The material was carefully given 2 washes, 10 minutes each, in sterile sea water.

2) Dehydration:

Two methods for removing water from the material were employed:

a) Air-drying: The glass slides were placed in a desiccator for 24 hours.

b) Critical point drying: specimens were taken through a dehydration series in ethanol as outlined for T.E.M. Once in 100% alcohol the material was covered in paper envelopes and placed in wire mesh baskets, which in turn are placed in the critical point dryer. The critical point of the liquid carbon dioxide occurs at 1072 pdr/sq. inch at 31°C.

3) Mounting:

The samples are then returned to a desiccator and then mounted with colloidal graphite or cellotape on the polished surface of a stubb.

4) Coating:

Stubs with specimens were coated with 20nm of gold and viewed with a Jeol JSM 840 scanning electron microscope.

3.6. Experiments to determine the influence of temperature, photoperiod, photon fluence and light quality on the growth and development of Feldmannia sp.

Feldmannia sp. was subjected to 4 treatments; photoperiod, temperature, photon fluence and light quality. For all treatments cultures were set up in the following way (Figure 8.):

Readings for each treatment were taken every 3-4 days using an Olympus inverted microscope. The following parameters were recorded: i) The number of cells per filament.

ii) The first appearance of sporangia.

iii) The numbers of immature, mature and empty sporangia.

The number of cells was counted until the first sporangia were formed. Further counting was considered too subjective since filaments obscured other filaments. Twenty-four readings were recorded from each Repli-dish. The number of readings was found to have standard errors within 10% of the mean for most treatments. All experiments were duplicated and in some instances triplicated. Since the effect of treatment on cell numbers and sporangia numbers was not constant over time, analysis of variance on all data would be meaningless. An indication of the differences in response between treatments could be obtained by way two way analysis of data collected on one day. The day where the difference between treatments was greatest, taken to be the last day of experimentation, was used. The results were statistically analysed using Duncan's multiple range test and qualitative changes during development under the various treatments was recorded photographically.

3.6.1. Photoperiod

Repli-dishes containing motiles were placed into one of six specialized photoperiod boxes (Figure 9.). Each box was fitted with a light and dark cycle timer and maintained constant temperatures of 20°C by means of fans. Thermometers in the boxes enabled constant checking of the temperature. Illumination was achieved with fluorescent tubes with photon fluence rates of 19$\mu Em^{-2}sec^{-1}$.
These boxes were set at a range of light/dark cycles; 18L:6D, 6L:18D, 12L:12D, 8L:7.5D:1.5L:7D, 14L:10D, & 10L:14D.

Readings were taken during the light phase of every light/dark cycle.

STEP 1

Plurilocular sporangia bearing filaments were placed in medium in a hanging drop slide.

STEP 2

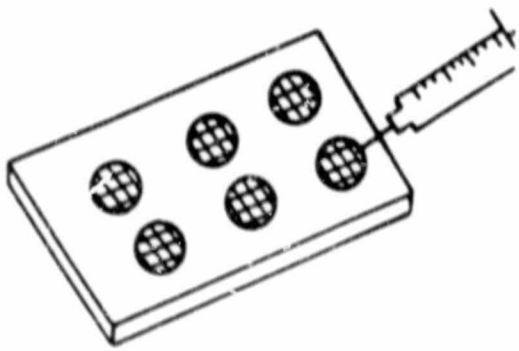
Within 2-4 hours motiles were released from sporangia and adult filaments removed

STEP 3

Medium containing motiles was sucked up with a syringe.

STEP 4

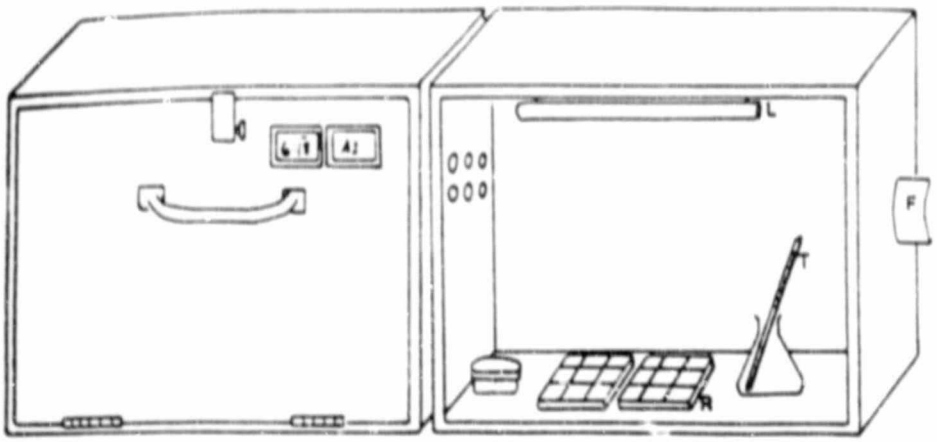
A few drops were placed into each compartment of a Repli-dish, the base of which was marked with a $1mm^2$ grid to ensure that the same germlings could be located again.



STEP 5

4ml of medium was added to each compartment and the Repli-dish was sealed with masking tape to reduce evaporation

Figure 8.: Proceedure for setting up cultures for experiments.



F-Fan R-Repli dish T-Thermometer

L-Cool flouresœnt light

Figure 9.: Boxes used for photoperiod treatments.

3.6.2. Temperature

Labcon growth cabinets were set at 10°C, 15°C, 20°C, 25°C, 30°C & 35°C respectively. Photon fluence rates in the growth cabinets ranged from 20 to 27$\mu Em^{-2}sec^{-1}$. The photoperiod for each cabinet was 14L:10D, which was selected since to is thought to resemble light/dark cycles most commonly experienced off the Natal coast. Possible fluctuations in temperature in the growth cabinets over a 24 hour period, was monitored with temperature hydrographs for a week. It was evident that slight fluctuations on either side of an average temperature occurred. (Figure 10.).

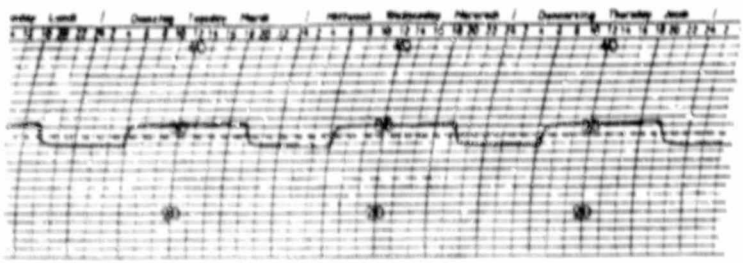


Figure 10 : Temperature fluctuations over a 3½ day period in an environmental cabinet set at 30°C.

Heat from the fluorescent tubes caused a gradual increase in temperature during the light period and when switched off the temperatures dropped, only to rise again during the next light period. Since this phenomenon was apparent in all of the growth cabinets, uniformity of conditions between treatments was thought to be maintained.

3.6.3. Photon fluence

A range of photon fluence rates were obtained by placing Repli-dishes under different fluorescent light source combinations in a growth cabinet. The cabinet was set at 20°C with a 16L:8D light/dark cycle. Fluctuations in temperature were recorded on a thermometer and temperatures never exceeded 2°C above and below 20°C.

One Repli-dish was placed at 14$\mu Em^{-2}sec^{-1}$ and another at 85$\mu Em^{-2}sec^{-1}$, using a single point light source. A third Repli-dish was subjected to light on three sides at 114$\mu Em^{-2}sec^{-1}$. Since in the last instance, more than one light source was used, each photon fluence reading reflects an average of readings taken in 6 directions, above, below and on all sides. An attempt was made to place the Repli-dishes sufficiently far away from the light source, so that discrepancies in temperature were reduced. One Repli-dish was placed in the dark.

As can be seen, the photon fluence and photoperiod were measured in separate experiments and use of the Mean Daily illuminance formula discussed in the literature review was not made. Mean Daily illuminance units would prove useful where the combined effects of photoperiod and photon fluence on growth and reproduction are tested.

3.6.4. Light quality

The spectral quality of green, blue and red cellophane paper was measured with a Varian, visible-light spectrophotometer (Figure 11.)

and found to resemble that of green, blue and red light. Repli-dishes containing zoospores were wrapped in this cellophane and placed on the same shelf in a growth cabinet set at 20°C with a 16L:8D. The temperature was constantly monitored and found never to exceed 3°C above and below 20°C. The photon fluence from a single source was 90 $\mu Em^{-2}sec^{-1}$ and the transparency of the different colours of cellophane paper was relatively uniform. Since the cellophane is transparent, it was possible to take readings without removing the cellophane. A Repli-dish, without cellophane was placed alongside those with cellophane as a control.

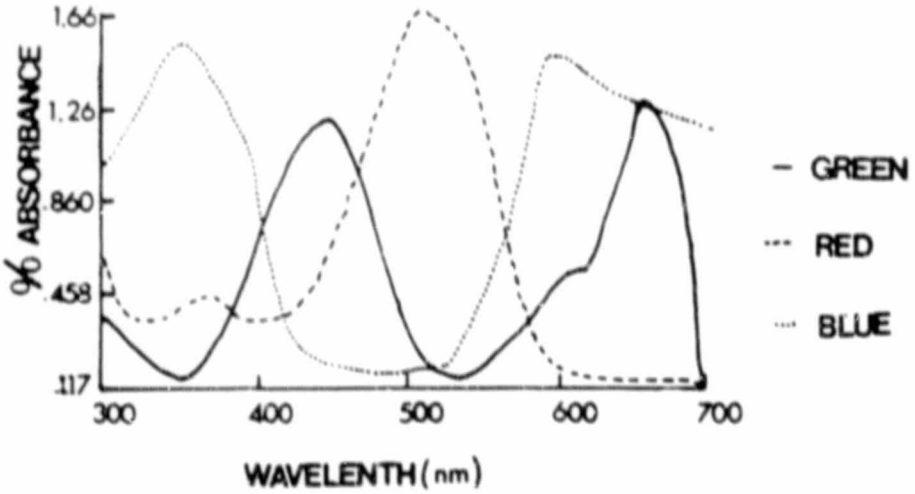


Figure 11.: The percentage absorption at different wavelengths of green, red and blue cellophane paper.

