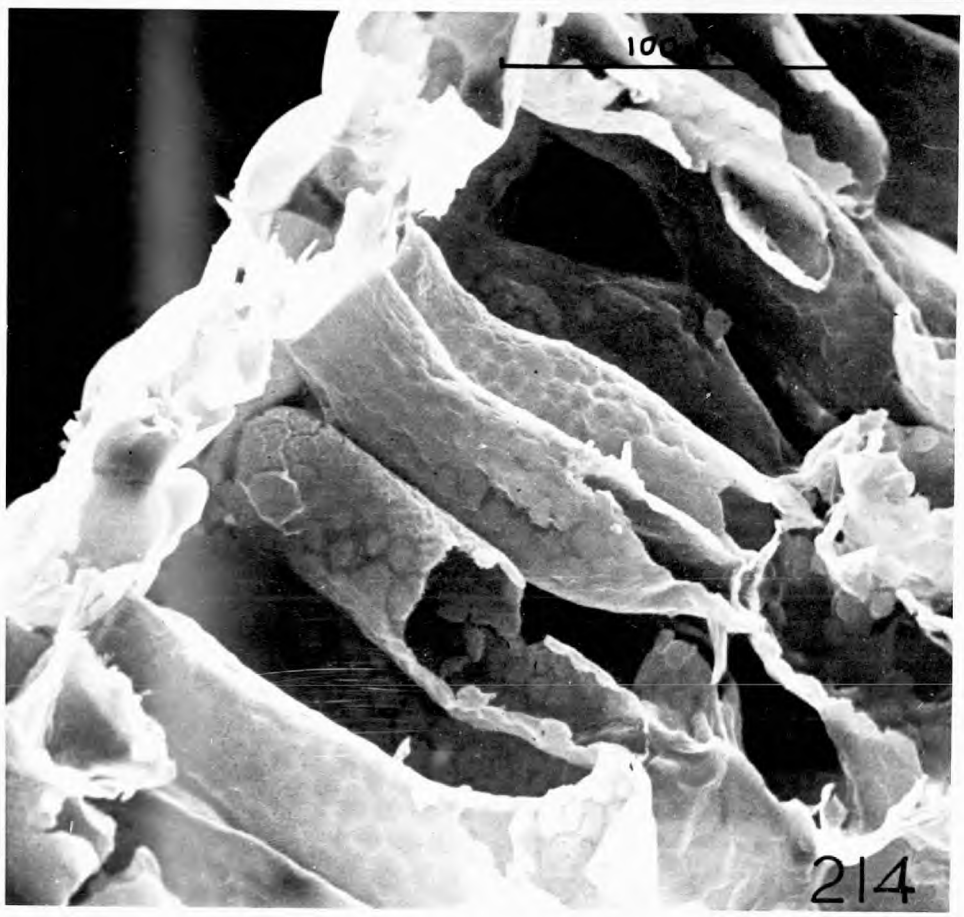

100µm


10µm
212

213. Section of Vicia faba leaf from a 2mm square after freeze-drying by method (i) (p. 56). Note the distortion and collapse of the cells, especially those of the spongy mesophyll. 1364/2b. D16.

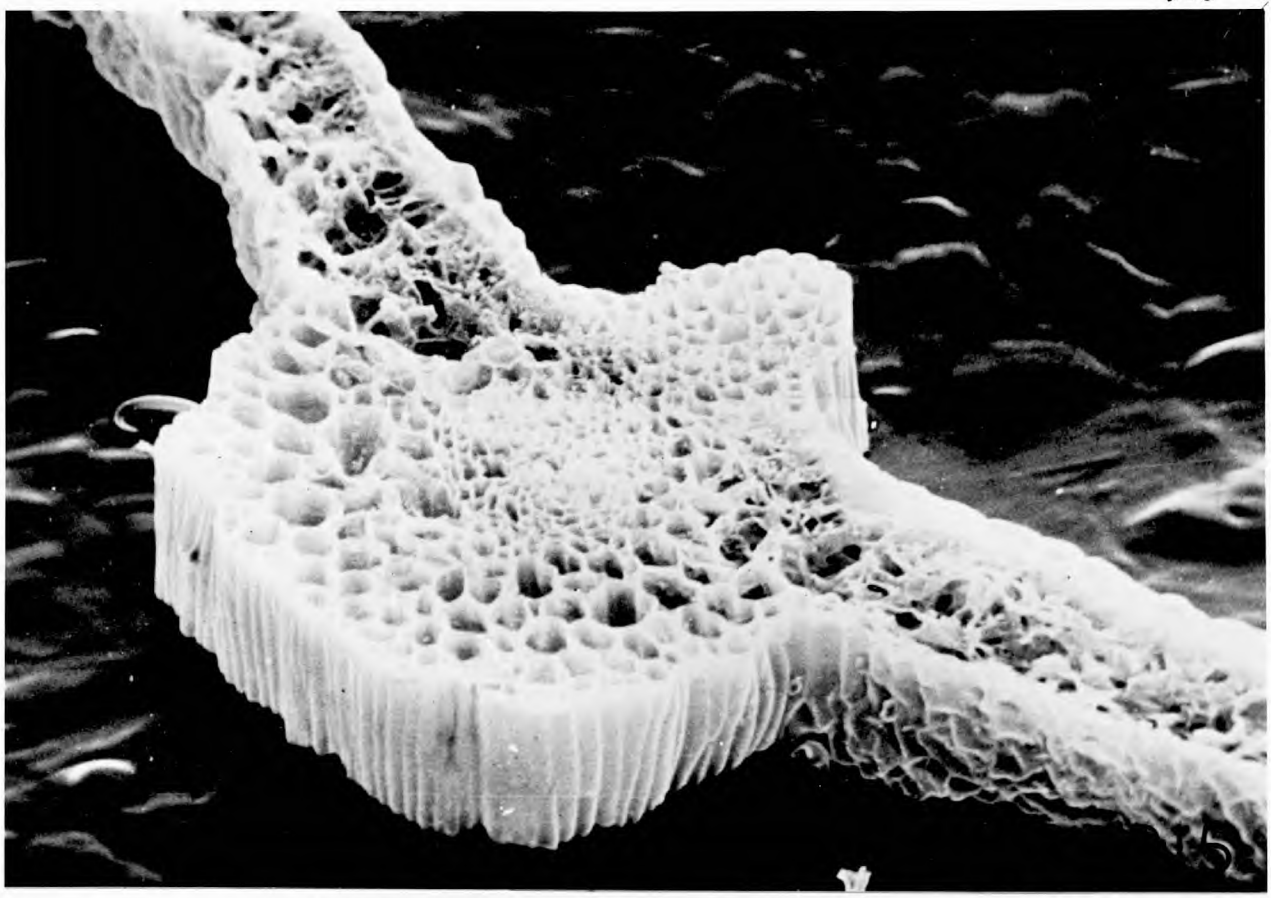
214. Section of V. faba leaf from a 2mm square freeze-dried by method (ii) (p. 56). Note the internal structure of the leaf in the region of the palisade. Cell walls appear curved over the chloroplasts. 1539/2b. A2A.

