

exciter filters used in this study ranged from 390-420, while in Kawai and Muller's studies the range extended from 400-440nm. The light emitted from the former filter was violet and the latter blue-violet. This discrepancy may have accounted for the lack of observation of autofluorescence in the Feldmannia sp. motiles. Use of a wide band interference filter emitting blue-violet light in the range of 390-440nm with a dichroic mirror FT460 and a barrier filter LP470 would establish more conclusively whether or not autofluorescence of zoospore flagella occurred in Feldmannia sp..

5.9. Zoospore fine structure

The structure of the zoospores of Feldmannia sp. studied in this dissertation do not deviate much from the ultrastructure of other zoids within the Ectocarpales. This supports the idea that the division is monophyletic (Clayton 1984). In Feldmannia sp. two chloroplasts occurred in each motile cell. One basally situated chloroplast was evident in a number of zoids (Baker & Evans 1973, Manton 1957), occasionally their tendency to produce lobes made it possible to view them in more than one profile.

Observations of settling motiles revealed that they are firmly attached to the substrate, most probably by an adhesive. Scanning electron micrographs of settled motiles revealed that the nature of this adhesive may be mucilaginous. The motiles were initially attached to the substrate by the anterior end, while more of the motile surface became attached with increasing time. The production of this mucilage is likely to occur in the dictyosome since vesicles concentrated in the anterior end of sectioned motiles appeared to be in close proximity to the dictyosome. Evidently there is some adhesive responsible for attachment of the germlings for a number of days, until the formation of rhizoids takes place. It is uncertain as to whether this adhesive is still produced after the cell wall is formed and further cell divisions take place. In other members of the Ectocarpales, when the rhizoids of the developing germlings make contact with the substrate, the rhizoids enlarge slightly, and secrete a yellow substance around the cell wall (Fletcher 1977). Slight enlargement of the tip of the rhizoid of Feldmannia sp. was

noted, however no yellow substance was associated with the rhizoids.

Attempts to embed Feldmannia sp. zoospores for sectioning revealed that they rapidly formed cell walls after release. If more than half an hour passed from the time of release to fixation, cell walls were already evident in sectioned material. Light microscope observations of the time taken from release to settlement of zoospores ranged from thirty minutes to two hours. Once settlement had taken place, cell wall formation did not necessarily commence immediately. In Ectocarpus (Baker & Evans 1973) cell walls were evident around zoospores one to two hours after settlement. It seems strange then, that in a number of embedding runs, only motiles with cell walls were obtained. Perhaps, these settled motiles were slightly heavier than the motiles without cell walls, and so less easily lost during the fixation procedure. If motiles were fixed before half an hour after their release, cell walls were present.

While zoospores of Feldmannia sp. did not show any substrate selection in their settling pattern, other brown algal spores appear to be sensitive to surface topography, particularly the ship-fouling species (Baker & Evans 1973). Zoospores were reported to remain motile for up to 24 hours (Wynne 1969 in Fletcher 1987, and Baker & Evans 1973) and could detect, and respond to, external stimuli such as light. Feldmannia sp. zoospores are positively phototactic and have not been seen to remain motile for longer than 2 hours. However, this may be the result of unnatural culture conditions.

5.10. The influence of temperature, photoperiod, photon fluence and light quality on the growth and development of Feldmannia sp.

The phenology of a species in the field may not be the same in all respects as that found in culture studies (Henry 1988). Environmental conditions in the laboratory include: constant temperatures, constant irradiance, often stagnant nutrient enriched medium and a lack of associated biota, while in the field varying temperatures, varying irradiance, well-mixed and low nutrient medium, associated biota of competitors and possible producers of growth affecting compounds may occur. Different phenological responses may also be the result of genetic variability in the species, particularly at the extremes of its geographical range. Despite all the differences between these two environments, responses found in culture studies must reflect to a large degree, the growth and reproductive capacities of a species in the field.

Feldmannia sp. grew successfully in a wide range of environmental conditions and this may reflect something of the extent of its natural geographic distribution. It grew most favourably in temperatures and photoperiods common to tropical and subtropical waters.

One of the chief objectives of exposing Feldmannia sp. to various environmental conditions, was to stimulate a change in the phase of the life cycle. It was anticipated that this change would take the form of production of unilocular sporangia. No such change took place and no unilocular reproductive structures were induced by any of the treatments. The significance of this is discussed in Section 5.1.

The experiments performed do however provide information on which treatments stimulate growth and reproduction and help to pinpoint at what stage of growth reproduction occurs, if at all. A count of the total number of sporangia on the last day of experimentation, provides an indication of the extent of the reproductive capacity of filaments grown at a particular treatment. The number of empty

sporangia may act as a more sensitive indication of the first formed sporangia.

Only general trends emerging from the experimental data can be used and there are a number of reasons for this:

- (i) There is variation between treatments, in the age of filaments and the number of cells at which reproduction occurs. While a certain amount of variation between treatments can be expected, in some instances the discrepancies between treatments was large, notably in the temperature and photoperiod experiments.
- (ii) It would be expected that growth of the same plant under the same experimental conditions, at short time intervals, would yield similar growth patterns. However, the pattern of growth and the slope of the graph varies markedly at different times on repeating experiments (Figures.15a - 17c), with the exception of the photoperiod experiment (Figures.14a & 14b).
- (iii) Unlike cell numbers, which are relatively uniform throughout a treatment, the numbers of sporangia varied markedly between filaments within a treatment. On a number of occasions, the filaments selected for recording had fewer sporangia than the surrounding filaments. Possibly a greater number of plants should have been observed and their sporangia number recorded.
- (iv) Since recordings were only made at 2-4 day intervals, the exact day of initiation of reproductive structures was often not established. The numbers of empty sporangia, however, may give an indication of which filaments were at a more advanced stage of reproduction. As mentioned in the results, the initial phases of sporangia production resembles early vegetative growth and so reproduction was only recorded once sporangia were already relatively mature.
- (v) There is always the possibility that conditions, other than those purposely varied for each treatment were not constant.

It was noticed that crowding of filaments may have to a certain extent, inhibited growth in some of the treatments. Since it was not possible to control the numbers of motiles syringed into each Repli-dish, crowding of filaments occasionally occurred. Plants grown under these conditions appeared to have fewer branches and a lower reproductive capacity than those not grown in crowded conditions. It is possible that both space and nutrients become limiting. While attempts to maintain salinity levels were made by sealing the Repli-dish dishes, it appeared that changes in salinity took place, as recorded in the temperature experiments (Figures.15a & 15b) ie. by evaporation and condensation. It is inevitable that the longer the duration of the experiment, the more prone to salinity effects the treatment would be. Here the photoperiod experiment may have been affected. Occasionally condensation on the inside lid of the Repli-dishes occurred, this too may have altered the salinity of the medium. Knoepffler-Péguy (1970) observed changes in the morphology of two *Feldmannia* sp. related to salinity levels. More saline conditions caused the dominance of the prostrate system, while reduced salinity resulted in the predominance of erect filament. Observations of *Feldmannia* sp. showed that a similar response of morphology to salinity levels may exist. It appeared, however, that the dominance of either the prostrate or erect system was related to the number of cells. Those filaments grown in high salinities had fewer cell numbers and less well developed erect branches than filaments grown in less saline conditions.

- (v) It appeared that reproduction was dependent on the cell numbers of the filaments viz, once a certain filament size was reached reproduction occurred. The filament size at which reproduction occurred varied markedly between experiments in some instances eg. Figures.15a & 15b, 17b & 17c, but appeared to be reasonably constant between treatments eg. Figures.14a & 14b, 15a & 15b, 17a, 17b & 17c.
- (vi) Counting of filaments, particularly mature filaments, was subjective. The erect filaments often extended out of the

focal range of the microscope and so cell number estimates based on the length of the filaments had to be made. Erect filaments also tended to obscure each other. The author is, however, confident that a high level of uniformity in counting was maintained, as filaments recounted yielded numbers very close to the originally recorded number.

The reasons behind the responses obtained under different environmental conditions will be discussed in turn.

The photoperiodic experiments did not initiate a change in the phase of generations of Feldmannia sp. and so no true photoperiodic response occurred (Luning 1981). If use is made of Dring's (1984) definition of a photoperiodic response in which a light break in the dark cycle is meant to induce a change of phase, then once again we can conclude that a photoperiodic effect was not exhibited in Feldmannia sp.. Rather filaments appeared to be responding to the amount of available light rather than to the length of the light period. Here we have assumed that the Feldmannia sp. is a sporophyte. If this alga was, however, a gametophyte then no alternations of generations would be observed regardless of the photoperiod.

Here it is necessary to point out that there may have been a flaw in the original experimental design. Dring (1984) pointed out that the experimental design should be such that the irradiance must be decreased in proportion to the increase in day-length, so that all treatments receive the same photon fluence and any differences in development between treatments can be attributed to day-length and not to the total amount of light received.

The results do suggest that greater day-lengths meet the requirements necessary for the induction of sexuality more rapidly than in short day treatments. According to Stifter (1959) light is required for photosynthesis and for photostimulation of respiration. Thus the requirements for growth are the same as those for reproduction. When filaments growing in short day-lengths were placed in 12L:12D light dark cycles, they became reproductive within 3 days. Here the

increase of available light satisfied the growth and reproductive requirements more rapidly than under short day-lengths. New medium was also added to these Repli-dishes and this may also have contributed to the surge in growth.

In order to establish whether a true photoperiodic response is evident in Feldmannia sp., it would be necessary to obtain clones that bear both unilocular and plurilocular sporangia. Here a change in phase would indicate the presence of a photoperiodic response.

Since Feldmannia sp. grows most favourably in long day-lengths (Figures. 14a & 14b) it is possible that it achieves maximum growth during the summer months.

The general trend emerging from the temperature experiments is that Feldmannia sp. grows and reproduces in a range of temperatures and most favourably in higher temperatures around 30°C. This is anomolcus when we consider that Feldmannia sp. was found growing on a relatively deep water host, where high water temperatures of 30°C would not be found.