IV. RESULTS

4.1. Light microscope observations

Feldmannia sp. was originally found growing epiphytically on its host Codiophyllum natalense (Rhodophyta, Cryotonemiaceae). Since Feldmannia sp. has not been relocated in the field, its epiphytic nature has not been confirmed.

Feldmannia sp. is a tufted, fine filamentous algae, ranging in height from 2-4mm (Plate 1 fig.2.). It most commonly occurs in clusters: each cluster comprising a number of intertwining plants. Associations of the algae to form a continuous covering on surfaces, makes it difficult to ascertain the limits of one individual plant.

Three morphological regions are recognised: the first formed, horizontally-spreading, prostrate region, consisting of barrel shaped cells; an erect region of filaments that arise at irregular intervals from the prostrate region and multicellular rhizoids, which also arise from the prostrate region in an opposite direction of growth to the erect filaments (Plate 1 figs.2.& 3., Figure.12).

The first cell formed in an erect filament is commonly larger and more rounded in shape than the other filament cells (Plate 1 fig.3.). Meristematic cells, found near to the prostrate region, are square in shape when viewed from the side, while the more distal cells are rectangle in shape (Plate 2 fig.2.). Both the prostrate and erect regions are indeterminate in growth but the erect regions appear to grow more profusely.

The cytoplasm in all three regions possesses a central nucleus and peripheral chloroplasts. Chloroplasts in the prostrate cells are more abundant and discoid in shape, unlike those in erect filaments. Here chloroplasts are sparse, particularly in the distal cells, and oval in shape. Pyrenoids associated with the chloroplasts, as well as cytoplasmic strands holding the nucleus in place in the centre of the cell, are evident in some cells (Plate 2 figs.3.-5.; Figure.12).

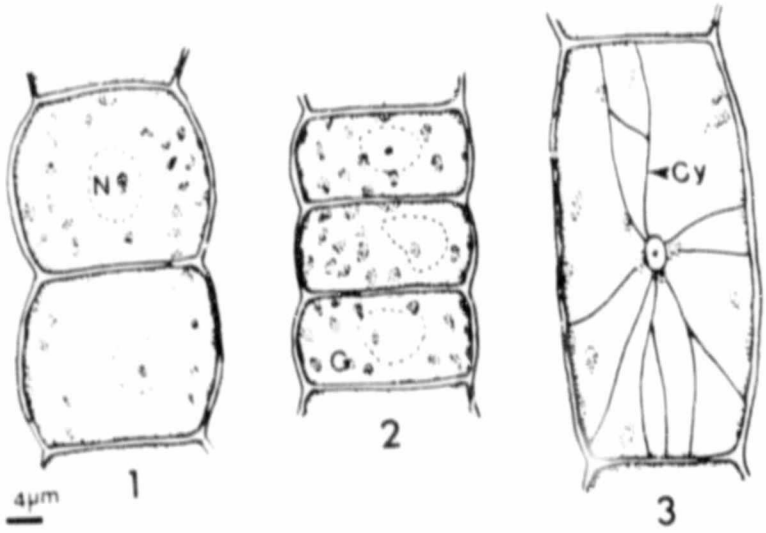


Figure 12.: Vegetative cells from three different morphological regions: 1. Prostrate, 2. Meristematic, 3. Erect filament. C=chloroplast, Cy=cytoplasmic strand, N=nucleus.

Feldmannia sp. rhizoids would, in an epiphytic association provide a means of attachment to the host without actually penetrating host tissue. Occasionally the rhizoid tips branch to form plug-like structures (Plate 1 fig.4.).

The prostrate and erect regions give rise to plurilocular sporangia in a number of localities along the filaments; directly on the prostrate region (Plate 3 fig.1.); below a meristematic region of an erect filament (Plate 1 fig.2.); at the tip of a short erect filament (Plate 1 fig.2.) and below secondary meristematic growth regions on erect filaments (Plate 1 fig.1.).

Sporangium form appears to vary within the lifetime of a single individual plant. The first formed sporangia are long, narrow and tapering (750-840µm in length) and develop below meristematic regions at the erect filament base. In more mature algae, erect filaments may become meristematic at the more distal ends. Sporangia produced

in these regions are comparatively shorter in length (240µm), more pair-shaped and produce fewer motiles. The angle from the point of origin is also wider in these smaller sporangia. The attachment to the filament may be either sessile, or by a subtending cell (Plate 3 fig.4.).

4.1.1. Growth of cultures

Subcultures of Feldmannia sp. were grown and maintained in culture as outlined in the materials and methods. After an initial lag phase in which the plants were adjusting to culture conditions, the growth was very rapid. Very rarely, contamination by a colourless unicellular algae and bacteria was observed, but this did not appear to interfere with the growth of the cultures.

Senescence was accompanied by a bleaching in the colour of the plant. It was possible to restore the growth of those filaments with a small trace of brown colouration by placing them into fresh medium, but once the filaments become clear, senescence was irreversible.

4.1.2. Development and reproductive differentiation

The time taken from settlement of zoospores to the production of sporangia on mature filaments, is between 14 and 17 days, when grown at 20°C, at light intensities of 100µEm^{-2}sec^{-1} and at a 16L:8D cycle. Unipolar germination of the zoospores is evident approximately 24 hours after release (Plate 4 figs.1.-9.) and the first formed cells are thought to constitute the prostrate region cells. There is a gradual increase in cell number until after 4-6 days an erect filament is produced. Rhizoids are not produced until much later indicating that some form of adhesion, possibly chemical, must be responsible for attaching the germlings to the substrate. The original zoospore cell is slightly larger and more rounded in shape than the newly formed cells and is always in evidence during the early stages of development. Occasionally the pattern of early sporeling development appears to resemble that of Feldmannia globifer

(Kutz) Hamel (Clayton 1974). Here cell elongation occurs on either side of the original zoospore cell, giving rise to the prostrate region in one direction and the rhizoids in the other.

A number of stages in the development of the sporangia were observed. Initially a vegetative cell produces a slight bulge to one side of the cell (Plate 5 fig.1.). The bulge increases in size and a cross wall separating it from the vegetative cell is formed (Plate 5 fig.2.). Until now, sporangium development resembles vegetative filament production. The bulge increases in size by a series of parallel and perpendicular divisions giving rise to a number of cubical locules (Plate 5 fig.4.). A subtending cell which does not undergo any vertical divisions is now in evidence. Further vertical and horizontal divisions take place and the sporangium increases in size (Plate 5 fig.5.). At maturity (Plate 5 fig.6.), red eyespots are evident in the cells within each locule. The sporangia do not have a set number of locules and there may be from 68 to 400 locules per sporangium. The final stage is reached when the zoospores have been released from the sporangium and only an empty sporangial case remains (Plate 6 figs.1.& 2.).

Zoospore release from the sporangium lasts from 4 to 7 minutes. The tip of the sporangium begins to bulge (Plate 7 fig.1.) and finally ruptures releasing a group of zoospores through a pore at the tip of the sporangium (Plate 7 fig.2.). The zoospores are surrounded by mucilage and remain stationary at the tip of the sporangium for a short period, before actively swimming free (Plates 7 & 8 figs.3.-10.). The zoospores continue to exude out of the sporangium in single file, moving up from the more basal loculi (Plate 8 figs.9.-15.), until no more remain (Plate 8 fig.16.). The release of zoospores from a sporangium can be viewed on the attached video tape.

The movement of the zoospores is erratic more specifically, they tend to swim in a straight line but stop when they come into contact with another zoospore or a filament. Once they have moved around the obstacle, they continue in a more or less straight line and remain motile for up to 2 hours before settling onto the substrate. The zoospores are positively phototactic and favour settlement near the

light source. Zoospores released in a Petri-dish partially covered with tin foil settled mostly in the uncovered region.

Observations of the settling patterns of zoospores revealed that in addition to settling at the bottom of a Repli-dish, zoospores often settled on adult filaments (Plate 9 fig.1.) and on one another (Plate 9 figs.1.-4.). The result of zoospores settling on top of one another, was bands of free floating germlings on the surface of the medium (Plate 9 figs.2.& 3.).

An attempt was made to determine whether Feldmannia sp. zoospores preferred settling on their own adult filaments, or those of other algae or even other surface types such as shells and driftwood. In this experiment, the zoospores appeared to have no particular substrate preference and settled on all the different algae, the driftwood, the shell and the base of the glass culture dish (Plate 10 figs.1.-3. & Plate 11 figs.4.-7.). Similar results were obtained when the experiment was repeated.

Following settlement, the zoospores attach themselves to the substrate. The fact that the rough critical point drying treatment used for scanning electron microscopy failed to dislodge zoospores attached to a glass slide, indicates that they are firmly attached to the substrate. The nature of the adhesive used for attachment was not determined, however, the copious amounts of mucilage associated with settled zoospores (Plate 29 fig.2) may form the means of attachment.