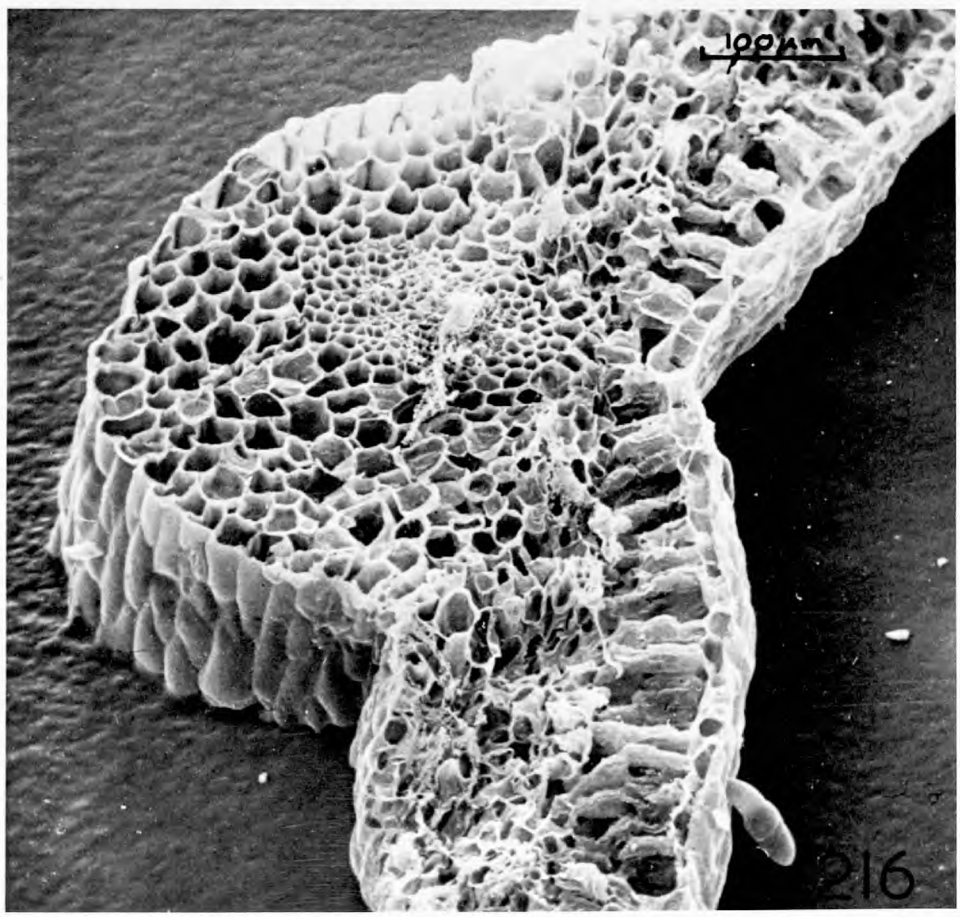

100μm


214

215. Hand section of P. vulgaris leaf freeze-dried by method (ii) (p. 56). Although cells of the mid-rib have remained rigid there is considerable collapse of the mesophyll cells. 1364/2a. A28.

216. Hand section of V. faba freeze-dried by method (i) (p. 56) over mid-rib region. 1539/3a. A27.



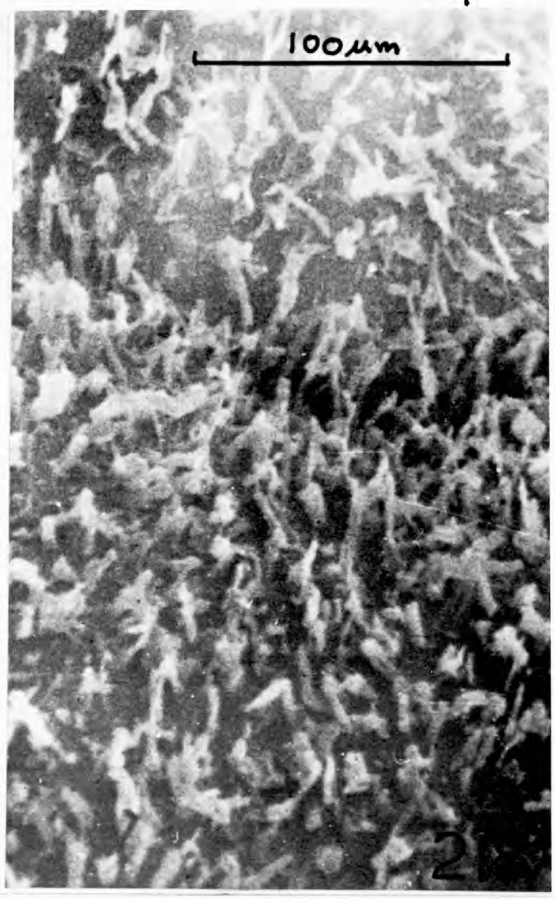

100μm
216

217. Piece of Prunus laurocerasus leaf, freeze-dried by method (ii) (p. 56), in a view into the vascular tissue showing the spiral thickening of the vessels. 1539/1b. B4A.