When the results obtained for the response of Feldmannia sp. under different temperatures is compared to that obtained in Giffordia mitchelliae (Ansler 1985), a number of similarities are evident. In both species, little growth occurred at 10°C, however, growth in culture was relatively good between 15-25°C. Corresponding to this, G.mitchelliae reached maximum size and abundance when similar sea water temperatures occurred at the collection sites. During late summer when temperatures exceeded 25°C, size and abundance decreased in the natural populations of G.mitchelliae. Growth of Feldmannia sp. did not appear to be limited by temperatures above 25°C and below 30°C (Figures. 15a & 15b). The temperature peaks in Natal coast waters would be slightly greater during the late summer than those in the California coastal waters where G.mitchelliae was collected and observed. This could mean that maximum size and abundance of Feldmannia sp. would be achieved at temperatures above those where the maximum size would be reached in G.mitchelliae.

Most rapid growth of Feldmannia sp. occurs under higher photon fluence rates (Figures 16a.- 16c.). This can be related to the photosynthetic rate which is stimulated to a greater extent at higher photon fluence rates. It appears, however, that the rapid increase in cell number at higher photon fluence rates is at the expense of reproductive capacity, reflected in the lower numbers of sporangia in most high photon fluence rate treatments. The bleaching effect of the filaments at high irradiances indicates that the pigment systems have been damaged in some way and so may influence the viability of the filaments. Hoffmann (1985) noted similar decolouration of ligulae in Glossophora kunthii at high irradiances and suggested that some specific developmental process was being affected resulting in decolouration.

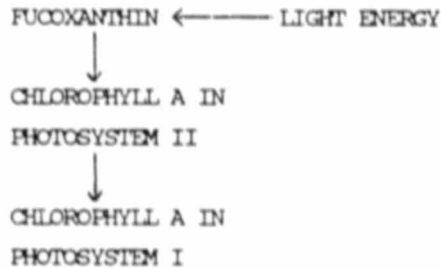
Greatest reproductive capacity appeared to be achieved at low photon fluence rates (Figures 16a.- 16c.). This may be expected since Feldmannia sp. was found growing epiphytically on a deep water algal host.

Of the light quality treatments, blue light induced the most rapid growth (Figures 17a.& 17b.). It has been shown that the wavelength of the filters used can be compared to the transmittance spectra of algal pigments to determine which pigments are most actively involved in photosynthesis.

There are a number of photosynthetic pigments in brown algae, each with maximum absorption peaks at different wavelengths (Saffo 1987). The absorption peaks of the red, blue and green cellophane paper used in this experiment were compared with the absorption peaks of photosynthetic pigments (Figures 18.- 20.) as determined by Saffo (1987)

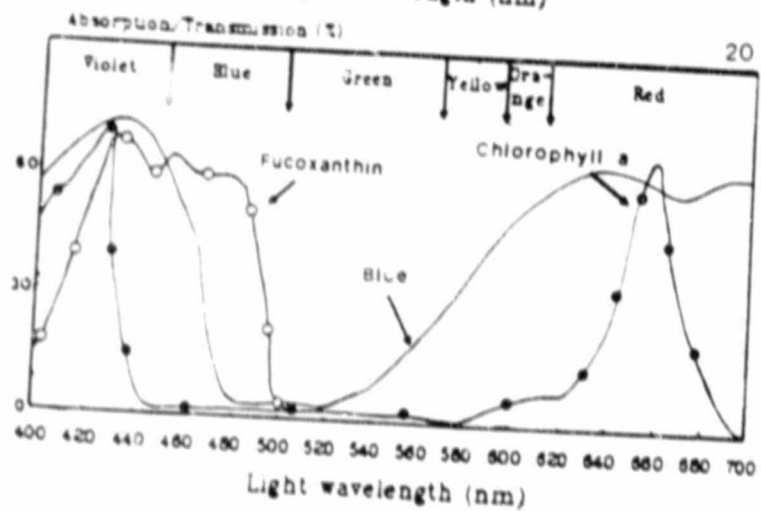
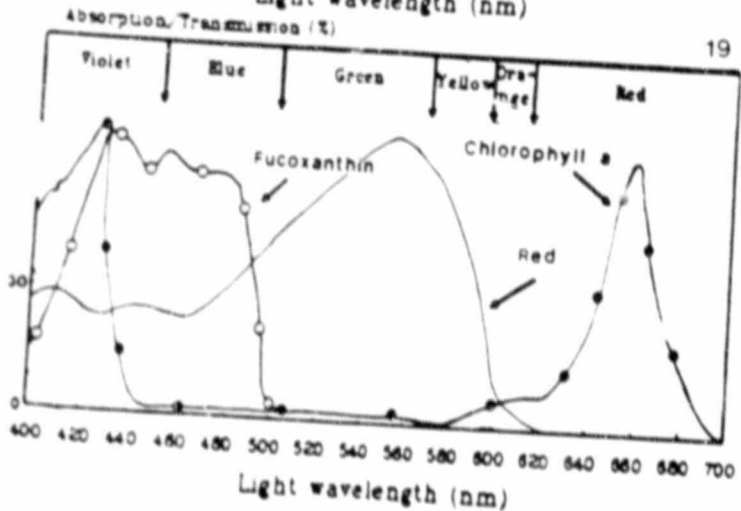
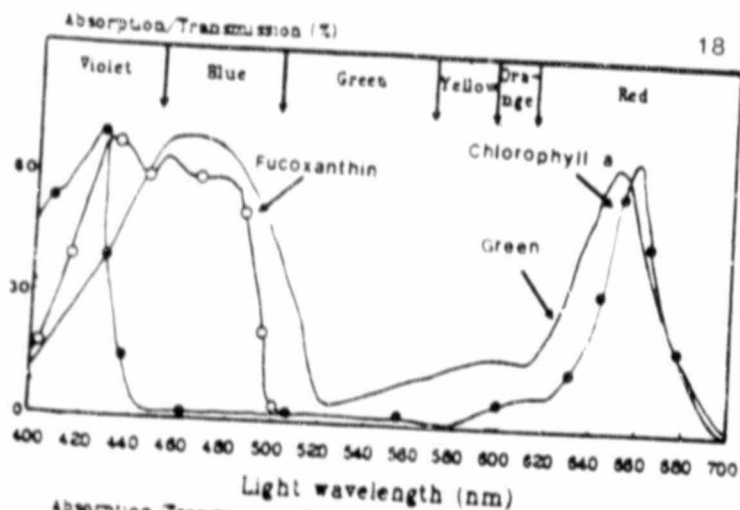
An attempt was made to determine the action of certain pigments in the photosynthetic process in Porphyridium cruentum (Ag.) Naeg. (Ley & Butler 1980). Since we have an indication of which pigments are being excited under the different filters, it may be possible to explain the effect of light quality on growth in Feldmannia sp. based on the work done on Porphyridium cruentum. In essence, it was found

that chlorophyll was organised into large photosystem I units and small photosystem II units and energy is exchanged between these units. It would be expected that the highest level of light energy available to the photosystem, would induce the fastest growth. The flow chart for energy transfer is summarised as follows:



Illumination of Feldmannia sp. with the blue and green filters resulted in the excitation of the pigment fucoxanthin and chlorophyll a, observed in the overlap of pigments and filter peaks in Figures 18 and 19. The red filter peak does not overlap with either of the two pigments, however, between 400 and 500nm there is some overlap at low absorption levels of the filter (Figure 20.). The faster growth of the alga grown under blue and green light may be explained by the direct excitation of either photosystem I or II by the chlorophyll a, or the less direct excitation of the photosystems by fucoxanthin. The weak overlap of the pigment absorption peaks with the red filter may explain the slower growth under this filter.

The influence of light quality on reproduction is not easy to explain. Since sporangia were recorded in all treatments in one experiment, their failure to form in other treatments must be due to some feature other than the light quality.



Figures 18.-20.: The absorption peaks of the green, red and blue filters (respectively), compared to the peaks of the pigments fucoxanthin and chlorophyll a.

VI. CONCLUSION

A number of features of the ultrastructure and development of Feldmannia sp. in culture have been observed in this dissertation. These can be summarised as follows:

- (i) It was established that the alga studied in this investigation belongs to the genus Feldmannia, however, identification to the species level was not conclusive. This alga had the greatest similarities to Feldmannia lebellii and F.globifer. Based on the presence of a pronounced prostrate system in this alga, not apparent in the other two species, it seems most likely that Feldmannia sp. is a new species.
- (ii) Autofluorescent white spots stain readily to the cell walls of Feldmannia sp. and fluoresces brightly under violet light. In this way it was possible to establish that growth occurs in secondary and apical meristematic regions. No autofluorescence of the flagella of motile cells was observed.
- (iii) The stages in the development of the plurilocular structures closely resemble those found in other members of the Ectocarpales (Knight 11929, Lofthouse & Capon 1975, Markey & Wilce 1976, Clayton 1984 & 1986). While the cell divisions of the sporangia do not follow a rigid pattern, there is a close resemblance to the two, six celled and nine rank stages identified in the development of Ectocarpus parvus (Lofthouse & Capon 1975).
- (iv) At the electron microscope level, information about the formation of the cell wall, functioning of Golgi and the formation of flagella is revealed. It appears that the first stage of cell wall formation is the production of a thin layer between two cells. It is onto this layer that fibrous-like material, released from Golgi derived vesicles, is packed. The role of plasmalemmasomes and/or lysosomes in cell wall formation is still unclear. The precursors of the flagella are the centrioles which give rise to the basal plates. The

flagella extends from the basal plate into a flagellar vesicle, until they have increased sufficiently in length to become confluent with the matrix surrounding each cell. Stages in the formation of mastigonemes were not evident. The Golgi could be apparently involved in a number of functions throughout zoosporegenesis and in the motile cells: cell wall formation, production of vesicles containing enzymes for cell wall breakdown and matrix formation in mature sporangia, and also the production of adhesives for attachment of motile cells.