4.1.3. Determination of regions of cell elongation

The optical brightner, Calcofluor White M2R binds readily to the cellulose in the cell walls of Feldmannia sp. filaments and fluoresces brightly, giving off a light blue colour, under violet light. Chloroplasts exhibit autofluorescence under violet light and appear red.

A cluster of germlings viewed directly after staining (Plate 12

fig.1), showed uniform uptake of Calcofluor M2R. One day after staining, the same cluster (Plate 12 fig.2) showed new growth and a large number of chloroplasts, which appear red, towards the centre of the cluster.

Mature filaments took up the optical brightner uniformly and fluoresced brightly under violet light (Plate 12 figs.3.& 5.). Large numbers of chloroplasts in the sporangia (Plate 12 fig.3.) and in the prostrate regions (Plate 12 fig.5.) detracted from the fluorescence in these regions. The cross walls of erect filaments fluoresced more than the surrounding filament portions, indicating concentrations of cellulose (Plate 12 fig 4.). One day after staining, buds developing on prostrate cells were visible (Plate 12 fig 6.). These buds did not fluoresce and were rich in chloroplasts. Three days after staining, cell elongation was evident at the base of erect filaments, where fluorescence was markedly reduced (Plate 13 fig.7.). From these meristematic regions large numbers of newly formed sporangia were evident (Plate 12 fig.8.).

The lack of fluorescence on either side of a fluorescent cell in the prostrate region, indicates yet another growth region (Plate 13 fig.9.). The distal ends of erect filaments also appeared to be capable of new growth and chloroplasts are concentrated at the tips (Plate 13 fig.10.).

Evidently there are four sites of cell elongation; the base of erect filaments, higher up on erect filaments, on prostrate cell regions and distal ends of erect filaments. The latter growth type is apical, while the other three are intercalary.

Unstained motile cells observed under the violet light excitation filter, were extremely dark and difficult to see, even at high magnifications. Slight red autofluorescence of chloroplasts was detected within the motile cell. There was no autofluoresence of the flagella.

4.1.4. Staining of carbohydrate in sporangia

The cell walls surrounding sporangia and between loculi were stained pink by Schiff Periodic acid, indicating the locality of carbohydrate material (Plate 14 figs.1.-4.). A thin layer of cytoplasm, adjacent to the cell wall, in the region of the matrix, also stained lightly (Plate 14 fig.1.). However this detail is not evident in the other photographs.

4.1.5. Determining the movement of dye through the sporangial wall

The blue colouration of motiles within sporangia of Feldmannia sp. indicated that the sporangial wall is semi-permeable to Toluidine blue. The vegetative portions of this alga were also stained blue, however, whether these regions are semi-permeable or not is not known.

4.2. Electron microscope observations

An important feature in interpreting sections is determining the plane of sectioning. With regards to the sporangia, the section plane could be determined with a high degree of certainty due to the shape of the sporangia and the orientation of embedded sporangia before sectioning. Establishing the plane of sectioned zoospores was possible by observing the outline of the cells and the orientation of organelles within each zoospore.

4.2.1. Ultrastructure of the vegetative thallus

Longitudinal sections through different regions of the thallus reveal the essential features of vegetative cell ultrastructure in Feldmannia sp. (Plate 15 figs.1.-4.). Few differences between the various vegetative regions exist. The shape of the cells in section is more or less rectanglular, with the shortest wall being the adjoining, cross wall. The exception is the meristematic cell (Plate 15 fig.2.) which is also rectangle, but here the longest wall

constitutes the cross wall of the cell.

The bulk of the cell volume is made up of a large vacuole, containing spherical bodies of granular and fibrillar material. This material may constitute cytoplasmic strands which hold the nucleus in the centre of the cell in place. The cytoplasm is restricted to the periphery of the cell and to a thin layer around the nucleus in prostrate, subtending sporangia and erect cells of the filament, while in meristematic regions, greater amounts of cytoplasm surround the nucleus.

The nuclei are usually spherical in section (Plate 15 fig.2.), however this is not evident in the more oblique sections of the prostrate region (Plate 15 fig.3.) and erect region (Plate 15 fig.4.) where only portions of the nucleus have been sectioned. The nuclei are approximately (3-4µm) in size and contain a centrally placed nucleolus. Each nucleus is surrounded by a double nuclear envelope and is often associated with dictyosomes. Large numbers of dictyosomes are evident around the nucleus of the meristematic cell. The general morphology of the mitochondria agrees with that already described from other brown algae (Bouck 1965, Oliviera & Bisulptura 1973, Russell 1974).

The elliptical chloroplasts are restricted to the periphery of the cell and orientated parallel to the long axis of the cell. Pyrenoids are found in close proximity to the chloroplasts. Other organelles found near to the chloroplasts are mitochondria and endoplasmic reticulum, which are not easy to resolve at these low magnifications. Spherical, electron-dense, osmiophilic bodies are also evident in the cytoplasm. They occur in noticeably larger amounts in the cytoplasm of the prostrate cell, than in the other cells. It is not known whether this is always the case.

The cell wall is fibrillar in nature and thicker in the subtending sporangial cell wall than in other regions. This could possibly be due to the plane of sectioning. Plasmodesmata are evident in the cell walls between two adjoining cells (Plate 15 fig.1.).

4.2.2. The Ultrastructure of zoospore production

A number of stages in the development of the sporangia and associated zoospores (used synonomously with motile cells) were identified at the light microscope level (Plate 5 figs.1.-6.). An attempt was made at examining the ultrastructure of these various stages and each will be discussed in turn.

In the early stages of zoospore production, divisions occurred mostly parallel to the long axis of the vegetative cell (Plate 16 fig.4.). A large, central nucleus, with nucleolus dominates the cytoplasm (Plate 16 figs.1.,2.& 4.). The nucleus has a granular appearance, since it is rich in DNA and is surrounded by a double nuclear envelope. Numerous dictyosomes, are found alongside the nucleus (peri-nuclear) and it is unsure whether the forming or mature face is closest the the nuclear envelope, since vacuoles are evident budding from either side of the dictyosome (Plate 16 figs.1.& 2.). Numerous spherical inclusions are evident adjacent to the nucleus (arrow, Plate 16 fig.2.). These appear similar in nature to the larger dictyosomal vesicles. Strands of endoplasmic reticulum, extending into the cytoplasm are also found within close proximity to the nucleus.

The cytoplasm is also granular and probably rich in RNA. Numerous small vacuoles, many containing fibrillar and granular material are found within the cytoplasm. Large, inclusions are often associated with these vacuoles and may occupy a large portion of the total volume of the vacuole (Plate 16 fig.1.). The chloroplasts, occurring at the cell periphery, the mitochondria, pyrenoids and osmiophilic bodies also occur in the cytoplasm. While the relative sizes of the these organelles may vary with the age of the developing zoospore, the ultrastructrural detail of these structures does not vary with age and this detail will be examined at a later stage.

At the 'intermediate' stage of development, the nucleus occupies a large proportion of the total cell volume and nuclear pores in the nuclear envelope are in evidence (Plate 17 figs.1.& 2.). The nucleolus is not always centrally placed, but may occur towards the edge of the nucleus (Plate 17 fig.1.).

The size of the chloroplasts (2µm by 0.6µm) has increased from the previous stage and genophores, represented by lighter regions at the poles of the chloroplasts, occur. The vacuoles now occupy a relatively small proportion of the cell volume. The cytoplasm appears to have pulled away slightly from the cell wall, resulting in a space on either side of the cell wall. This cavity contains numerous globules which may either be remains of cytoplasmic material, products required for cell wall formation, or possibly even an artifact.

Numerous perpendicular and parallel divisions have taken place, giving rise to many cubical and rectangle locules (Plate 17 fig.2; Plate 18 fig.1.). The surrounding sporangial wall comprises a dense and less dense fibre layer and is far thicker than the cell walls between loculi. No plasmodesmata between adjoining loculi are in evidence.

At maturity, sporangia contain large numbers of locules (Plate 19 fig.1.). The newly formed cell walls, associated with the most recent cell divisions are thinner than the older cell walls. There is no set pattern of cell division, however, 'compartments', separated by older, thicker cell walls and containing approximately 8 cells are evident.

A number of ultrastructural changes take place in the mature sporangium. The cell contents shrinks further away from the cell walls, leaving, in some instances, large spaces on either side of the cell wall, containing granular material. Flagella in every locule are in evidence for the first time, and the chloroplasts contain intraplastidial eyespots. Osmiophilic bodies are in greater abundance than in the previous stages.

Just prior to release, the zoospores round up and the cell wall material becomes very thin, possibly indicating a breaking down prior to release (Plate 19 fig.2.). At this stage mitochondria are abundant in the zoospores.

Detail of a mature locule (Plate 20 fig.1.), reveals paramural bodies in the space left by the contracting cytoplasm. These bodies may consist of many concentric membranes, or alternatively a number of membranes are loosely arranged within a surrounding membrane (Plate 23 fig.3.). It is uncertain as to whether these paramural bodies occur inside or outside the plasmalemma. The occurrence of fibrillar and granular material within the space left by the contracting cytoplasm may indicate that it constitutes a matrix.

Once again the nucleus is centrally placed (Plate 20 fig.1.) and possess distinct nuclear pores (Plate 20 fig.3.). A large stalked pyrenoid extends from the chloroplast (Plate 20 fig.1.). The mitochondrion (Plate 20 fig.2.) is elongated to ovoid in form and surrounded by a double membrane, the inner of which forms tubular cristae, which have slightly constricted bases. The cristae are numerous and closely packed in the lumen. The peri-nuclear, dictyosomes (Plate 20 fig.4.), are orientated with their forming face closest to the nuclear envelope and the mature face furthest away. Many vacuoles occur in association with the mature face. In mature sporangial cells these vacuoles contain fibrous-like substances (Plate 21 figs.1.& 2.), similar to the fibres located in the matrix surrounding the cell.