218. Piece of of leaf of P. sativum, freeze-dried by method (i) (p. 56), showing the wax crystals on the upper epidermis. 1295/5a. D16.

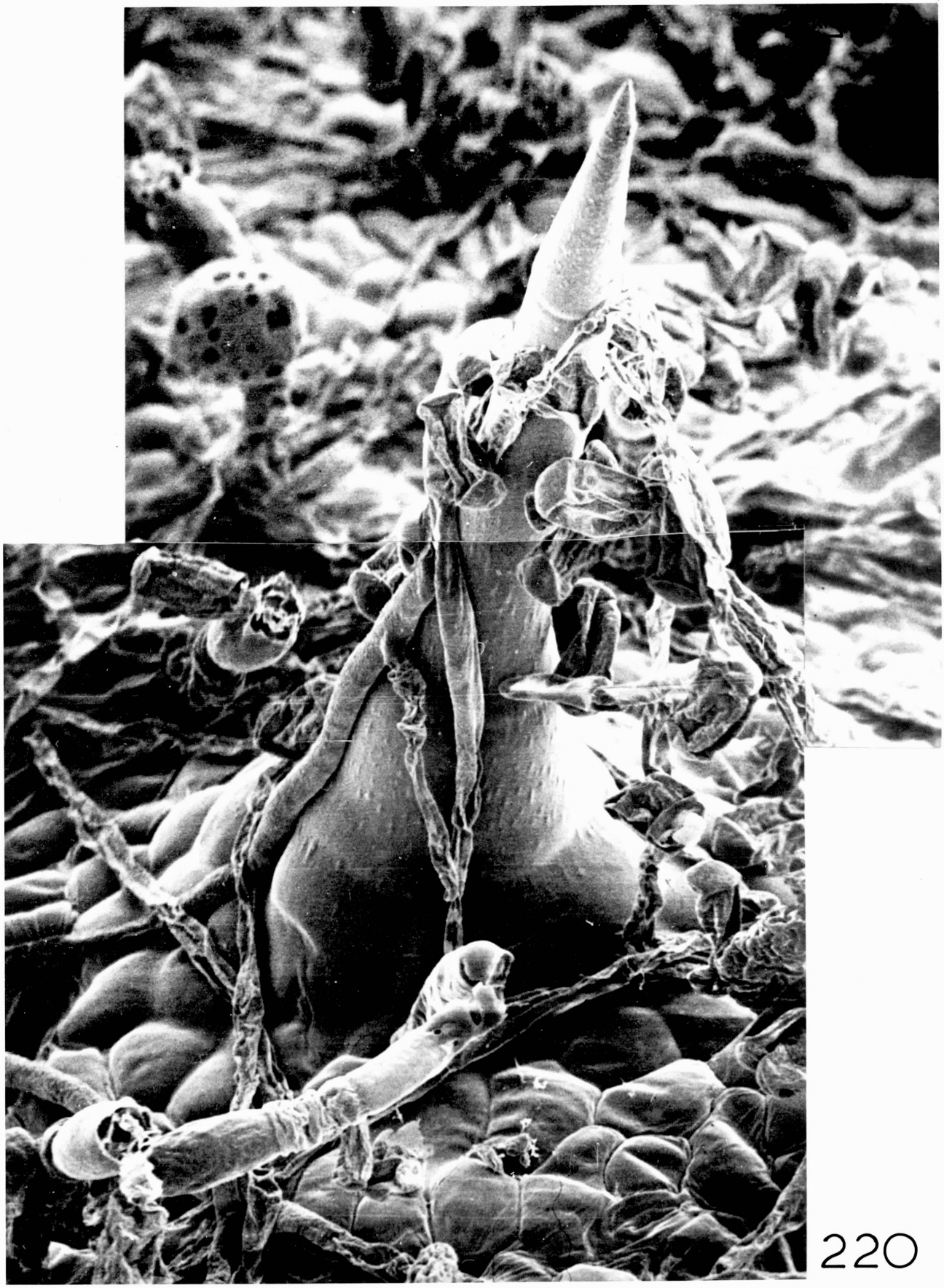
219. Piece of P. laurocerasus, freeze-dried by method (ii) (p. 56), showing stomata on the lower epidermis and long crystals of wax over the leaf surface. 1539/1b. B6A.


217


100μm


100μm
219

220. A photomontage of a hair on the surface of a cucumber leaf, freeze-dried by method (ii) (p. 56), infected by Erysiphe cichoracearum. Collapsed hyphae and conidia surround the hair. 1793. K31, 32.



220

2. RACE IDENTIFICATION

There has been no work carried out in this country on the identification of races of U. appendiculatus. The differential varieties of French beans for distinguishing the English races of rust were thus not in existence.

Differential varieties were obtained from the United States for the purpose of obtaining a resistant variety, as several attempts at screening English cultivars failed to find one that was resistant. The American cultivars were kindly supplied by Dr. W.J. Zaumeyer of the United States Department of Agriculture, Beltsville, Maryland, 20705.

A pure isolate of the fungus was prepared by a modified version of the 'Dry Twist' method devised by Fleishmann et al. (1966).