- (v) The ultrastructure of motile cells is also consistent with observations of other members of the Ectocarpales (Manton 1957, Baker & Evans 1973, Clayton 1984). Large numbers of vesicles at the anterior ends of the motile cells, are thought to be associated with mullage formation required for attachment to a substrate. The motile cells attach to the substrate by their anterior ends and do not appear to have any substrate preference.
- (vi) It is most likely that the pressure responsible for expelling motile cells from the sporangia is due to swelling of the mullage surrounding each motile cell. The presence of PAS stain in this mullage indicates that it is carbohydrate in nature and capable of swelling when hydrated. This supports the observation of Ruth (1976) on *Chorda tomentosa*.
- (vii) *Feldmannia* sp. 1. tolerates a wide range of environmental conditions and grows most favourably in water temperatures of 20-30°C, photoperiods of 14L:10D and 16L:8D, and photon fluences of $114 \mu\text{E m}^{-2} \text{sec}^{-1}$. Often the treatment that induces the most rapid increase in cell numbers detrimentally affects the reproductive capacity, particularly in the case of high photon fluence. Next to white light, *Feldmannia* sp. appears to grow most favourably in blue light, followed by green light and then red light. This may be explained by comparing the absorption peaks of the different filters with the absorption peaks of the pigments fucoxanthin and chlorophyll a. The greater overlap of peaks of blue and green

light resulted in greater excitation of photosystems I and II. This led to more energy being available for growth under these two filters than for the alga grown under the red light conditions. The influence of light quality on reproduction is unclear.

- (viii) No unilocular structures were induced during the various experiments. There are a number of possible reasons to explain this. The stimulus necessary to trigger a change in phase may be extremely subtle and it is possible that the experimental conditions used in this thesis did not cover the stimuli needed to induce unilocular formation. Alternatively, the original stock plant may have been a gametophyte bearing plurilocular gametangia, and therefore would never be able to produce unilocular structures, regardless of the environmental conditions. Finally, it is possible that unilocular structures are never formed in the life cycle of Feldmannia sp.. This could only be established once more wild material is collected and exposed to a wider range of experimental conditions.

While a detailed study of Feldmannia sp. has been made there are a number of deficiencies where further research could be undertaken:

- (i) It would be necessary to establish conclusively whether this is indeed a new species by scouring all the literature on the Ectocarpales classification. If this alga has not yet been described then a full description and species name must be made and published.
- (ii) The geographical distribution of the taxon along the east South-African coast should be determined. Collection of wild material may yield unilocular reproductive structures, which would provide more information on the life cycle of this species.
- (iii) Further sectioning of mature sporangia would reveal more information on the stages in cell wall and flagellar formation.
- (iv) Carbon and gold palladium coating of motile cells and more

Careful negative staining, may also reveal more of the detail of the mastigonemes of the flagella.

It is hoped that this thesis provided some practical information that may be useful to further studies on members of the order Ectocarpales. It seems that there are large similarities in morphology, ultrastructure and development between members of this group and in this respect many of the findings of this thesis are not new. It is always more difficult to draw conclusions and derive explanations for responses in experimental studies. It is hoped that this dissertation provides some indication of the responses to a range of environmental conditions and inspired speculations as to why they occurred.

This was clearly a laboratory based study and an obvious omission in obtaining a complete understanding of the life cycle of Feldmannia sp., is studies on the ecology of the alga in its natural environment. Perhaps if this alga were relocated in the field, a new dimension in the factors controlling growth and reproduction would emerge.

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VIII. APPENDIX

8.1. Culture media

Preparation of sterilized sea water

- i) Sea water was double filtered through Whatman's No. 1 filter paper.
- ii) One part distilled water was added to nine parts of filtered sea water and sterilized in an autoclave by steaming at 70°C for 20 minutes at 105KPa. The water was stored in sterile glass bottles and allowed to cool to room temperature before use.

Preparation of Soil extract

- i) One part soil (obtained from Garsfontein!) was boiled in a pressure cooker with two parts distilled water.
- ii) The mixture was then left to stand overnight allowing the sand and silt to settle. The liquid supernatant was double filtered through Whatman's No.1 filter paper.
- iii) The brown fluid was autoclaved for 20 minutes at 150KPa dispensed into screw cap vials and stored in the refrigerator.

Preparation of P.E.S.

TABLE 2.: Provosoli enrichment solution ingredients
(Stein 1973)

Three solutions are made up:

I. <u>Salts stock solution:</u> (add 10ml ⁻¹ culture)	
To 1000ml of double filtered sea water add:	
a) NaN ₃	5,609g ⁻¹
b) Na -glycerophosphate	0,7877g ⁻¹
c) Fe EDTA	264,3g ⁻¹
d) Tris - Buffer (pH 7,8)	7,995g ⁻¹
II. <u>Trace metals:</u> (add 10ml ⁻¹ culture)	
e) ZnS ₄ 7H ₂ O	0,023g ⁻¹
f) MnS ₄ 4H ₂ O	0,1628g ⁻¹
g) CuS ₄ 7H ₂ O	0,04778g ⁻¹
h) FeC ₃ 6H ₂ O	0,04865g ⁻¹
i) Na ₂ EDTA	1,0013g ⁻¹
j) H ₃ BO ₃	1,1438g ⁻¹
III. <u>Vitamin stock solutions:</u> (add 2ml ⁻¹)	
These are added separately to the cultures	
Vitamin B ₁₂ (cyanocobalamin)	0,00016g ⁻¹
Biotin	0,00008g ⁻¹
Thiamin - HCL	0,002g ⁻¹

8.2. Electron microscopy

The preparation of fixatives involved in primary fixation

- a) One part 6% glutaraldehyde to nine parts sterilized sea water for eight to 15 hours at room temperature.
- b) One part 4% glutaraldehyde to one part 0,25% sucrose to eight parts 0,1M sodium cacodylate for 2¹/₂ hours at 4°C.
- c) One part 3,5% glutaraldehyde to one part 1% caffeine to eight parts 0,1M sodium cacodylate buffer, pH 7, overnight at 4°C.

Preparation of the sodium cacodylate buffer

32 grams of sodium cacodylate was dissolved in sea water to give a final volume of 1 litre, 0,2M sodium cacodylate buffer pH 6.8-7.2.

Preparation of glutaraldehyde

To make up the different concentrations of glutaraldehyde, 12,5ml of 0,2M sodium cacodylate buffer was added to :

- a) 6% glutaraldehyde : 6ml of 25% glutaraldehyde in distilled water
- b) 4% " : 4ml "
- c) 3,5% " : 3,5ml "

All of the above solutions were made up to 25ml with distilled water.

Post fixation:

Algae were washed in the following

- a) sterilized sea water (x3)
- b) i. 0,1M sodium cacodylate buffer pH 6.8-7.2 and 60% sucrose
- ii. " " 40% sucrose
- iii. " " 20% sucrose
- iv. 0,1M sodium cacodylate buffer only.
- c) 4 washes in sodium cacodylate buffer.

Preparation of 2% OsO₄

1 mg of crystalline OsO₄ was dissolved in 25ml of distilled water to give a 4% solution of OsO₄. To obtain a 2% OsO₄ solution, 1ml of the 4% OsO₄ was diluted with a) 1ml sterilized sea water, b) and c) 1ml 0,1M sodium cacodylate buffer.

Embedding and polymerization

Impregnation of material with Spurr's resin:

- i) 3 parts ethanol to 1 part Spurr's resin for 1 hour
- ii) 1 part ethanol to 1 " "
- iii) 1 part ethanol to 3 parts Spurr's resin for 1 hour
- iv) 100% Spurr's resin for a) and c) 2 hours
- b) 15 hours
- v) fresh 100% Spurr's resin for a) 2 hours
- b) 6 hours
- c) 24 hours

Preparation of Spurr's resin: (Spurr, 1967)

Vinylcyclohexane Dioxide (VCD or ERL 4206)	10 grams
Diglycidyl Ether of Polypropylene glycol (DER 736)	6 grams
Nonenyl succinic Anhydride (NSA)	26 grams
Dimethylaminoethanol (DMAE)	0,4 grams

The Spurr's constituents were mixed thoroughly and added in the order listed above, as DMAE is a catalyst.

Uranyl acetate preparation

A 2% aqueous solution (i.e. 1 gram of uranyl acetate in 50ml of distilled water) was made up and 1ml of 95% ethanol was added to decrease the surface tension. Since uranyl acetate is light sensitive, it is stored in a volumetric flask wrapped in aluminium foil to exclude light.

Lead citrate preparation

30ml of distilled water was added to 1,33 grams of lead nitrate and 1,74 grams sodium citrate in a 50ml volumetric flask. The solution was then shaken intermittently over a period of 30 minutes. The white precipitate was cleared by adding 8ml of 1N sodium hydroxide. The solution was then made up to 50ml by adding distilled water.

8.3.1. Embedding and polymerisation of motiles

- i. 3 parts ethanol : 1 part Spurr's resin for 20 minutes
- ii. 1 part " : 1 "
- iii. 1 part " : 3 parts "
- iv. 100% Spurr's for 30 minutes
- v. Fresh 100% Spurr's for 30 minutes

Procedure for making Formvar - coated viewing grids

A solution of formvar was made by adding 2grams of powdered formvar to 100ml chloroform. In order to remove water absorbed from the atmosphere by the chloroform, micromesh pellets were added to the solution. Dust-free, unused glass slides were dipped into the formvar solution in a Coplin jar, allowed to dry and their edges scraped clean with a sharp blade. The formvar film was then floated off the slide onto water, the surface of which had been cleaned with a sweep of lens tissue, in an evaporating dish. After grids had been distributed on the formvar film (which had a silver to gold interference colour) they were collected on cleaned pieces of wire gauze or photographic negative.

8.3. Staining Techniques

Schiff's Reagent: (de Tomasi 1936 in O'Brien & McCully 1981)

1 gram of basic fuchsin was dissolved in 200ml boiling water. This was agitated for 5 minutes, cooled to exactly 50°C and then filtered. 20ml N HCL was added to the filtrate. When cooled to 25°C, 1 gram metabisulphite was added. The solution was left to

stand in the dark for 14 hours. 2 grams of activated charcoal was added, agitated for 1 minute and then filtered. It was necessary to keep the filtrate in the dark at 0°C and to allow it to reach room temperature before use.

8.4. Calcofluor White M2B

Address of supplier: Sigma Chemical Company
P.O.Box 14508
St Louis
USA.