Chloroplast structure varies little between the different stages of zoospore production (Plate 22 figs.1.-7.). The thylakoids are arranged in groups of threes and run parallel to the long axis of the choroplast. Occasionally single lamellae interconnect lamellae bands (Plate 22 fig.6.). A girdle lamellae, within the stroma, encircles the rim of each chloroplast. Distinct light regions at the chloroplast poles denote areas of DNA accumulations or genophores and ribosomes also contributes to the granular appearance of the stroma (Plate 22 fig.7.). Small osmiophilic bodies are located in the chloroplast stroma.

The pyrenoid, having a uniform, granular appearance, is attached to the chloroplast by a narrow stalk (Plate 22 figs.2.& 6.), and generally protrudes towards the centre of the cell. The chloroplast and pyrenoid are surrounded by the chloroplast envelope and the

chloroplast endoplasmic reticulum. While the stalk is not always evident, the presence of the endoplasmic reticulum and chloroplast envelope around the pyrenoids, shows that they do in fact form part of the chloroplast. The chloroplast endoplasmic reticulum is closely appressed to the chloroplast on the side furthest from the nucleus, but on the opposite side this membrane, comes away from the chloroplast envelope and forms a space. This space contains numerous membrane profiles.

Eyespots are visible in the chloroplasts of mature sporangial cells (Plate 22 figs.4.& 5.). Each eyespot is composed of a single layer of lipid-like globules and appears to occur in a depression on the side of the chloroplast, often in close proximity to the flagella (Plate 22 fig.4.).

Lysosomes, containing many smaller vesicles, some electron-dense, are evident in the cytoplasm of relatively mature sporangial cells (Plate 23 figs.1.& 2.). Occasionally these were found closely appressed to cell walls and may play a role in cell wall formation. The surrounding cytoplasm is very granular (Plate 23 fig.4.). Paramural bodies (Plate 23 fig.3.), in this instance, occur external to the plasmalemma.

A number of stages in the development of cell walls is recognised. The order in which they are discussed may not be strictly correct since these observations were made at random, using sections of sporangia of different ages.

Initially a thin layer of cell wall material is deposited perpendicularly to the older, existing walls (Plate 24 fig.1.). Later, the vacuoles on either side of the developing wall become evident (Plate 24 fig.2.) and are free of any inclusions. Numerous smaller vacuoles appear to be in close proximity to the dictyosome and it is not clear whether or not these are precursors of the larger vacuoles. Two large nuclei, with nucleoli in each adjacent cell are evident. At this stage, the developing cell wall is not entire, but segmented in parts (Plate 24 fig.4.) and membranous in nature. At a seemingly later stage of development, vacuoles decrease in size and

globular material is deposited in the region of the developing cell wall (Plate 24 fig.3.). Mitochondria are located close to the developing cell wall at all stages. In some instances vacuoles rich in fibrous-like substances apparently released their contents near the region of cell wall formation (Plate 25 figs.1.& 2.).

The globules evident in earlier stages of cell wall development are in evidence on one side of the locule wall (Plate 26 figs.1.& 2.) and a mature sporangial wall (Plate 26 fig.6.). Mature sporangial walls (Plate 26 figs.3.& 6.) are composed of a number of fibrous layers, which may be laid down parallel or perpendicular to the long axis of the cell and may be tightly or loosely woven. As many as 4 layers were evident (Plate 26 fig.6.). Unlike these outer sporangial walls, the locule walls are composed of a single layer of fibres, laid down in a haphazard fashion (Plate 26 figs.1.,2.& 3.). Plasmodesmata occur between subtending sporangia cells and mature locules (Plate 26 fig.4.) and between adjacent vegetative cells (Plate 26 fig.5.).

At an 'intermediate' stage of zoospore formation, a centriole and associated microtubular root system, the first signs of flagella formation, were evident (Plate 27 fig.1.).

In mature sporangia, flagella develop in separate flagellar vesicles which are confluent with the matrix as they contain matrix-like globules and fibres (Plate 27 fig.2. & Plate 28 fig.1.). In section the flagella often occur in groups of three within the flagellar vesicle. It is thought that the flagellar vesicles are external to the plasmalemma, since in many sections the flagella are not enclosed in the cytoplasm. The flagellar vesicle cavity, appears to be filled with granules and fibres, similar to that found in the matrix.

Two types of flagella are produced; a smooth flagellum and a whiplash flagellum bearing mastigonemes (Plate 27 fig.4.). The smooth flagellum is often more closely appressed to the cytoplasm than the tinsel flagellum (Plate 27 fig.3.). Sections through the tinsel flagella reveal that during zoospore development, mastigonemes emerge from one side of the flagella only (Plate 28 fig.3.). The 9

& 2 arrangement of microtubules, characteristic of most flagella, is present in the flagellar shaft region (Plate 28 fig.4.).

The region of insertion of the flagella is thought to be somewhere between the nucleus and the chloroplast (Plate 27 fig.6. & Figure.5.), however, this was not clearly established. The intraplastidial eyespot is also thought to be associated with this insertion region (Plate 27 fig.3. & Figure.5.).

4.2.3. The ultrastructure of the zoospores

Little detail of zoospores structure can be resolved at the level of the light microscope (Plate 29 fig.4.). Only the nucleus and flagella can be seen at this magnification.

The presence of two flagella per zoospore was confirmed by negatively stained zoospores (Plate 29 fig.1.). Neither of the flagella had mastigonemes and these were probably lost during specimen preparation. An impression of the possible arrangement of mastigonemes on the tinsel flagellum is evident in Figure 13. The flagella emerged laterally, half way along the side of the zoospore. The tip of the shorter of the two flagella, had a thinner extension, which could be an acronema, although the possibility of an artifact here cannot be ruled out.

Scanning electron micrographs of zoospores (Plate 29 fig.2.) showed the presence of rod-shaped bacteria attached to the surface of the zoospore. Larger rod-shaped bacteria were present in the mucilage, thought to be a different mucilage to that exuded from the sporangium at the time of zoospore release. It is clear that zoospores attach to the substrate by their narrower, anterior ends.

The ultrastructure of the zoospore (Plate 29 fig.3. & Figure 13.) is not unlike that of mature sporangial cells. Once again the nucleus is centrally placed and surrounded by a double nuclear membrane, possessing pores (Plate 30 fig.1.). Endoplasmic reticulum is found in close proximity to the nucleus. Two large chloroplasts each with

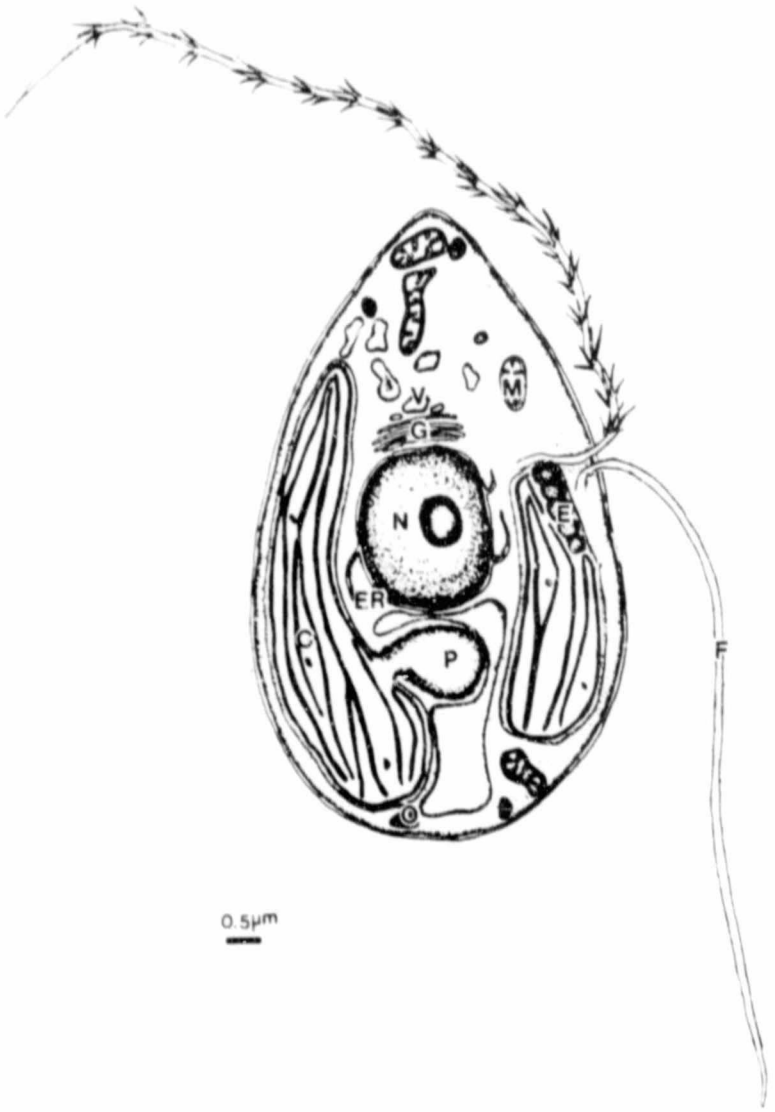


Figure 13.: Diagramatic representation of the arrangement of organelles of a Feldmannia sp. zoospore.
(Flagellar roots have been ommitted and the helical arrangement of the mastigonemes on the tinsel flagellum is based on assumption only.)
C= Chloroplast, E= Eyespot, ER= Endoplasmic reticulum,
F= Flagellum, G= Golgi, M= Mitochondrion,
N= Nucleus, O= osmiophilic body, P=Pyrenoid,
V= Vacuole.

stalked pyrenoids (assumed) are located in the basal region of the zoospore. The eyespots (Plate 30 fig.3.) are in close association with the flagella and the tinsel flagellum is located in a slight depression of the zoospore. The orientation of this micrograph is upside down, since the tinsel flagellum is usually located higher up than the smooth flagellum. The anterior region of the zoospores contains vacuoles (Plate 29 fig.3.) and a tangential section through this anterior region reveals an abundance of mitochondria interspersed in the vacuoles (Plate 30 fig.2.).