Uredospores collected from a 'Pinto' plant were spread on a cellophane sheet placed on 4% tap water agar in a Petri dish. Individual uredospores were picked up on a pig's eyelash fixed to a cocktail stick (this was used instead of the needle used by Feishmann et al., 1966, to reduce damage to the leaf suface) under a dissecting microscope at x160 magnification. The pig's eyelash with a single uredospore was streaked across the under surface of a primary leaf of a 'Pinto' bean and the plant incubated as described (p. 44). Plants were isolated to prevent cross contamination.

Uredospores produced from a single sorus were collected, diluted with talc and inoculated onto 'Pinto' beans.

The uredospores thus derived were used to inoculate the American differential varieties Pinto 111, Golden Gate Wax, Seminole, # 3, # 643, # 765, # 780, # 814 and also, as a control, Sutton's Pinto. Three replicates of each variety were used and kept throughout in a greenhouse. Fourteen days after inoculation the diameter of 60 sori per cultivar were measured. All cultivars except # 643 were measured on the upper suface.

The results are shown in the following table:

DIFFERENTIAL VARIETY	DIAMETER OF SORUS IN µm	REACTION GRADE H & Z*	D & V**
Sutton's Pinto	685	10	5
Pinto 111	700	10	5
Golden Gate Wax	415	7	4
Seminole	350	5-6	4
3	715	10	5
643 under surface	215	3	3
765	nf. & 150	1 & 2	2 & 3
780	665	10	5
814	nf. & 100	1 & 2	2 & 3

* Harter & Zaumeyer (1941). ** Davison & Vaughan (1963).
nf. - necrotic fleck with no uredospores produced.

As for Kievitsboon Koekoek in a previous experiment (p. 204) cultivars 765 and 814 gave two different reactions on the same leaf.

The reactions given by the American differential varieties to the rust do not correspond to any of the known races in the U.S.A. (Zaumeyer, 1969: personal communication) and so it may be regarded as a new race.

3. LIST OF MAJOR SUPPLIERS

Chemicals
General: BDH Chemicals Ltd., Poole, Dorset.
General E.M.: TAAB Laboratories, 52 Kidmore End Rd., Emmer Green, Reading.
Resins: G.T. Gurr Ltd., Carlisle Rd., The Hyde, London, N.W.9.
Plantvax: UniRoyal International, 3 Tilney St., London, W.1.

Diamond Knives
Ge-Fe-Ri, Via Maritima, Frosinone, Italy.
Instituto Venezolano De Investigaciones Cientificas, Caracas, Venezuela.
Gelatine Capsules: Park, Davis & Co., Hounslow, Middx.
Grids: Smethurst Highlight, Sidcot Heaton, Bolton, Lancs.
Growth Cabinet: R.E.A. Bott Ltd., 28 Wigmore St., London, W.1.

Microscopes
EM6B: GEC-AEI (Electronics) Ltd., Scientific Apparatus Division, P.O. Box 1, Harlow, Essex.
Olympus: A. Gallenkamp & Co.Ltd., Technico House, Christopher St., London, E.C.2.
Reichert: Shandon Scientific Co., 65 Pound Lane, London, N.W.10.
Scanning E.M.: Cambridge Instrument Co. Ltd., Chesterton Rd., Cambridge.

Microtomes
Reichert: Shandon Scientific Co.
Sorvall: V.A. Howe & Co. Ltd., 46 Pembridge Rd., London W.11.
LKB: LKB Instruments Ltd., LKB House, 232 Addington Rd., Selsdon, South Croydon, Surrey, CR2 8YD.
Pasteur Pipettes: Harshaw Chemicals Ltd., P.O. Box 4, Daventry, Northants.

Photographic
Agfa-Gevaert Ltd., Great West Rd., Brentford, Middx.
Ilford Ltd., 53/54 Berwick St., London, W.1.
Kodak Ltd., Kodak House, Kingsway, London, W.C.2.
Seeds: Sutton & Sons Ltd., Reading, Berks.
A.R. Zwaan & Zn, Voorburg, Netherlands.
Vacuum Coating Unit: Balzers High Vacuum Ltd., Northbridge Rd., Berkhamstead, Herts.
Vellin Tissue: General Paper Box Manufacturing Co., Treforest Trading Estate, Pontypridd, Glam.
Vermiculite: Pan Britanica Industries Ltd., Waltham Abbey, Essex.

4. BUFFER

The following sodium phosphate buffer was used routinely. The stock solution was made up at twice the final molarity for diluting 1:1 with glutaraldehyde, sucrose or sucrose and osmium tetroxide.

Na_2HPO_4 2.84g / 100ml water = 0.2M

NaH_2PO_4 $2H_2O$ 3.12g / 100ml water = 0.2M

pH	ml Na_2HPO_4	ml NaH_2PO_4
6.5	16.0	34.0
7.0	30.5	19.5
7.5	42.0	8.0

Based on the buffer devised by Milonig (1961, 1962, 1964)

5. EPON MIXTURES

Based on the formula devised by Luft (1961). The following mixtures were found to give good results.

Hard Mix: B

Epikote 812	-	120.70g		-	100.00ml
BDMA	-	4.00ml	200ml or	-	3.78ml
MNA	-	108.05g		-	89.00ml

Medium Mix: 1A:1B

Epikote 812	-	115.00g		-	91ml	
BDMA	-	2.75g		-	3ml	
MNA	-	57.00g	200ml or	-	47ml	always add
DDSA	-	61.80g		-	62ml	DDSA last.

Soft Mixes: (a) 3A:1B

Epikote 812	-	103.5g	
BDMA	-	4.0ml	
MNA	-	28.5g	200ml
DDSA	-	92.3g	

(b) 4A:1B

Epikote 812	-	101.2g	
BDMA	-	4.0ml	200ml
MNA	-	22.8g	
DDSA	-	98.8g	

BDMA - benzyldimethylamine, DDSA - dodecenyl succinic anhydride, MNA - methyl nadic anhydride.