VOLUME II

PLATE 1

Figs.1.-4. Light microscope features of Feldmannia sp..

- Fig.1. Sporangia growing on meristematic region of erect filaments (EF). Rhizoids (R) are often associated with these regions.
- Fig.2. Feldmannia sp. showing arrangements of prostrate (P) and erect filaments (EF) with sporangia growing mostly at the base of the erect filaments.
- Fig.3. Arrangement of prostrate (P) and erect filaments (EF) in greater detail. Note the presence of rhizoids (R) and the rounded shape of the first cell in each erect filament.
- Fig.4. Detail of the rhizoids (R) with a multiple tip, probably for attachment.

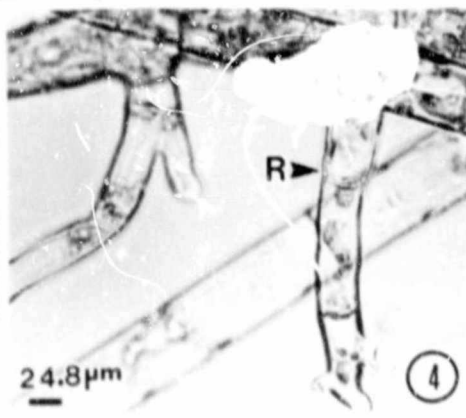
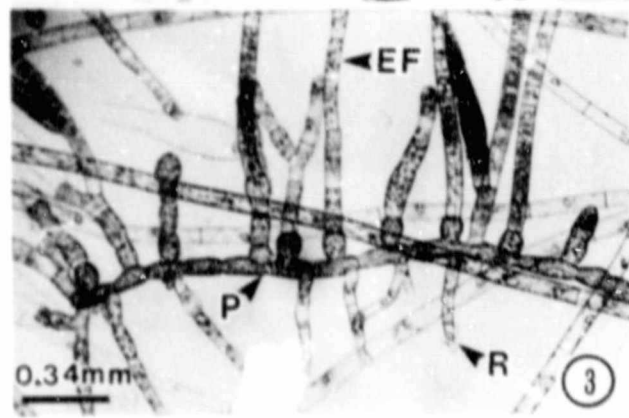
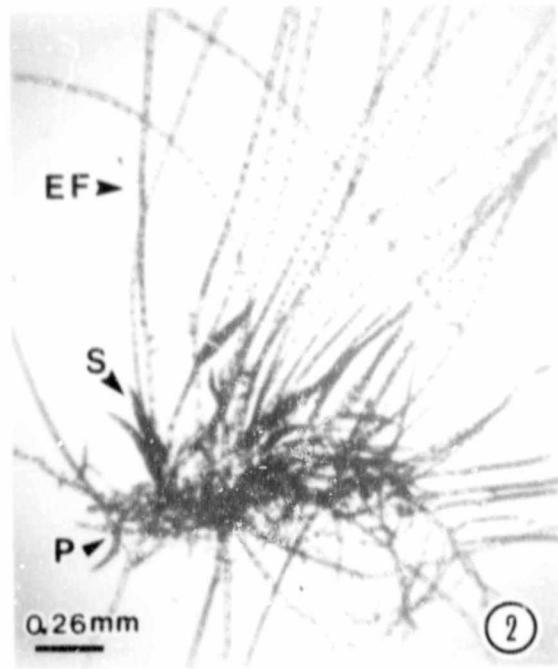


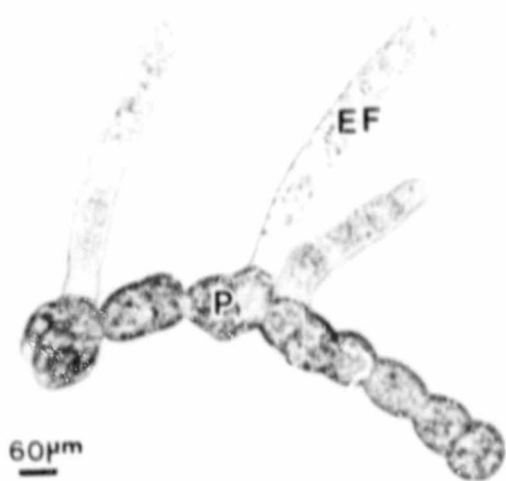
PLATE 2

Figs.1.-5. Light microscopic detail of vegetative portions of Feldmannia sp..

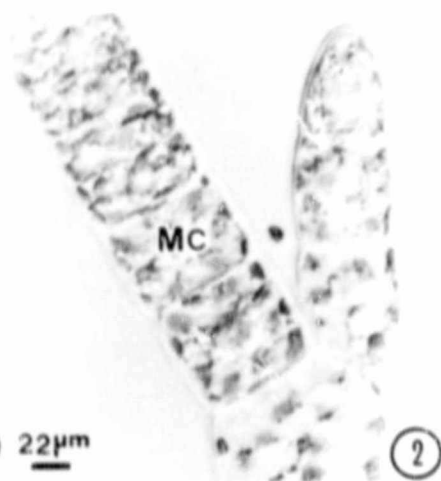
Fig.1. Globular appearance of the prostrate region (P) bearing erect filaments (EF).

Fig.2. The meristematic vegetative cells (Mc) are square when viewed from the side, and appear closely packed.

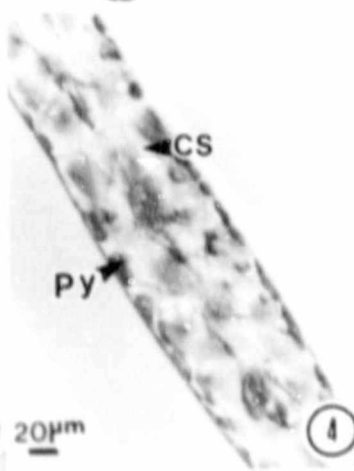
Figs.3.-5. A through focus of a portion of an erect filament revealing cytoplasmic strands (CS), chloroplasts (C) and pyrenoids (Py).



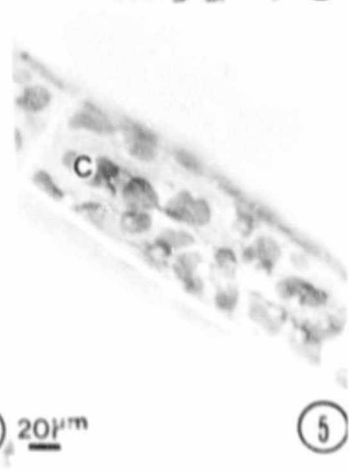
① 22µm



③ 20µm



④ 20µm



⑤

PLATE 3

Figs.1.-4. Sporangia are attached to different positions on the thallus.

Fig.1. A sporangium attached to the prostrate region (P).

Fig.2. A sporangium located at the tip of an erect filament.

Fig.3. A sporangium emerging from an erect filament below a meristematic region (MR).

Fig.4. Sporangia possessing a rounded, vegetative, subtending cell (Sc), which is in turn attached to an erect filament.

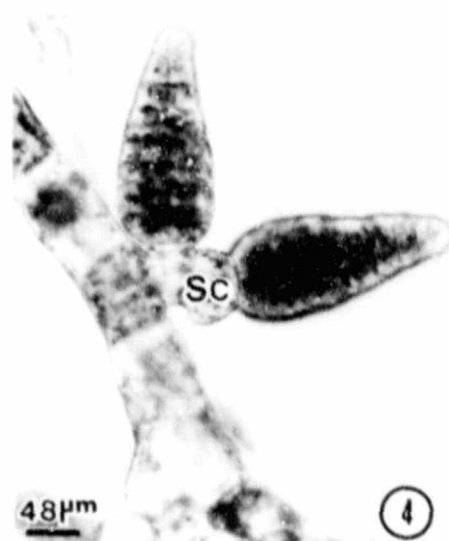
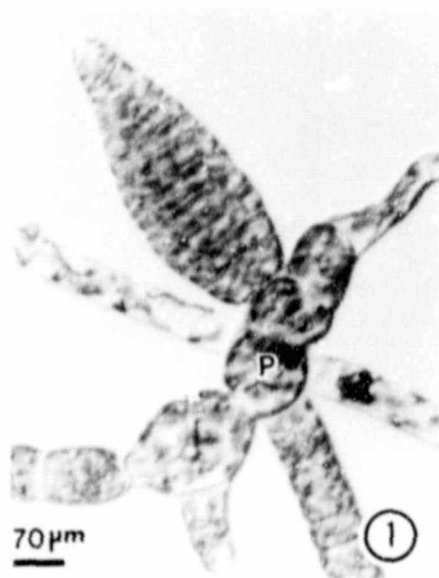


PLATE 4

- Figs.1.-9. The development of a Feldmannia sp. germling.
- Figs.1.-5. The elongation of the prostrate system.
- Fig.6. An erect filament is in evidence (arrow).
- Figs.7.-9. Further development of germling into a mature plant.
- Figs.10.-12. The overall morphology of adult Feldmannia sp. plants under red, blue and green light.
- Fig.10. The predominance of the creeping, prostrate system of germlings grown under red light.
- Fig.11. A predominance of erect filaments and the presence of sporangia on a germling grown under blue light.
- Fig.12. A predominance of erect filaments on a germling grown under green light.