The blebs and bulges extending from the plasmalemma around the zoospores are probably related to a fixation artifact.

Cell walls form rapidly around settled zoospores (Plate 31 figs.1.-4.), which are once again shown to attach to the substrate by their anterior ends (Plate 31 fig.4.). The cell walls appear to be slightly thicker at this region of attachment. The vacuoles occupy a large portion of the cell volume. Unusual bone-shaped, paramural bodies were evident inside the cytoplasm and alongside the cell wall (Plate 31 fig.3.).

4.3. Experiments to determine the influence of temperature, photoperiod, light intensity and light quality on the growth and development of Feldmannia sp.

Feldmannia sp. was found to be a good system for experimental studies; it grows quickly, it is small so that a large number of plants can be used per treatment and it has clearly defined cells that can be measured with relative ease.

The percentage germination of zoospores in each experiment was approximately 99%, with the exception of those zoospores which remained inactive in extreme environmental conditions. The occasional presence of a colourless flagellate contaminant in very few of the Repli-dishes, did not appear to influence the growth and development of the plants.

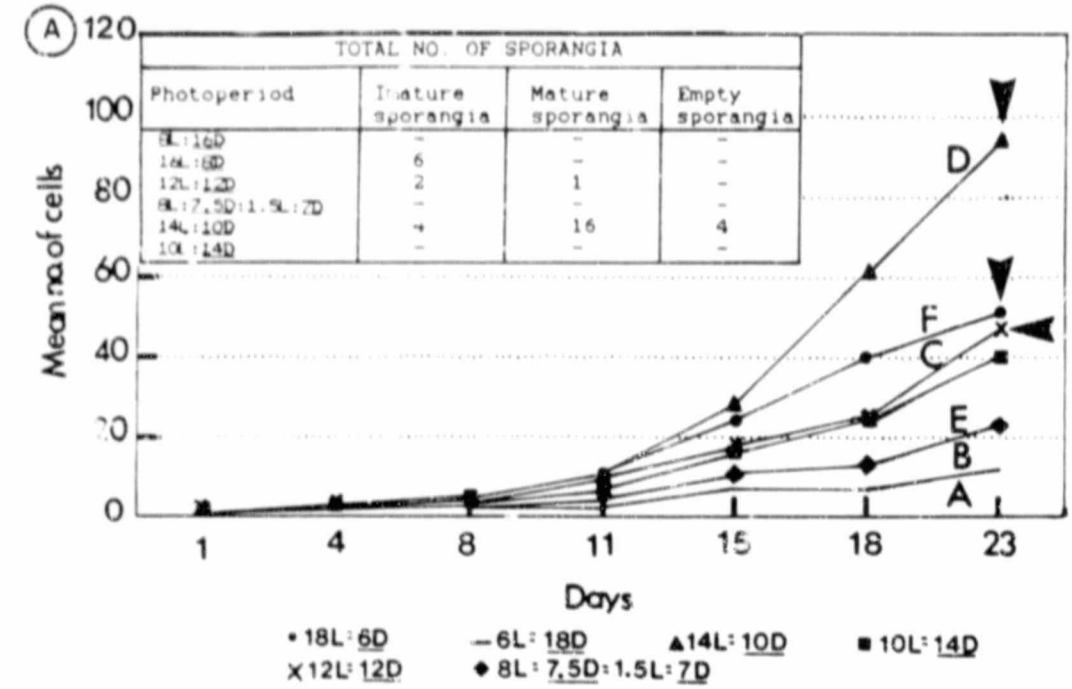
Growth followed a general trend in all of the environmental conditions tested. Initially there was a lag phase, lasting approximately 6 days, during which the alga established itself in the new culture medium. Thereafter, growth was rapid until finally a growth plateau (or stationary phase of growth) was reached. Reproduction occurred during the rapid phase of growth. The time taken to become reproductive and then reach a plateau depended on the environmental variable. No unilocular reproductive structures were ever formed during the course of this investigation.

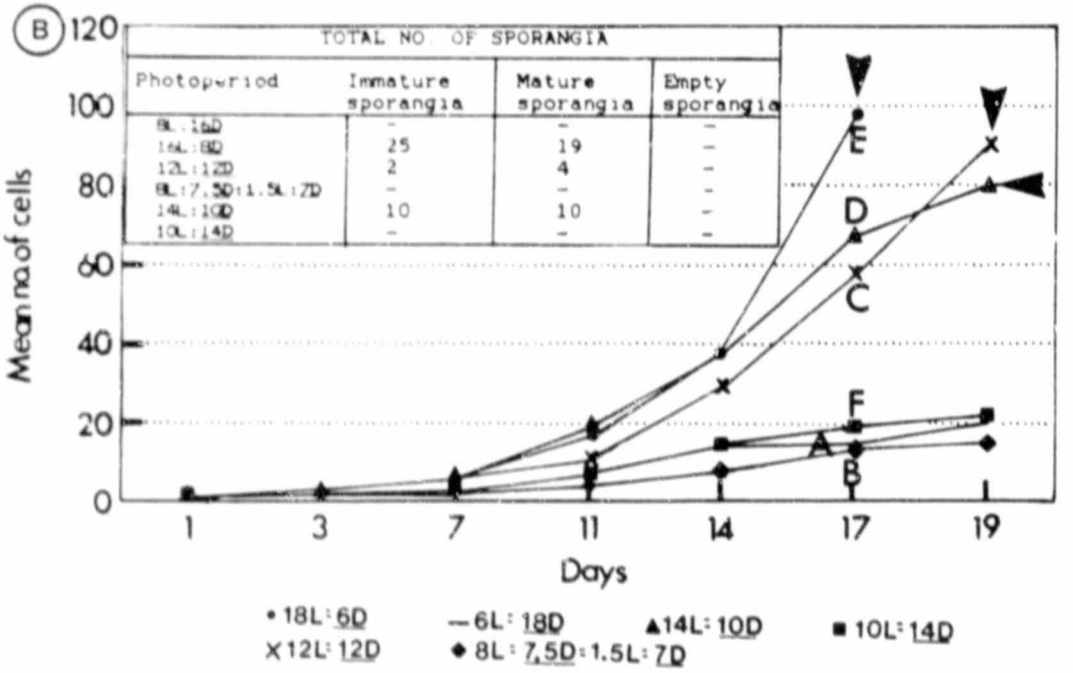
The results of each experiment are recorded graphically (Figs.14.-17c). The total number of sporangia, recorded on the last day of experimentation, is tabulated as an insert on each graph. The low numbers of sporangia on these tables is to be expected in view of the number of samples recorded. An arrow indicates the first observation of a reproductive phase. However, since readings were only taken at 2,3 or 4 day intervals, the number of each type of sporangium, namely immature, mature and empty, may provide a better indication as to which plants became reproductive first. Here we assume that the plants with the most empty sporangia, were the first to become reproductive.

The time in days is recorded along the X-axis and the growth as an increase in mean cell number along the Y-axis. Letters of the alphabet above readings on, usually, the last day of experimentation reflect the significance of variance for each treatment according to Duncan's grouping test. Means with the same letter are not significantly different while different letters show significant differences at the 0.01 confidence limits. For example, the value at letter A is significantly different from the value at B, while A is not significantly different to another point A.

4.3.1. Photoperiod

In essence the longer the day-length, the sooner the alga become reproductive (Figs. 14a & 14b). The light/dark cycles of 18L:6D, 14L:10D, and 12L:12D were first to become reproductive and had





Figures 14a & 14b: The influence of photoperiod on growth and reproduction in Feldmannia sp..

greater numbers of cells than the shorter light/dark cycle treatments. More specifically, while in Fig.14a the 18L:6D light/dark cycle was first to produce sporangia and had the greatest effect on the mean cell number average, in Fig.14b, 14L:10D appeared to be the most favourable photoperiod for growth. Both of these treatments had high sporangial numbers. By looking at the numbers of empty sporangia in Fig.14b, it seems possible that 14L:10D was the first treatment to become reproductive.