The mixtures were stored in 10ml aliquots in 15ml polythene-capped glass vials at -20°C.

6. REYNOLDS' LEAD CITRATE STAIN (1963)

'Analar' grade chemicals are used throughout. The lead salts should be from fairly fresh stocks, otherwise the solution will be turbid and it will stain poorly.

(i) Add 1.33g $Pb(NO_3)_2$ and 1.76g $Na_3(C_6H_5O_7).2H_2O$ to 30ml water in a 50ml volumetric flask and shake vigorously for 1min and allow to stand for 30min, with intermittent shaking during this time.

(ii) 8.0ml N NaOH (4%) is added and the suspension diluted to 50ml with water. After mixing by inversion and on the complete solution of the precipitate the stain is ready for use.

The pH of the stain should be 12.0 ± 0.1. Any turbidity can be removed by centrifugation and the solution can be stored in glass or polythene bottles. The stain is stable for about six months.

N.B. It is advisable to use freshly boiled distilled water, to remove the carbon dioxide, for making up the stain.

7. SCHEDULE FOR CLEANING GRIDS

A perforated porcelain thimble is used for all operations in a beaker containing the reagents and vibrated in an ultrasonicator.

1. Vibrate in chloroform.
2. Rinse in acetone.
3. Vibrate in 15% 'Quadralene' in water.
4. Rinse in tap water.
5. Vibrate in distilled water.
6. Rinse in distilled ethanol.
7. Vibrate in distilled ethanol.
8. Rinse in distilled ethanol.
9. Leave covered to dry.

8. FILMING GRIDS

Use 0.6% formvar in chloroform.

1. Clean 3in x 1in microscope slides. The method of cleaning may be varied until the best results are obtained. If the slides are too clean some difficulty may be encountered in removing the film, or if they are too dirty poor films will result. Successful methods have ranged from merely polishing the slides with a vellin tissue to cleaning thoroughly with a cleansing powder (e.g. 'Vim'), washing under the tap, rinsing and then storing in distilled water. The slides were finally dried for use by blotting off excess water between sheets of filter paper (Whatman No. 50) and wiping with a vellin tissue.

2. Cleaned slides are held in tweezers and dipped into formvar solution, withdrawn slowly and evenly and allowed to dry before scoring an area about 2in x $\frac{3}{4}$in around one face of the slide. The scored surface is moistened by breathing heavily onto it and the slide is then gently lowered at an angle (about 45°) into a bowl of water. The surface of the water having been swept with a glass rod, coated with silicone fluid, to remove any surface film. The formvar film should float off from the slide.

3. Grids are placed onto the surface of the film, dull side down, and the whole film with grids picked up on nylon gauze.

4. Filmed grids are dried and stored in a desiccator.

9. PHOTOGRAPHIC MATERIALS

Plates

Light microscopy - Ilford R40

Electron microscopy - Ilford N50

Special purpose - Ilford N30, Kodak Scientific

Film

Ilford HP3, FP4, Pan F

Paper

Agfa Rapidoprint, used with an Agfa processor

Agfa Brovira range of contrast grades

Developers

Plates - N50, R40 - Kodak D19b followed by a Patterson-Johnson 'Acustop' stop bath

N30, R40 - Ilford ID 11

Film - Ilford ID 11

Paper - Ilford Bromophen

Fixer

Plate, film and paper - Ilford Ilfofix

10. LONG ASHTON NUTRIENT SOLUTION

Modified from Hewitt (1952).

Final pH 5-6

KNO_3	- 50.5000	g per 100l diluted solution	
$Ca(NO_3)_2$ (hydrated)	- 110.0000	" " " "	"
$NaH_2PO_4.2H_2O$	- 20.8000	" " " "	"
$MgSO_4.7H_2O$	- 36.9000	" " " "	"
$MnSO_4.4H_2O$	- 0.2230	" " " "	"
$CuSO_4.5H_2O$	- 0.0240	" " " "	"
$ZnSO_4.7H_2O$	- 0.0290	" " " "	"
H_3BO_3	- 0.1860	" " " "	"
$(NH_4)_6Mo_7O_{24}.4H_2O$	- 0.0035	" " " "	"
Fe-EDTA	- 0.1500	milli equivalents per l	

The aluminium, gallium, cobalt and nickel salts used by Hewitt (1952) were omitted and ferric citrate was replaced by iron-ethylene-diaminotetra-acetic acid (Fe-EDTA) to give 0.15milli equivalents of iron per litre of diluted nutrient solution.

Salts of K, Ca, Na, Mg and B were made up separately to 200ml with water. Salts of Mn, Cu, Zn and Mo were combined and made up to 200ml with water. 5ml of each of these stock solutions were taken and added to 2.455l water in a Winchester to which was also added 15ml of the stock Fe-EDTA, to give

the final diluted nutrient solution.

The Fe-EDTA was prepared by making up solutions of:

$FeCl_3.6H_2O$	1.35g	made up to 300ml with water
Na_2EDTA	3.70g	" " " " " "

The two solutions were slowly mixed together and the pH adjusted to 5.5 with 1N NaOH. This stock solution was stored in a refridgerator.

REFERENCES

AKAI, S. (1959). In Plant Pathology I, 391 (Eds., HORSFALL, J.G. & DIMOND, A.E.), Academic Press, New York.

ALLEN, R.F. (1923a). J. agric. Res. 23, 131.

ALLEN, R.F. (1923b). J. agric. Res. 26, 571.

ALLEN, R.F. (1926a). J. agric. Res. 32, 701.

ALLEN, R.F. (1926b). J. agric. Res. 33, 201.

ALLEN, R.F. (1967). J. agric. Res. 34, 697.