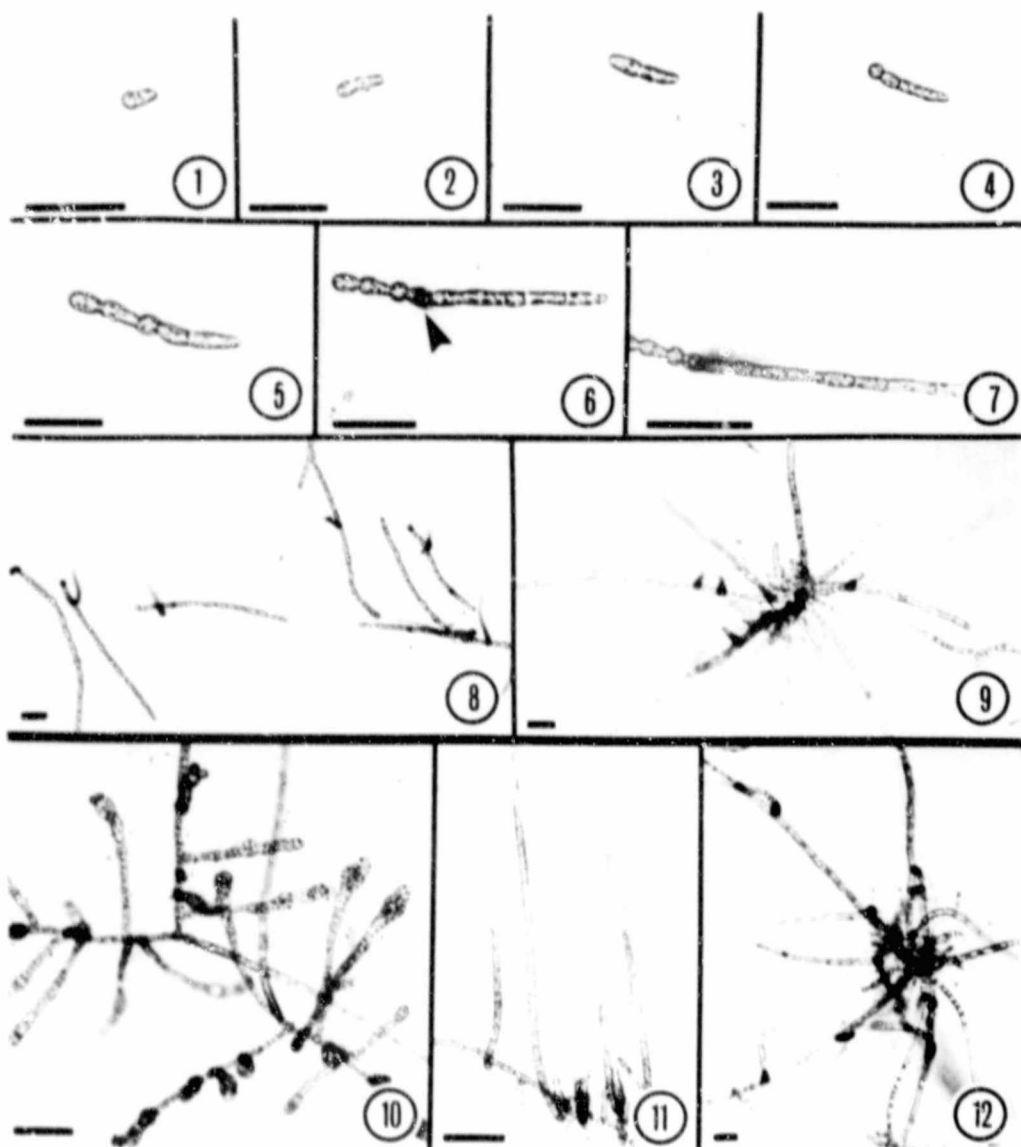


PLATE 5

Figs.1.-7. Light microscope observations of stages in the development of sporangia from a vegetative filament.

Fig.1. Bulging to one side of a vegetative filament.

Fig.2. The sporangium consists of one cell only and is separated from the vegetative cell by a cross wall.

Fig.3. Young sporangia with a few locules and a subtending cell (Sc).

Fig.4. Further divisions have taken place and more locules are evident in this sporangium.

Fig.5. An 'intermediate' sporangium approaching maturity.

Fig.6. Mature sporangium, with rounded up zoospores at the sporangium tip.

Fig.7. Empty sporangium after zoospore release.

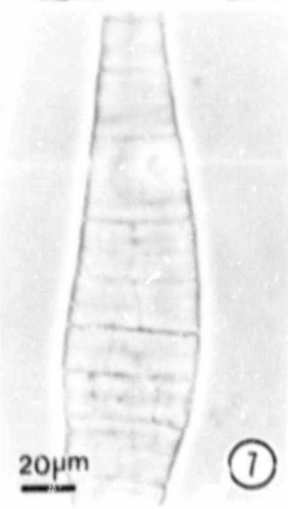
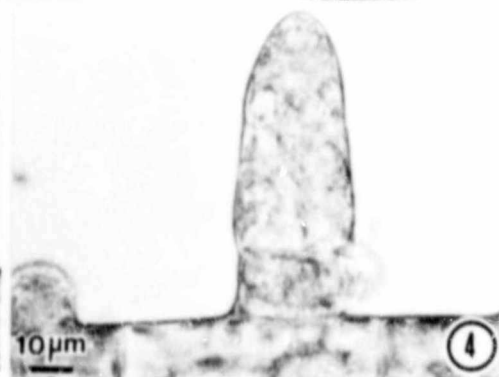
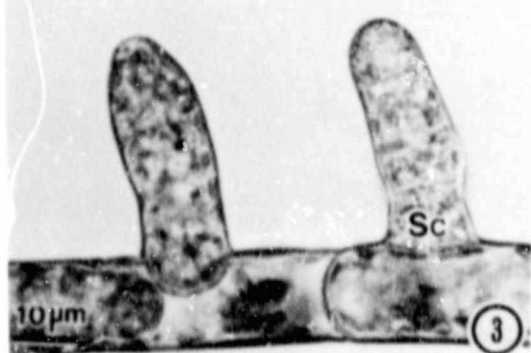
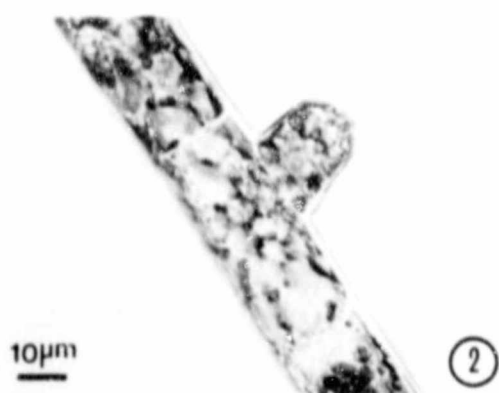
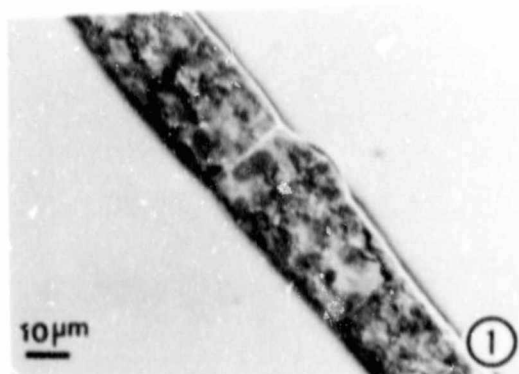


PLATE 6

- Figs.1.& 2. Light microscope micrographs of sporangia that have partially released their contents.
- Fig.1. Flagellate motiles (Mo) are evident within a sporangium. The adjacent sporangium shows the pore (Q) through which the motiles escape. Note that the flagella (F) trail behind as they move through the pore.
- Fig.2. Rarely, zoospores (Z) germinate within the sporangium. The pattern of the cell arrangement before release is still sculptured in the remaining sporangial wall.

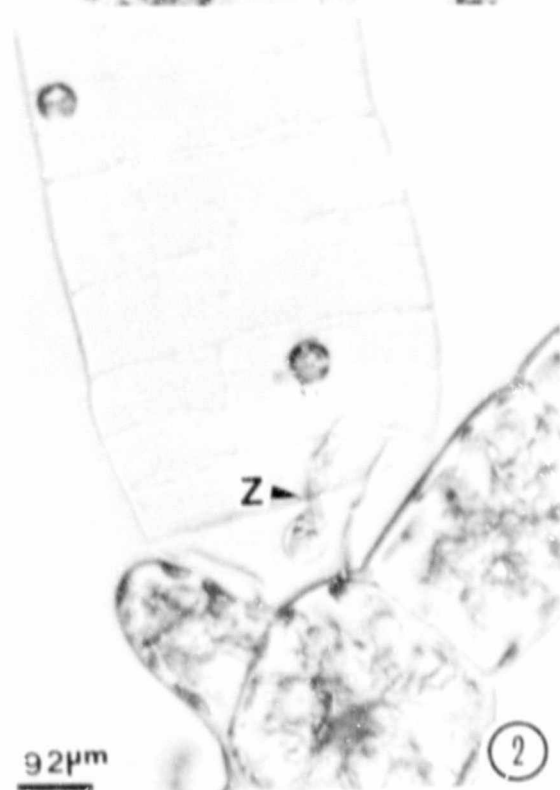
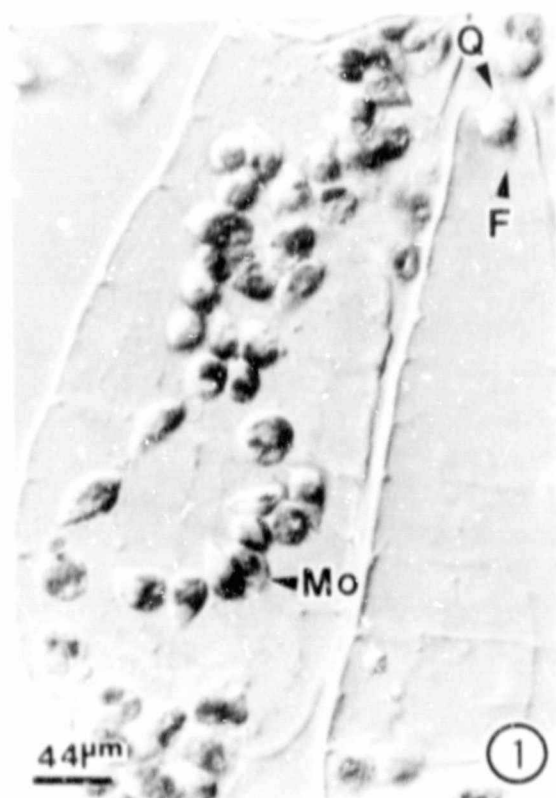


PLATE 7

Figs.1.-8. Stages in the release of motiles from a plurilocular sporangium.

Fig.1. Sporangium prior to release.

Fig.2.-8 Rupture of the sporangium tip and the release of motiles. The released motiles remain stationary at the sporangium tip and are entangled in a mucilage.