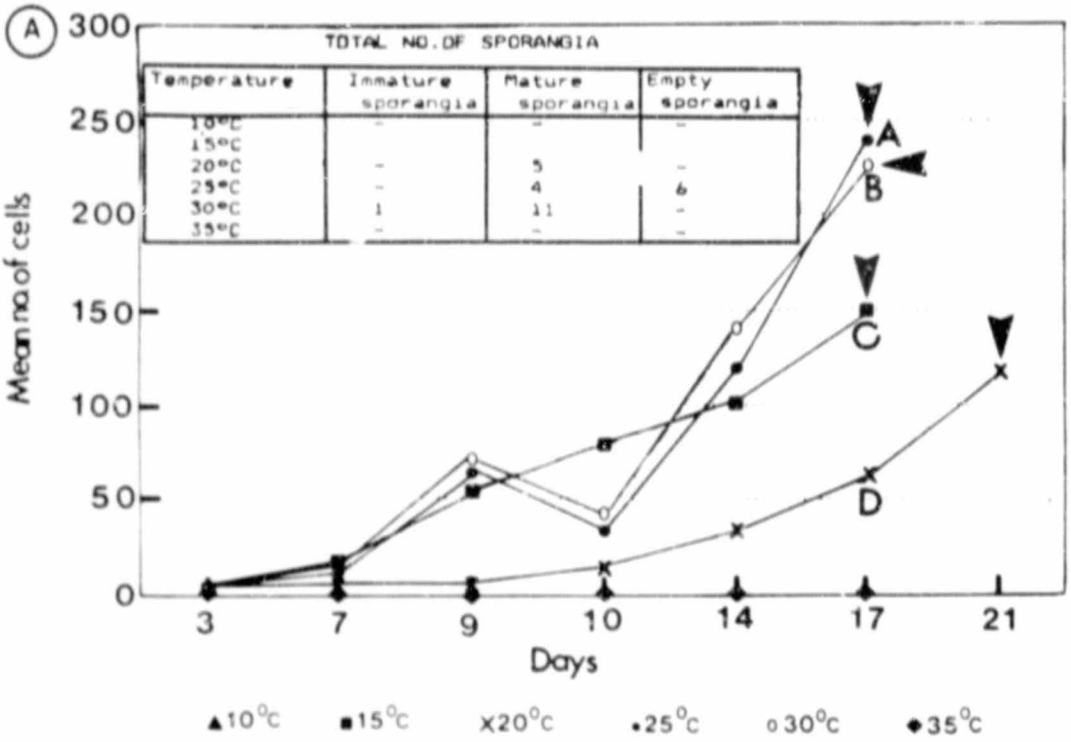
None of the short day treatments in Figs.14a & 14b viz. 6L:18D, 10L:14D and 8L:7.5D:1.5L:7D became reproductive even after 27 days. However, when these Repli-dishes had the media changed and were placed in conditions of 12L:12D, they all became reproductive within 3 days.

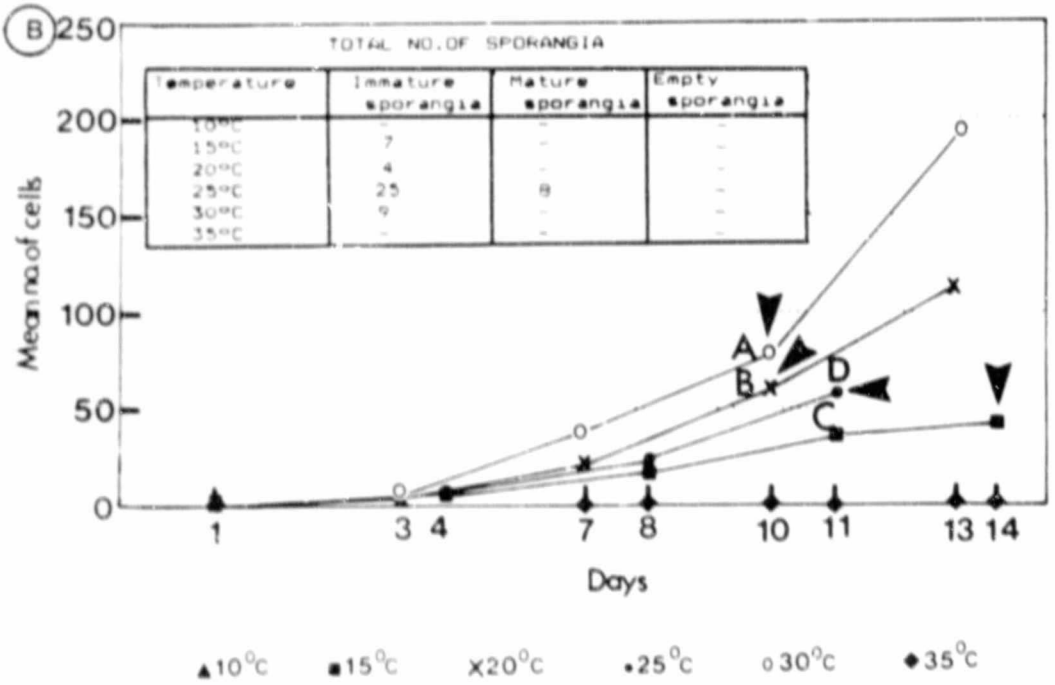
The mean cell numbers and the age of the filaments at which reproduction occurred, appeared to vary between repetitions of the same experiment (Figs.14a & 14b). In Fig.14a sporangia were in evidence after 17 to 19 days and mean cell numbers ranged from approximately 100 to 78. In Fig.14b the first indications of reproduction were apparent after 23 days of growth and mean cell numbers at this stage ranged from 95 to 45. In Fig.14a the mean cell numbers of the treatments that became reproductive were markedly greater than in those treatments that were not reproductive. This difference in mean cell numbers between reproductive and non reproductive treatments is not distinctive in Fig.14b.

No morphological differences were observed between treatments.

4.3.2. Temperature

It is evident from Figs.15a and 15b, that while extreme temperatures of 35°C and 10°C inhibit growth, higher temperatures ranging from 15°C to 30°C are most favourable for growth. The filaments with the greatest cell number means were the first to become reproductive and also had the highest total number of sporangia. If the assumption that the treatment with the highest number of empty sporangia is the first to have been reproductive is used, then those





Figures 15a & 15b: The influence of temperature on growth and reproduction in Feldmannia sp..

filaments grown at 25°C must have become fertile before the other temperature treatments. Unfortunately, the total number of sporangia at 15°C in Fig.15a was not recorded.

The time taken to become reproductive, and the mean number of cells at which reproduction is first observed, varied between the experiments represented by Figs. 15a and 15b. The filaments in Fig.15b became reproductive after 10 to 11 days, from a cell number mean of 40 to 70 cells, whereas in Fig.15a reproduction was evident after only 17 days, with cell number means from 120 to 240. These large discrepancies make it difficult to make any sound conclusions from the data.

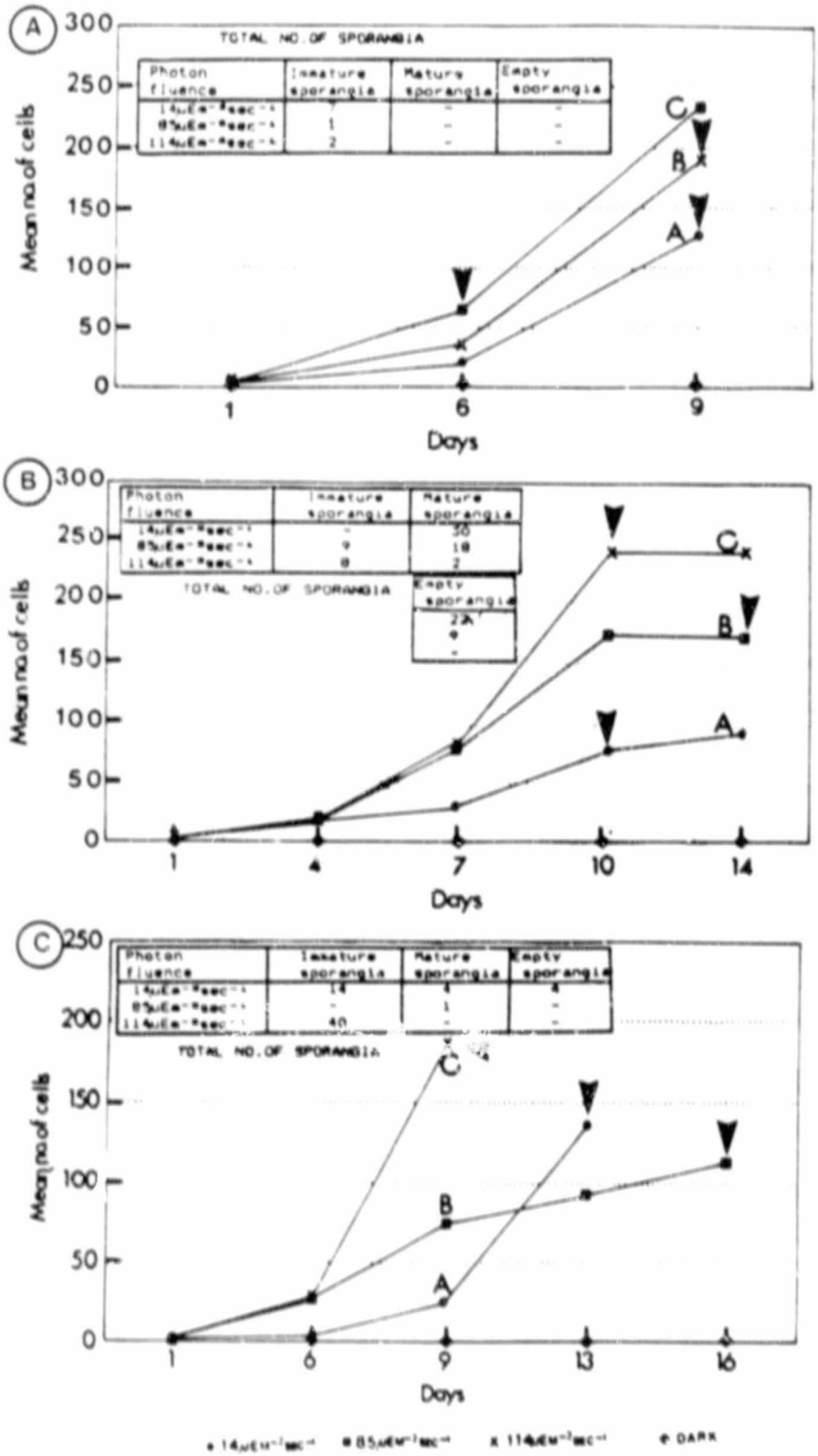
As indicated by the letters of the alphabet, indicative of Duncan's test grouping, all treatments had significantly different mean cell numbers on the same day of experimentation.