ALLEN, R.F. (1928). J. agric. Res. 36, 487.

ARONSON, J.M. (1965). In The Fungi I, 49 (Eds., AINSWORTH, G.C. & SUSSMAN, A.S.), Academic Press, New York.

de BARY, A. (1863). Annls. Sci. nat., ser. 4, 20, 5.

de BARY, A. (1870). Beitrage zur Morphologie und Physiologie der Pilze, Dritte Rheihe.

BECKING, J.H., DE BOER, W.E. & HOUWINK, A.L. (1964). Antonie van Leeuwenhoek 30, 343.

BERLIN, J.D. & BOWEN, C.C. (1964). Am. J. Bot. 51, 445.

BLACKWELL, E.M. (1953). Trans.Br. mycol. Soc. 36, 138.

BOYER, M.G. & ISAAC, P.K. (1964). Can. J. Bot. 42, 1305.

BRACKER, C.E. (1964). Phytopathology 54, 889 (Abst).

BRACKER, C.E. (1967). Ann. Rev. Phytopathology 5, 343.

BRACKER, C.E. (1968). Phytopathology 58, 12.

BRANTON, D. (1966). Proc. Nat. Acad. Sci. 55, 1048.

BUTLER, E.J. (1907). Mem. Dep. Agric. India bot ser. 2, 1.

CARO, L.G. & PALADE, G.E. (1964). J. Cell Biol. 20, 473.

CLOWES, F.A.L. & JUNIPER, B.E. (1968). Plant Cells, Blackwells, Oxford.

COFFEY, M.D., BOSE, A. & SHAW, M. (1969). Can. J. Bot. 47, 1291.

COLLEY, R.H. (1918). J. agric. Res. 15, 619.

CORNER, E.J.H. (1935). New Phytol. 34, 180.

CROSS, C.F., BEVAN, E.J. & BEADLE, C. (1918). Cellulose, Longmans, London.

CURRIER, H.B. (1957). Am. J. Bot. 44, 478.

CURRIER, H.B. & SHIH, C.Y. (1968). Am. J. Bot. 55, 145.

CURRIER, H.B. & STRUGGER, S. (1956). Protoplasma 45, 552.

CUTTER, V.M. (1959). Mycologia 51, 248.

CUTTER, V.M. (1960). Mycologia 52, 726.

DALTON, A.J. (1961). In The Cell II, 603 (Eds., BRACHET, J. & MIRSKY, A.E.), Academic Press, New York.

DAVISON, A.D. & VAUGHAN, E.K. (1963). Phytopathology 53, 456.

DAVISON, E.M. (1968). Ann. Bot., Lond., (N.S.) 32, 613.

DEKHUIJZEN, H.M. & SCHEER, C. VAN DER (1967). Neth. J. Pl. Path. 73, 121.

DEKHUIJZEN, H.M. & SCHEER, C. VAN DER (1969). Neth. J. Pl. Path. 75, 169.

DEVERALL, B.J. (1967). Ann. appl. Biol. 59, 375.

DICKENSON, S. (1949a). Ann. Bot., Lond., (N.S.) 13, 89.

DICKENSON, S. (1949b). Ann. Bot., Lond., (N.S.) 13, 219.

DICKENSON, S. (1949c). Ann. Bot., Lond., (N.S.) 13, 337.

DICKENSON, S. (1949d). Ann. Bot., Lond., (N.S.) 13, 345.

DICKENSON, S. (1955). Ann. Bot., Lond., (N.S.) 19, 161.

DUFF, G.H. (1918). Phytopathology 8, 289.

ECHLIN, P. (1968). Jl R. microsc. Soc. 88, 407.

ECHLIN, P., GODWIN, H., CHAPMAN, B. & ANGOLD, R. (1966). Proc. 6th Int. Congr. Electron Microsc. Kyoto 2, 317.

EHRLICH, H.G. & EHRLICH, M.A. (1962). Am. J. Bot. 49, 665 (Abst.).

EHRLICH, H.G. & EHRLICH, M.A. (1963a). Am. J. Bot. 50, 123.

EHRLICH, H.G. & EHRLICH, M.A. (1963b). Phytopathology 53, 1378.

EHRLICH, M.A. & EHRLICH, H.G. (1966). Can. J. Bot. 44, 1494.

EHRLICH, M.A., EHRLICH, H.G. & SCHAFER, J.F. (1968a). Am. J. Bot. 55, 1020.

EHRLICH, M.A., SCHAFER, J.F. & EHRLICH, H.G. (1966). Phytopathology 56, 878 (Abst.).

EHRLICH, M.A., SCHAFER, J.F. & EHRLICH, H.G. (1968b). Can. J. Bot. 46, 17.

ESAU, K., CHEADLE, V.I. & GILL, R.H. (1966). Am. J. Bot. 53, 765.

ESCHRICH, W. (1961). Z. Bot. 49, 153.

ESCHRICH, W. & CURRIER, H.B. (1964). Stain Techn. 39, 303.

FEDER, N. & O'BRIEN, T.P. (1968). Am. J. Bot. 55, 123.

FEDER, N. & WOLF, M.K. (1965). J. Cell Biol. 27, 327.

FELLOWS, H. (1928). J. agric. Res. 37, 647.

FRAYMOUTH, J. (1956). Trans. Br. mycol. Soc. 39, 79.

FREDERICK, S.E., NEWCOMB, E.H., VIRGIL, E.L. & WERGIN, W.P. (1968). Planta 81, 229.

FLEISHMANN, G., KHAIR, J. & DINOOR, A. (1966). Can. J. Bot. 44, 1009.

FREY-WYSSLING, A. & MÜHLETHALER, J. (1965). Ultrastructural Plant Cytology, Elsevier, London.

FROMME, F.D. (1915). Am. J. Bot. 2, 82.