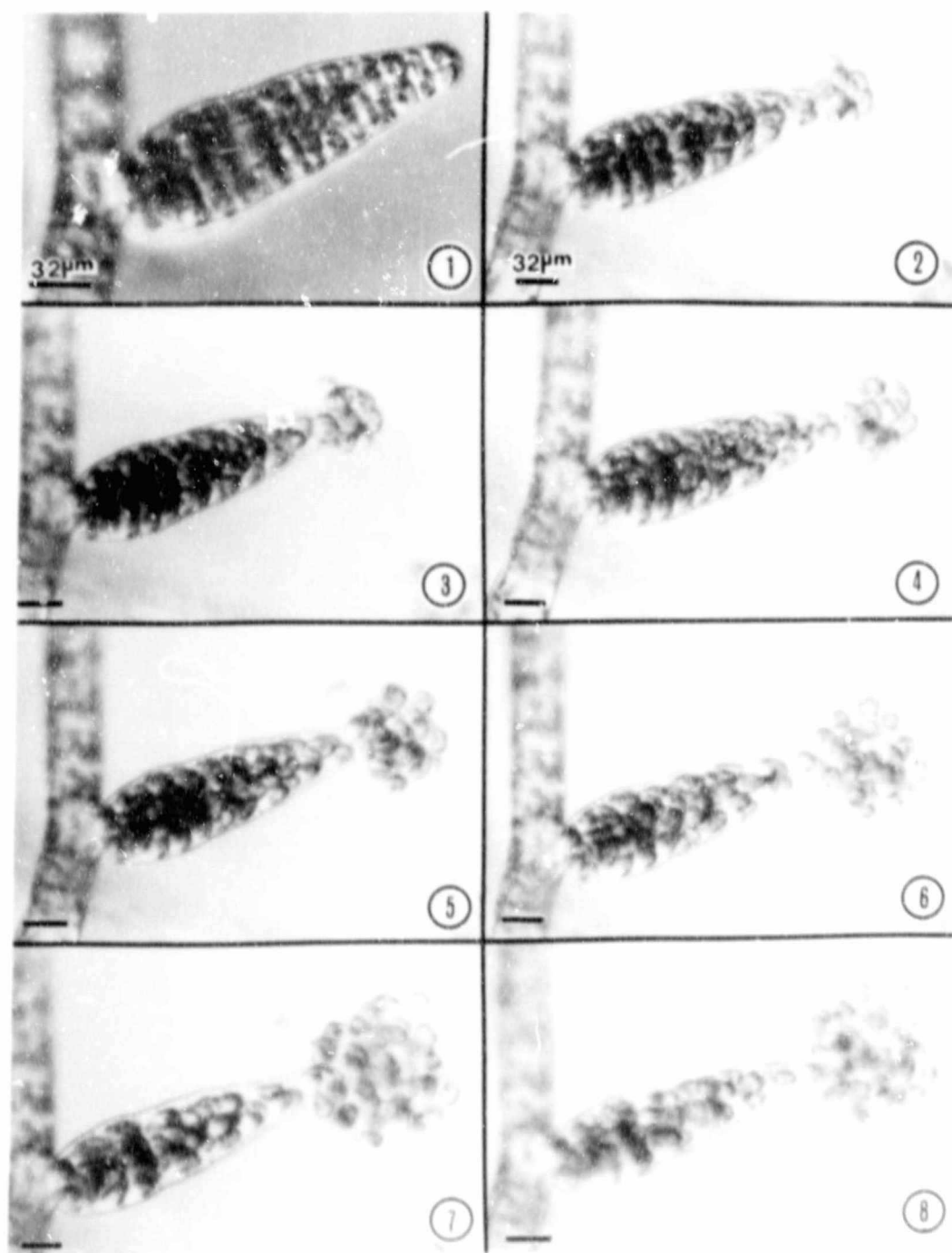


PLATE 8

- Figs.9.-16. Further stages in the release of motiles from a plurilocular sporangium.
- Figs.9.-10. While most motiles are released, the motiles at the tip disentangle themselves from the mucilage and swim free.
- Figs.11.-15. The last remaining motiles stream out of the sporangium.
- Fig.16. All the motiles have been released after approximately four minutes.

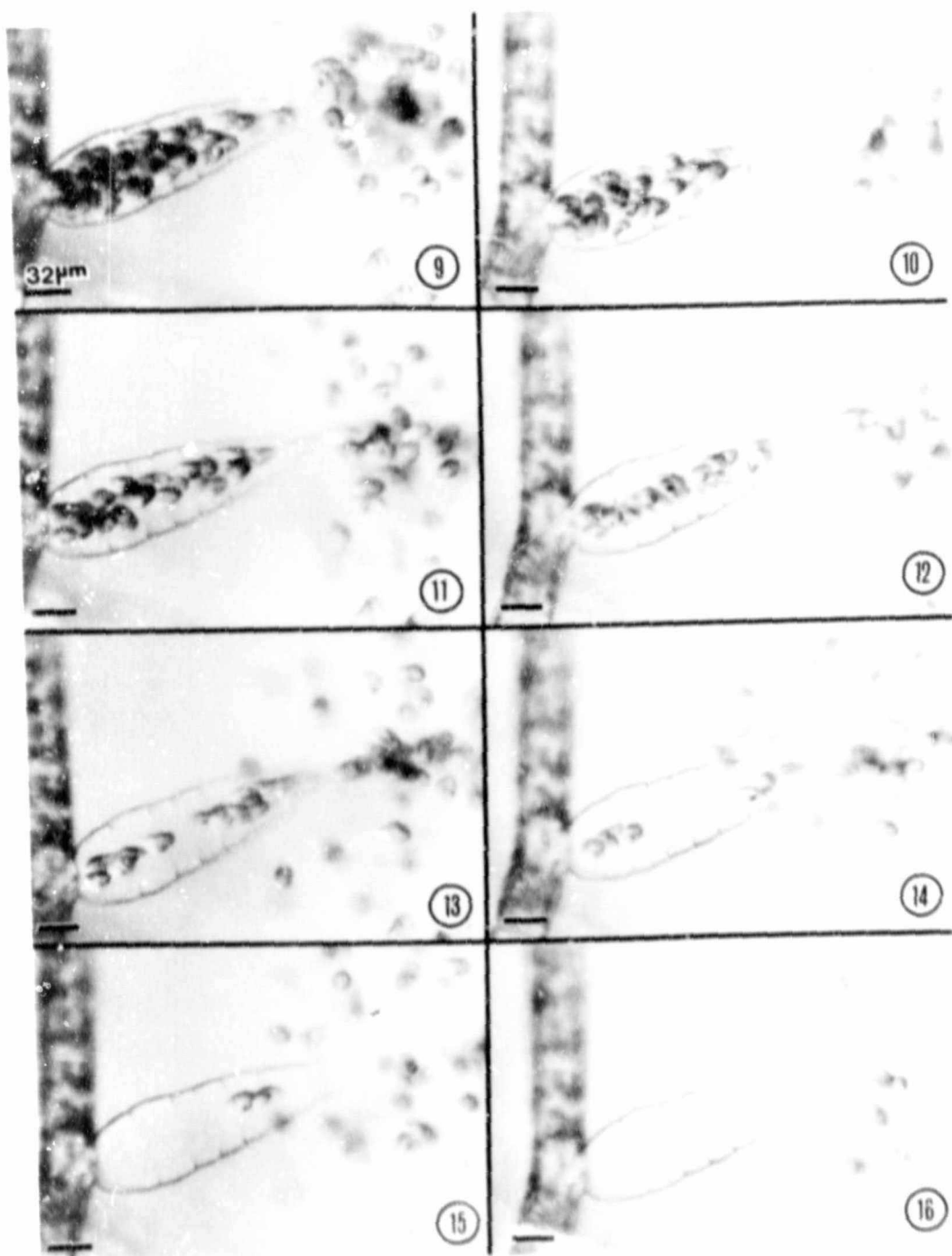


PLATE 2

Figs.1.-3. Light microscope observations of germlings.

Fig.1. Motiles attach themselves to an adult filament (E) or each other.

Fig.2. A dense band of germlings.

Fig.3. The surrounding filaments appear to be free of attached germlings.

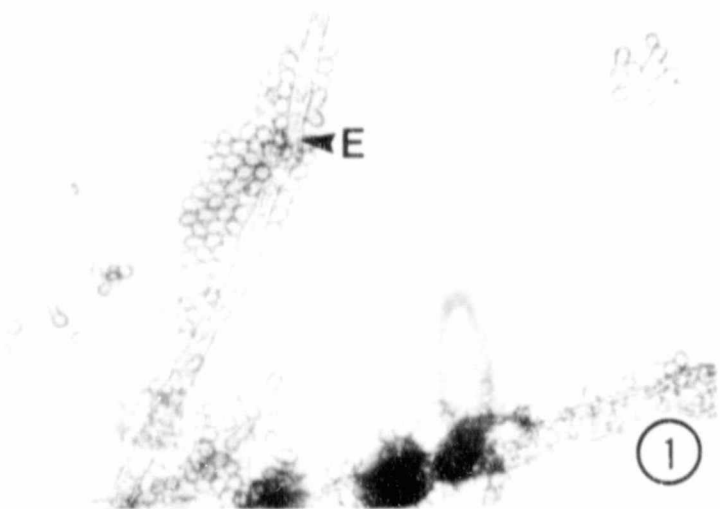
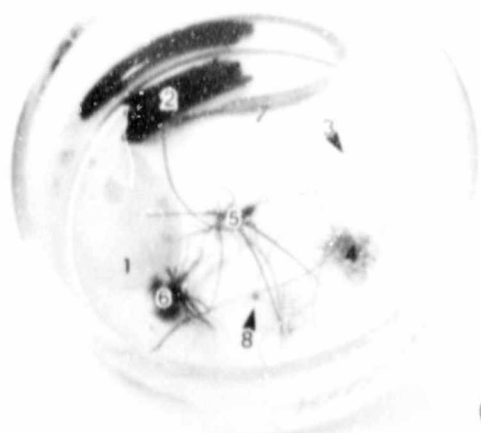