The salinity of the medium for the experiment in Fig.15a was measured before and after the experiment to determine whether any evaporation was taking place. The salinity before the experiment was 30 0/00, while after the experiment, it ranged from 32 0/00 to 46 0/00, which was recorded in the medium at 30°C. Clearly evaporation was taking place, but the saline conditions at 30°C did not appear to inhibit growth to any great extent in this instance.

Interestingly in a preliminary experiment where whole filaments as opposed to zoospores were placed under the same temperature conditions, those at 10°C also failed to grow, while the plants at 35°C grew slowly. This may suggest that the temperature tolerance zoospores is different to that of mature filaments.

4.3.3. Photon fluence

From Figs. 16a and 16b it is evident that Feldmannia sp. grows more rapidly in high photon fluence rates (85 & 114$\mu Em^{-2}sec^{-1}$) and not at all in the dark (where zoospores failed to germinate). In Fig. 16c cell numbers of low photon fluence rates (14$\mu Em^{-2}sec^{-1}$)



Figures 16a - 16c: The influence of photon fluence on growth and reproduction in Feldmannia sp..

exceeded those of 85$\mu Em^{-2}sec^{-1}$. Filaments became reproductive under all treatments, but it was obvious that highest number of sporangia occurred at low photon fluence rates (14$\mu Em^{-2}sec^{-1}$). In Fig.16a, high photon fluence rates of 114$\mu Em^{-2}sec^{-1}$ appear to have inhibited sporangia formation, however this is not the case in Fig.16c, where large numbers of immature sporangia were recorded.

Once again there appears to be variation in the time taken to become reproductive. In Fig.16a reproduction is observed occurring as early as after 6 days of growth, while in Fig.16b, the first indication of reproduction occurred after 10 days of growth. In Fig.16b a plateau of growth was reached after 10 days, not evident in the other experiments, however, a plateau may have been observed if further readings had been taken over a longer time period.

There also appears to be large variation in mean cell numbers at which reproduction occurs. In Fig.16a reproduction was evident in filaments with mean cell numbers of 60 at photon fluence rates of 39$\mu Em^{-2}sec^{-1}$. At the other extreme, reproduction was first apparent at photon fluences of 114$\mu Em^{-2}sec^{-1}$ at mean cell numbers of 240.

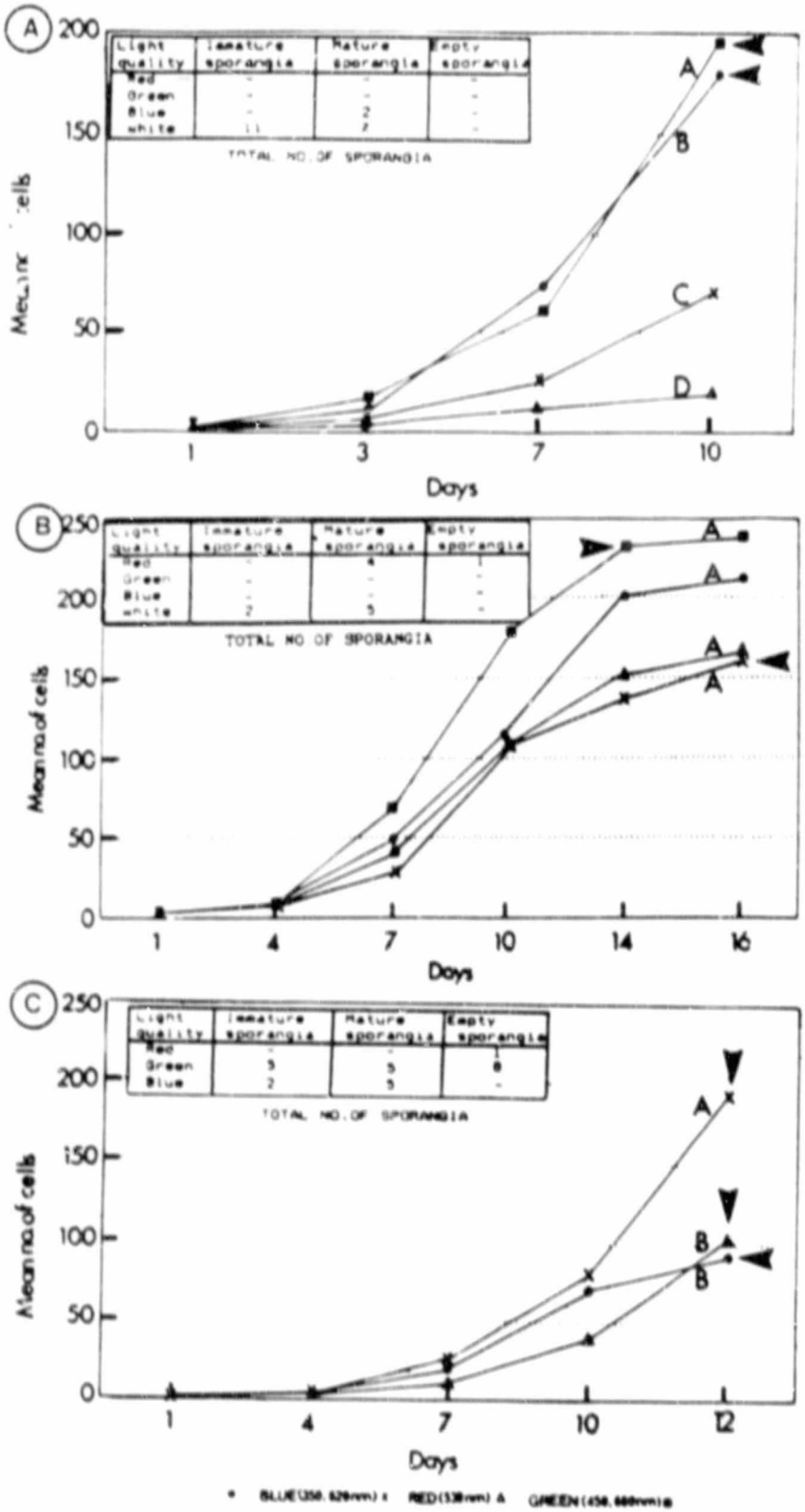
As indicated by the letters of the alphabet, indicative of Duncan's test grouping, all of the treatments have significantly different mean cell numbers on the last day of the experiment.

Filaments became bleached after a few days of exposure to high photon fluence rates. Prolonged exposure rendered the filaments completely white and ultimately killed the plants.

4.3.4. Light quality

Feldmannia sp. grew most favourably, in terms of increase in mean cell numbers, in white light and rapidly became reproductive under these conditions (Figs.17a & 17b).

Mean cell numbers of the plants grown in blue light (350, 620nm)



Figures 17a - 17c: The influence of light quality on growth and reproduction in Feldmannia sp..

were larger than those in red (520nm) and green (450, 660nm) light (Fig.17b) and significantly larger in Fig.17a. In Fig.17c, filaments grown in green light had significantly higher (at the 0.001 probability level) cell numbers on the last day of the experiment when compared with plants grown in red and blue light. Severe condensation on the inside lids in the latter experiment, are thought to have possibly altered the salinity and may well have influenced the pattern of growth. Only the growth trends of the other two experiments will be considered. Fig.17a was included to illustrate that in this instance all of the treatments became fertile.

In Fig.17a, only the filaments grown in white and blue light became reproductive, while in Fig.17b, only those in white and red light were fertile. Similar observations were made up to 6 days after experimentation. In Fig.17c, all treatments induced the onset of a reproductive phase, indicated by the production of plurilocular sporangia.

Once again there is a discrepancy between repetitions of the same experiment, in the time taken for the onset of a reproductive phase and the mean cell numbers at which reproduction occurs. Reproduction was first apparent after 10 days in Fig.17a, while sporangia were first evident after 14 days of growth in Fig.17b. Sporangia occurred on filaments with mean cell numbers of 90 (blue light) in Fig.17c, while they were first evident in Fig.17b when cell numbers were as high as 230 (white light).

Some differences in filament morphology were apparent. Germlings grown under red light tended to adopt a creeping habit, with a large number of filaments remaining prostrate (Plate 4 fig.10.). Green and blue light treated germlings (Plate 4 figs.11.& 12.) tended to have an equal number of erect to prostrate filaments similar to those found in filaments grown under white light conditions.

V. DISCUSSION

5.1. The life history of Feldmannia sp.

It is unclear as to which phase of the life cycle, in an alternation of generations, the Feldmannia sp. studied in this thesis belongs, since there was no evidence of meiosis, fusion of motiles or unilocular reproductive structures.