FULLER, M.S. & BARSHAD, I. (1960). Am. J. Bot. 47, 105.

GAY, J.L. & GREENWOOD, A.D. (1966). Colston Pap. 18, 95.

GIBSON, C.M. (1904). New Phytol. 3, 184.

GREENWOOD, A.D., LEECH, R.M. & WILLIAMS, J.P. (1963). Biochim. Biophys. Acta 78, 148.

GUYOT, A.L. (1957). Encycl. mycol. 29, 481.

HARDING, H., WILLIAMS, P.H. & McNABOLA, S.S. (1968). Can. J. Bot. 46, 1229.

HARRIS, R.H. (1964). Ann. Mag. nat. Hist: ser. 13, 7, 65.

HARRIS, R.H. (1968). Med. biol. Ill. 18, 180.

HART, H. (1926). Phytopathology 16, 185.

HARTER, L.L., ANDRUS, C.F. & ZAUMEYER, W.J. (1935). J. agric. Res. 50, 737.

HARTER, L.L. & ZAUMEYER, W.J. (1941). J. agric. Res. 62, 717.

HEATH, I.B. (1969). Ph.D. Thesis, University of London.

HESLOP-HARRISON, J. (1966). Endeavour 25, 65.

HEWITT, E.J. (1952). Tech. Comm. Commonw. Bur. Hort. Plantn. Crops 22.

HILU, H.M. (1965). Phytopathology 55, 563

HIRATA, K. & KOJIMA, M. (1962). Trans. Mycol. Soc. Japan 3, 43.

HOHL, H.R. (1960). Phytopath. Z. 40, 317.

HUNT, P. (1968). Trans. Br. mycol. Soc. 51 103.

HUTCHINS, H.L. & LUTMAN, B.F. (1938). Phytopathology 28, 859.

ITO, K. (1949). Bull. Govt. Forest Exp. Stn Meguro 43, 1.

KISLEV, N., SWIFT, H. & BOGORAD, L. (1965). J. Cell Biol. 25, 327.

LAMPORT, D.T.A. (1965). In Advances in Botanical Research 2, 151 (Ed. PRESTON, R.D.), Academic Press, London.

LAUNDON, G.F. & WATERSON, J.M. (1965). C.M.I. Descriptions of Pathogenic Fungi and Bacteria, No. 57.

LEDBETTER, M.C. & PORTER, K.R. (1963). J. Cell Biol. 19, 239.

LUFT, J.H. (1961). J. biophys. biochem. Cytol. 9, 409.

MADELIN, M.F. (1968). In The Fungi III, 253 (Eds. AINSWORTH, G.C. & SUSSMAN, A.S.), Academic Press, New York.

MAHESHWARI, R., ALLEN. P.J. & HILDEBRANDT, A.C. (1967a). Phytopathology 47, 855.

MAHESHWARI, R., HILDEBRANDT, A.C. & ALLEN, P.J. (1967b). Can. J. Bot. 45, 447.

MAINS, E.B. (1917). Am. J. Bot. 4, 179.

MANGIN, L. (1892). Bull. Soc. bot. Fr. 39, 260.

MANGIN, L. (1895). Bull. Soc. Hist. nat. Autun 8, 55.

MANOCHA, M.S. & SHAW, M. (1966). Can. J. Bot. 44, 669.

MANOCHA, M.S. & SHAW, M. (1967). Can. J. Bot. 45, 1575.

MARCHANT, R. & ROBARDS, A.W. (1968). Ann. Bot., Lond., (N.S.) 32, 457.

MARRYAT, D.C.E. (1907). J. agric. Sci., Camb. 2, 129.

MARTIN, J.T. (1964). Ann. Rev. Phytopathology 2, 81.

McKEEN, W.E., SMITH, R. & BHATTACHARYA, P.K. (1969). Can. J. Bot. 47, 701.

McKEEN, W.E., SMITH, R. & MITCHELL, N. (1966). Can. J. Bot. 44, 1299.

MILONIG, G. (1961). J. appl. Phys. 32, 1637.

MILONIG, G. (1962). Proc. 5th Int. Conf. Electron Microsc. Philadelphia 2, 8.

MILONIG, G. (1964). Symposium of Electron Microscopy, Modena, April, 1963, 347, Roma, Consiglio Nazioale della Ricerche.

MONNERON, A. (1966). Proc. 6th Int. Congr. Electron Microsc. Kyoto 2, 27.

MOOR, H. (1965) Elektronenmkroconishe Praparationstechnik Gefrierätzung, Messrs. Baltzers, A.G., Liechtenstein.

MOOR, H. & MÜLHETHALER, J. (1963). J. Cell Biol. 17, 609.

MOORE, R.T. (1963a). Mycologia 55, 663.

MOORE, R.T. (1963b). J. Bacteriol. 86, 866.

MOORE, R.T. (1965). In The Fungi I, 95 (Eds. AINSWORTH, G.C. & SUSSMAN, A.S.), Academic Press, New York.

MOORE, R.T. & McALEAR, J.H. (1961a) Mycologia 53, 194.

MOORE, R.T. & McALEAR, J.H. (1961b) Phytopath. Z. 42, 197.

MOORE, R.T. & McALEAR, J.H. (1962) Am. J. Bot. 49, 86.

MOSS, E.H. (1926). Ann. Bot., Lond. 40, 813.

NICKERSON, W.J.(1963). Bacteriol. Rev. 27, 305.

NORTHCOTE, D.H. (1969). Proc. Roy. Soc. B 173, 21.

O'BRIEN, T.P., FEDER, N. & McCULLEY, M.E. (1964). Protoplasma 59, 368.

O'BRIEN, T.P. & McCULLEY, M.E. (1969). Plant Structure and Development, Collier- McMillan, Toronto.

PEACHEY, L.D. (1958). J. biophys. biochem. Cytol. 4, 233.