PLATE 10

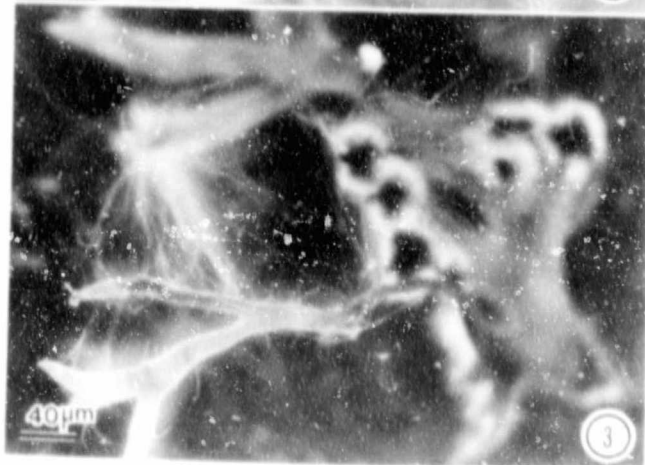
- Figs.1.-3. Light microscope observations of the attachment of Feldmannia sp. to various substrates.
- Fig.1. An overall view of an evaporating dish, filled with medium, containing a 1) shell, 2) piece of driftwood and the following algae: 3) Schottera micariensis, 4) Pterocladia caloglossoides, 5) Chaetomorpha crassa, 6) Cryptonemia luxurians, 7) Botryocladia madagascarensis and 8) Feldmannia sp..
- Fig.2. Fine strands of Feldmannia sp. are evident attached to Chaetomorpha crassa.
- Fig.3. Feldmannia sp. evident on the laminae of the dimorphic Cryptonemia luxurians.



1



2



3

PLATE 11

- Figs. 4.-7. Light microscope observations of the substrate preferences of Feldmannia sp. continued.
- Fig.4. Numerous Feldmannia sp. growing on the surface of driftwood.
- Fig.5. Feldmannia sp. filaments attached to Schoterra micariensis.
- Fig.6. A few filaments of Feldmannia sp. attached to Botryocladia madagascarensis.
- Fig.7. A shell, acting as a substrate to Feldmannia sp.

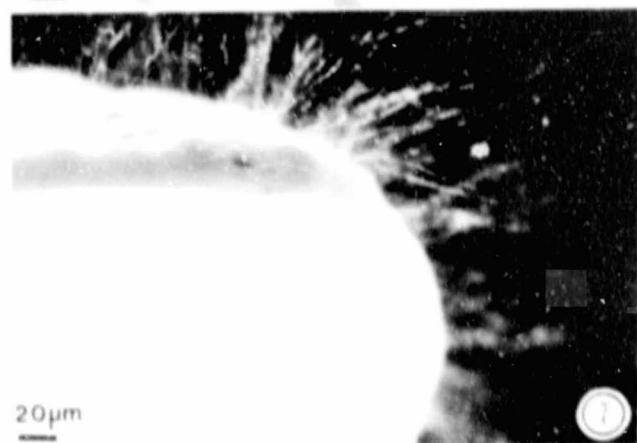
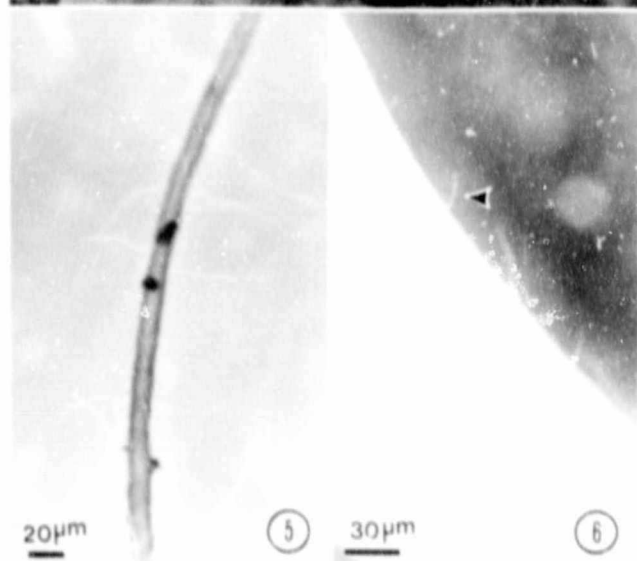
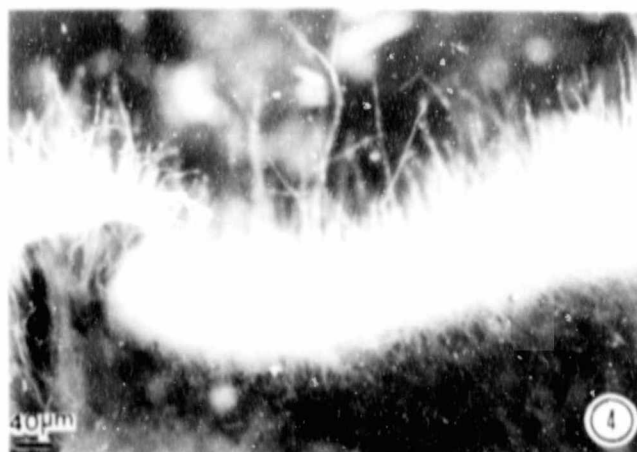


PLATE 12

- Figs.1.-6. Ultraviolet fluorescence of filaments stained with Calcofluor White M2R.
- Fig.1. A clump of germlings after 20 minutes of staining with Calcofluor White M2R.
- Fig.2. The same germlings one day after staining, reveals large numbers of autofluorescing chloroplasts in the central region of the prostrate filament portion (arrow).
- Fig.3. Mature filaments after 20 minutes in Calcofluor White M2R.
- Fig.4. The erect portion of filaments also after 20 minutes of staining, showing a greater intensity of staining at the cross wall regions (arrow).
- Fig.5. Portion of a filament a day after staining. Large numbers of autofluorescing chloroplasts occur in the prostrate region as well as the base of erect filaments.
- Fig.6. Budding (Bu) probably associated with erect filament formation evident on prostrate portion after one day of staining.

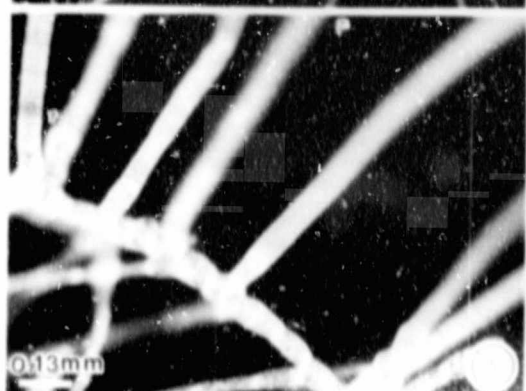
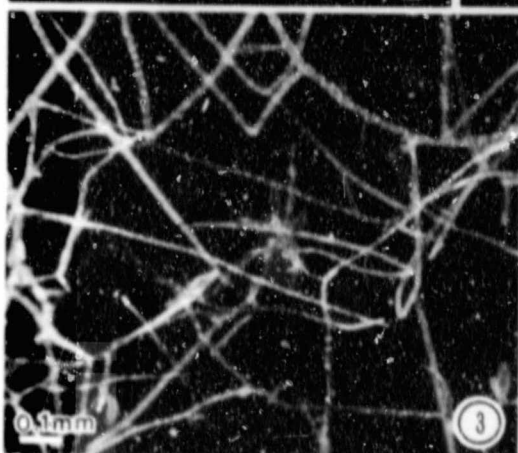
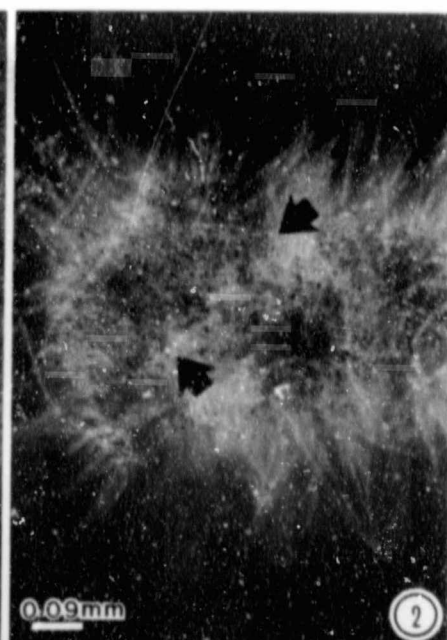
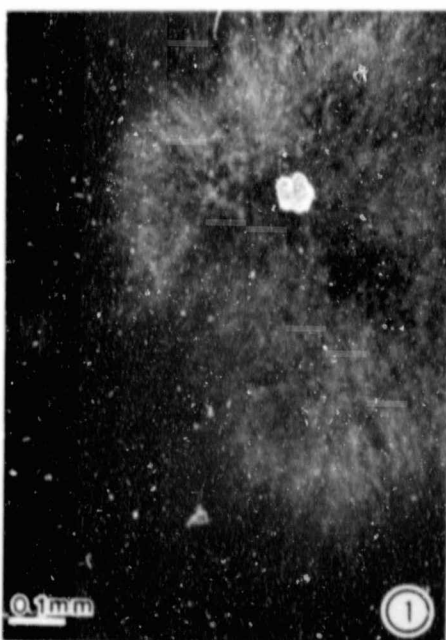


PLATE 13

Figs.7.-10. Ultraviolet fluorescence of filaments three days after staining with Calcofluor White M2R.

Fig.7. Absence of fluorescence in the basal portion of an erect filament indicates the presence of a meristematic region (MR).

Fig.8. The development of sporangia (S) on a meristematic region is evident.

Fig.9. Intercalary division on either side of a fluorescent cell.

Fig.10. The tips of erect filaments showing an absence of fluorescence and therefore indicating another site of elongation.

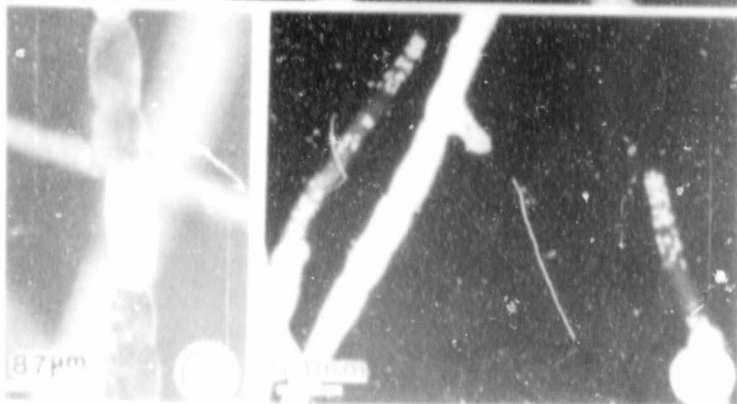
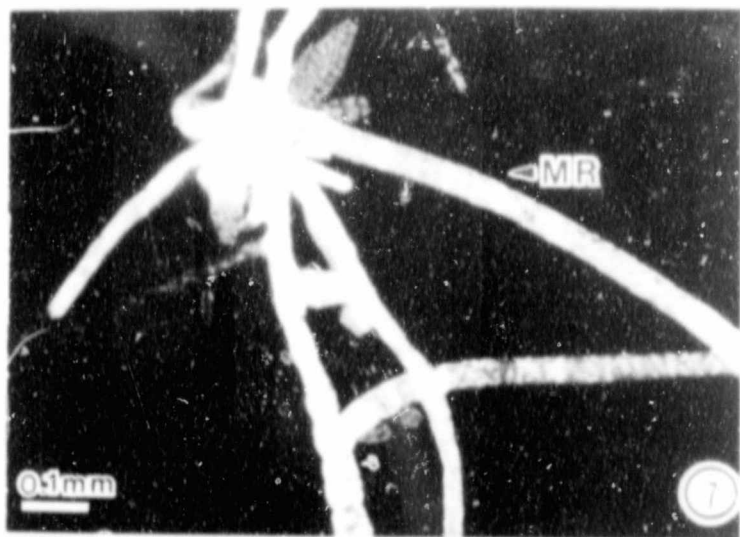


PLATE 14

Figs.1.-4. PAS staining of monitor sections of sporangia.

Fig.1. Longitudinal section through a sporangium reveals uptake of stain in the sporangial and locule walls. Lighter pink staining is evident on the edges of the cytoplasm in some locules (arrow).

Figs.2.-4. Longitudinal sections through sporangia showing the presence of PAS stain in the sporangial and locule cell walls.

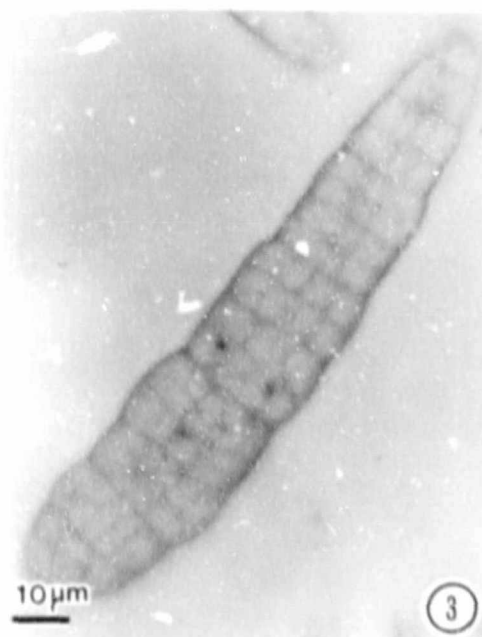
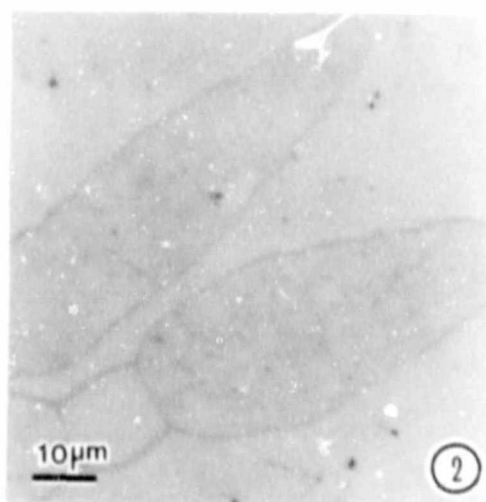
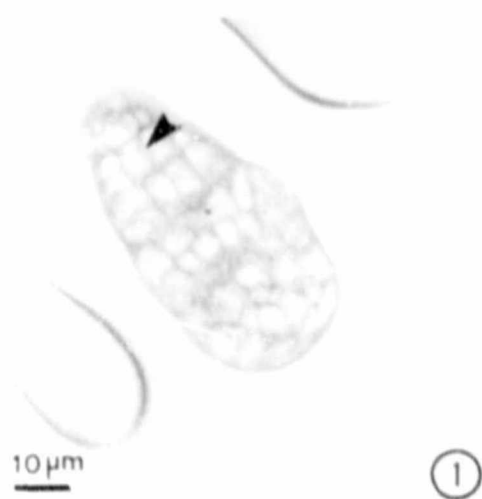


PLATE 15

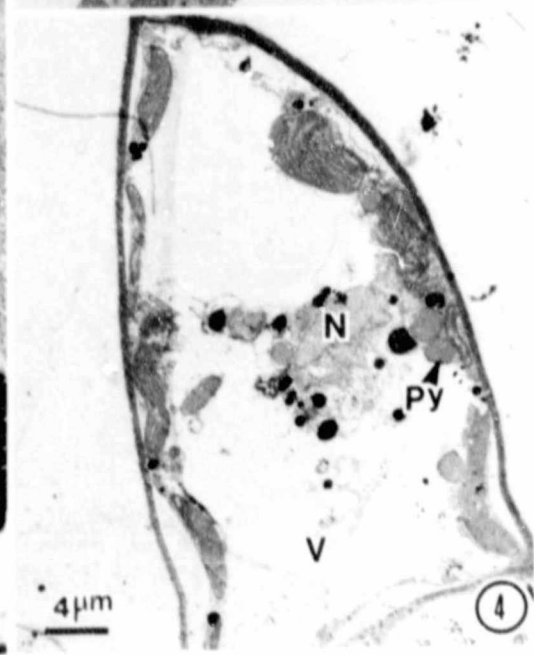
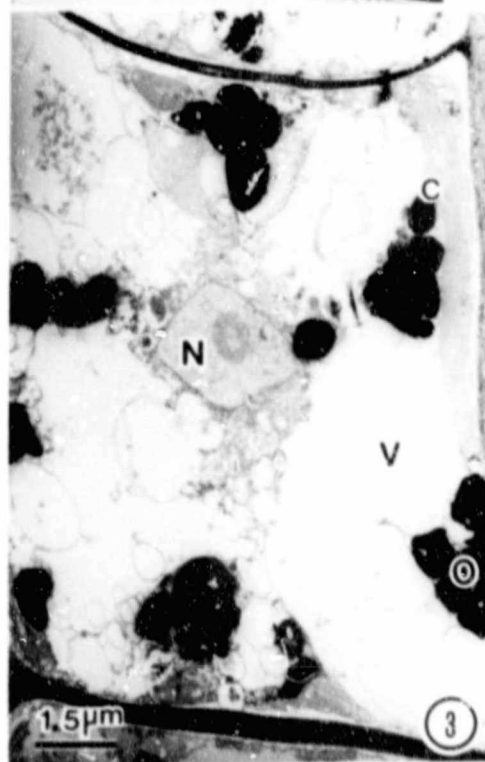
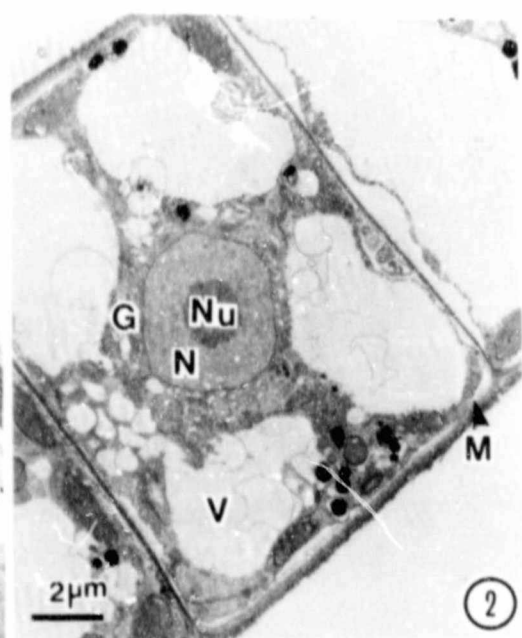
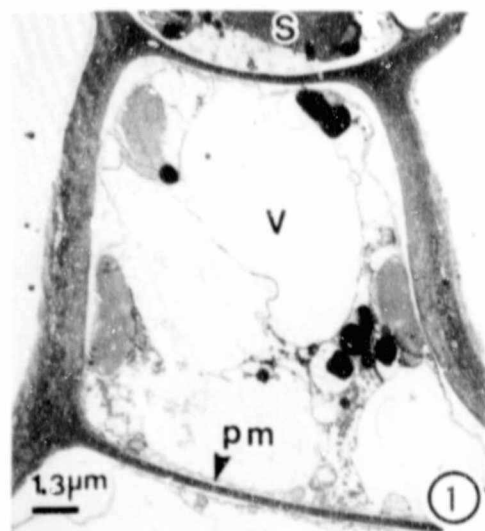
Figs.1.-4. Ultrastructure of vegetative regions of Feldmannia sp..

Fig.1. The vegetative cell subtending the plurilocular sporangium (S), with a large vacuole (V) and plasmodesmata (pm).

Fig.2. The square meristematic cell has a large central nucleus (N) with nucleolus (Nu) and a large Golgi system (G) associated with it. The vacuoles do not appear to occupy as much of the cell volume as in other vegetative regions. There are a large number of mitochondria (M) evident.

Fig.3. The vegetative cell from the prostrate region has a central nucleus (N), large vacuoles (V) and concentrations of osmiophilic bodies (O). Chloroplasts (C) are restricted to the periphery of the cell.

Fig.4. A portion of an erect filament has a central nucleus (N) and a large vacuole (V). Pyrenoids (Py) associated with chloroplasts are also evident.



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