PEASE, D.C. (1964). Histological Techniques for Electron Microscopy, Academic Press, New York.

PERSON, C. (1959). Can. J. Bot. 37, 1101.

PERSOON, C.H. (1796). Observationes Mycologicae I, 16.

PEYTON, G.A. & BOWEN, C.C. (1963). Am J. Bot. 50, 787.

PICKETT-HEAPS,J.D. & NORTHCOTE, D.H. (1966). J. Expt. Bot. 17, 20.

REICHLE, R.E. & ALEXANDER, J.V. (1965). J. Cell Biol. 24, 489.

REYNOLDS, E.S. (1963). J. Cell Biol. 17, 208.

REVE, J.P., NAPOLITANO, L. & FAWCETT, D.W. (1960). J. biophys. biochem. Cytol. 8, 575.

RICE, M.A. (1927). Bull.Torrey Botan. Club 54, 63.

RICE, M.A. (1934). Bull.Torrey Botan. Club 61, 155.

ROBERTSON, J.D. (1964). In Cellular Membranes in Development (Ed. LOCK, M.I.), Academic Press, New York.

ROBERTSON, N.F. (1965). In The Fungi I, 613 (Eds., AINSWORTH, G.C. & SUSSMAN, A.S.), Academic Press, Newy York.

ROELOFSEN, P.A. (1959). The Plant Cell Wall, Gebrüder Borntraeger, Berlin-Nikolassee.

RUBIO-HUERTOS, M. & CALONGE, F. DE DIEGO (1965). Microbiol. Espan. 18, 127.

SABATINI, D.D., BENSCH, K. & BARRNETT, R.J. (1963). J. Cell Biol. 17, 19.

SAPPIN-TROUFFY, P. (1896). Le Botaniste 5, 44.

SAVILE, D.B.O. (1954). Mycologia 46, 736.

SCHMELING, B. VON & KULKA, M. (1966). Science 152, 659.

SHAW, M. (1963). Ann. Rev. Phytopathology 1, 259.

SHAW, M. (1964). Phytopath. Z. 50, 159.

SHAW, M. & MANOCHA, M.S. (1965a). Can. J. Bot. 43, 747.

SHAW, M. & MANOCHA, M.S. (1965b). Can. J. Bot. 43, 1285.

SHU, P., TANNER, K.G. & LEDINGHAM, G.A. (1954). Can. J. Bot. 32, 16.

SIDMAN, R.L., MOTTLA, P.A. & FEDER, N. (1961). Stain Technol. 36, 279.

SIEGEL, S.M. (1962). The Plant Cell Wall, Pergamon Press, London.

SMITH, D.C. & DREW, E.A. (1965). New Phytol. 64, 195.

SMITH, G. (1900). Bot. Gaz. 29, 153.

SMITH, O.F. (1938). J. agric. Res. 57, 671.

STAKMAN, E.C. (1915). J. agric. Res. 4, 193.

STANBRIDGE, B. & GAY, J.L. (1969). Trans. Br. mycol. Soc. 53, 149.

TEMMINK, J.H.M. & CAMPBELL, R.N. (1969). Can. J. Bot. 47, 421.

THATCHER, F.S. (1939). Am. J. Bot. 26, 449.

THATCHER, F.S. (1943). Can. J. Res. C 21, 151.

THOMAS, P.C. & ISAAC, P.K. (1967a). Can. J. Bot. 45, 287.

THOMAS, P.C. & ISAAC, P.K. (1967b). Can. J. Bot. 45, 1473.

TILNEY, L.G. & PORTER, K.R. (1967). J. Cell Biol. 34, 327.

TUPY, J. (1959). Biologia Pl. 1, 192.

TUREL, F.L.M. (1969). Can. J. Bot. 47, 821.

UNGER, F.J.A.N. (1936). Einfl. d. Bodens. 216.

WALKER, W.S. & BISALPUTRA, T. (1967). Can. J. Bot. 45, 2103.

WANG, D. (1961). Can. J. Bot. 39, 1595.

WARD, H.M. (1902). Ann. Bot., Lond. 16, 233.

WELLS, K. (1964). Mycologia 56, 327.

WHALEY, W.G., MOLLENHAUER, H,H. & LEECH, J.H. (1960). Am. J. Bot. 47, 401.

WHITNEY, H.S., SHAW, M. & NAYLOR, J.M. (1962). Can. J. Bot. 40, 1533.

WILLIAMS, P.G. & LEDINGHAM, G.A. (1964). Can. J. Bot. 42, 1503.

WILLIAMS, P.G., SCOTT, K.J. & KUHL, J.L. (1966). Phytopathology 56, 1418.

WILLIAMS, P.G., SCOTT, K.J., KUHL,J.L. & MACLEAN, D.J. (1967). Phytopathology 57, 324.

WILLIAMS, P.H. & McNABOLA, S.S. (1967). Can. J. Bot. 45, 1665.

WILLIAMS, P.H. & YAKAWA, Y.B. (1967). Phytopathology 57, 682.

WILSON, M. & HENDERSON, D.M. (1966). British Rust Fungi, Cambridge University Press.

WINGARD, S.A. (1935). Phytopathology 25, 39.

WOOD, R.K.S. (1967). Physiological Plant Pathology, Blackwells, Oxford.

YARWOOD, C.E. (1961). Phytopathology 51, 22.

YOUNG, P.A. (1926). Am. J. Bot. 13, 502.

ZALOKA, M. (1965). In The Fungi I, 377 (Eds., AINSWORTH, G.C. & SUSSMAN, A.S.), Academic Press, New York.

ZAUMEYER, W.J. & THOMAS, H.R. (1957). U.S. Dept. Agriculture Tech. Bull. No. 868.

ZIMMER, D.E. (1965). Phytopathology 55, 296.