There are two alternative phases to which this algae could belong, viz. the sporophyte or the gametophyte generation, which both bear plurilocular reproductive structures. Plurilocular-bearing sporophytes have been the only generation found in a number of studies on related species in the Ectocarpales (Pedersen 1974, Clayton 1984), and based on this nomenclature, the reproductive structures in this thesis have been referred to as sporangia. Anomalous, however, is the inability of the so-called sporophyte to produce unilocular sporangia in a range of environmental conditions. This contrasts with the findings of Müller (1963), where Ectocarpus sp. sporophytes produced unilocular sporangia below 14°C, plurilocular sporangia above 19°C and both types at 16°C. Either the so-called sporophyte does not bear unilocular sporangia in its life cycle regardless of the environmental conditions, as found in Sorocarpus micromorus (Pedersen 1974), or the original stock plant was a gametophyte. (A unilocular structure would never be formed on a gametophyte plant.) In the absence of gametes of the opposite sex, it is possible for the gametophyte to regenerate itself by parthenogenesis.

Before concluding that a unilocular organ is entirely absent from the life cycle of Feldmannia sp., much closer examination of this species in the wild and culture is necessary. Clayton (1988) points out that a loss of any sexual reproduction may have evolved in species inhabiting particularly stable environments. Similarly, in this instance, there is the possibility that absence of environmental parameters, that induce unilocular formation, may prevail in the area in which Feldmannia sp. grows naturally. There is also the possibility that environmental parameters other than those used in

this dissertation, cause the formation of unilocular reproductive structures.

The ploidy of the adult plant could be determined using techniques such as DAPI fluorescence, where the intensity of fluorescence of reproductive structures, could be compared amongst different populations. The intensity of fluorescence is related to amount of DNA in the nucleus ie. diploid nuclei would give off greater fluorescent intensities than haploid nuclei. Thus, the fluorescence of diploid sporangia on the sporophyte generation would be greater than that of haploid gametangia on the gametophyte generation. Unfortunately, no further populations were collected in this study and so this technique could not be performed.

It is evident that more of the Feldmannia sp. needs to be collected in the field before any conclusions about its life cycle can be made. In Clayton's (1974) review on the Ectocarpales, she found that, morphologically, cultured algae closely resembled their wild parents. One may find, however, that their behaviour may differ from behaviour found in the field.

5.2. Taxonomic considerations

Uncertainties also surround the classification of the taxon studied in this investigation. In essence, the characteristics of this alga correspond to those of Feldmannia, as described by Hamel (1939), Knoeffler-Péguy (1970), Clayton (1974), Abbott and Hollenberg (1976) and Pedersen (1983). There are, however, some deviations from the pattern of germination of zoospores in this study to those observed by Clayton (1974) and Pedersen (1983) in other Feldmannia sp.. These workers describe bipolar germination of zoospores, with two germination tubes forming asynchronously; one developing into the prostrate system and the other a rhizoid. A similar pattern of germination was evident in Giffordia granulosa (Fletcher 1981). While the above pattern of germination has been observed in Feldmannia sp. in this study, more commonly, unipolar germination of zoospores was observed. Here, the cell corresponding to the original

motile cell does not take part in the formation of erect filaments, but rather a prostrate region was formed first, giving rise to rhizoids and erect filaments only later.

This phenomenon of displaying two germination patterns is unusual, since algae typically have only one pattern. The production of rhizoids early on in development, would serve to ensure attachment to a substrate. The alga studied in this project, however, is securely attached to the substrate in the absence of rhizoids, suggesting that some other means is responsible for ensuring attachment.

It is also uncertain as to which species of Feldmannia the plant studied in this thesis belongs. In agreement with Clayton (pers. comm.) the species that it most closely resembles are F.lebelli (Crouan & Crouan) Hamel and F.globifer/ F.simplex (Crouan & Crouan) Hamel. A comparison of the features of both of these species with the Feldmannia sp. studied in this investigation, are tabulated in Table 2.

There are a number of features which are common to Feldmannia sp. and F.lebelii and F.globifer. However, as Clayton (pers. comm.) suggests, the absence of a pronounced prostrate system in both F.lebelli and F.globifer indicates that Feldmannia sp. is neither of these two species. Also, Feldmannia sp. appears to reach greater size at maturity than F.lebellii and F.globifer. A thorough investigation of the available literature on the taxonomy of the Ectocarpales suggests that the species of Feldmannia investigated in this dissertation may well be new. Since the emphasis of this thesis has not been on the classification, a new name has not be assigned to this species and this is why it has been referred to as Feldmannia sp. throughout.

Organelle fine structure has more recently been used as a taxonomic tool. More specifically, use has been made of the pyrenoid (Dodge 1973), eyespot (Dodge 1973), chloroplast (Evans 1966,1968; Hori 1971,1972; Aseni et al. 1977; all in Clayton & Shankly 1987) and flagellar structure (Hibberd 1979 in Moestrup 1982, Moestrup 1982) in providing an indication of the phylogeny of a particular group of

algae. The Feldmannia sp. studied in this project was found to be typically Phaeophyceae with respect to its ultrastructure.

One limitation in using organelle ultrastructure in algal classification is that phycologists attempt to define clear-cut boundaries into which related groups are expected to fit. I believe that it would be more advisable to use ultrastructural characteristics as an indication of the possible association of a taxon within a group and its relationship to other groups. In doing so, then, some overall picture of its evolutionary status may emerge. Phycologists may also expect to find greater variation in organelle structure in those groups that occupy a greater variety of habitats. For example, the Phaeophyceae are largely marine, commonly occupying similar depths along the shore. Consequently, the organelle fine structure does not appear to vary much in this group. The Dinophyceae, on the other hand, occupy marine, estuarine and fresh waters, and variation in organelle structure within this group is evident (Dodge 1973). Phycologists should possibly take the adaptations of alga found in different niches into account before imposing a rigid classification scheme on them.

Calcofluor White M2R staining of filaments revealed that there are a number of regions of filament growth. The presence of such a diversity of growth regions may be indicative of an evolutionarily advanced member of the Ectocarpales, however, more research on the Ectocarpales is needed to establish this. The advantage of numerous growth regions, is that the the alga's chances of survival, in terms of grazing and colonisation of areas, are higher.

The use of optical brightners is by no means a new technique, and they have been used effectively in studies of regions of cell elongation in Griffithsia C.Agardh, Callithamnion Lyngbye, and Antithamnion Nageli (Waaland & Waaland 1975 in Molloy 1987). Calcofluor White ST has also been used to study the thecal plate arrangement in armoured dinoflagellates (Fritz & Triemer 1985).

Table 2.: Table of comparison of F.lebelli (Womersley 1987), F.globifer (Clayton 1974) and Feldmannia sp..

Feature	F.lebelii	F.globifer	Feldmannia sp.
Thallus	Tufted 1-8mm Rhizoids pene-	Tufted 2cm Occassionally	Tufted 2-4mm
prostrate system	trate host not well developed	endophytic not well developed	well developed
Maximum filament diameter	24µm	53µm	60µm
Meristem location	single meristems	subtending, long unbranched filaments, 2° meristems occur	at base of erect filaments, 2° meristem. on mature plants
Sporangia 1. shape	elongate & tapering	lanceolate - globose	elongate & taper-ing-1°meristem lanceolate to globose-2° meristems
2. attachment	usually single celled stalk occassionally sessile	stalked	usually single celled stalk occassionally at tip of erect filament
Maximum sporan-ial length	160µm	170µm	840µm
Unilocular sporangia	stalked, ovoid	stalked, ovoid rare	-
Distribution	Europe,	Europe,	Natal South

5.3. The ultrastructure of vegetative regions

The vegetative cells of Feldmannia sp. possess the characteristic ultrastructural features of the Phaeophyceae viz: The chloroplasts contain thylakoids arranged in groups of threes in central and peripheral bands; prominent stalked pyrenoids; perinuclear dictyosomes; and plasmodesmata are found in the cell walls (Bouck 1965, Baker & Evans 1973, Oliviera & Bisulptra 1973, Russell 1973). The nucleus is also similar to those already studied in other members of the Ectocarpales (Baker & Evans 1973, Oliviera & Bisulptra 1973, Markey & Wilce 1976a & 1976b).

There was also not much variation between regions of the thallus. The meristematic cells were more reminiscent of younger cells, with smaller vacuoles and more conspicous nuclei than the other vegetative regions.

5.4. The development of sporangia and the release of motile cells

Sporangia developed most commonly below the meristematic regions, on erect filaments, close to the prostate system. More mature filaments were capable of producing sporangia at secondary meristematic regions on erect filaments. These sporangia were smaller in size, and had a larger angle in relation to the filament axis. Knight (1929) noted a similar development of two sizes of sporangia in Ectocarpus siliculosus, and suggested that the smaller size of the more recently produced sporangia was due to the reduced availability of reserves of the entire plant. In the alga studied in this project, the smaller sporangia were associated with mature cultures, and formed only after the larger sporangia, near to the prostrate system, had released their contents. Often bleaching of the filaments, indicative of senescence, was already underway. Thus the number of photosynthetically active cells for the production of energy rich compounds, would have been reduced. It is plausible then, that the reserves available to the sporangia associated with the secondary meristem would be reduced, and may influence the size of the sporangia and the number of motiles they produced.

Rhizoids developed in the region of secondary meristematic growth and secondary sporangia production, giving rise to a new plant which still remained attached to the parent plant. Whether the reserves became partitioned into this component or not is unknown. Morphologically there was no evidence to support this.

Lofthouse and Capon (1975) observed a rigid pattern of sporangial development in E.parvus, involving 2, 6 celled and 9-rank stages. At maturity, the sporangia were described as having 9 ranks with 18 sub-ranks, suggesting that the number of zoospores produced was constant. While similar stages were recognised in Feldmannia sp., the pattern of development appeared to be more flexible, and this is why use of the terms early, intermediate and mature sporangia were used. At maturity, evidence of previous divisions was sculptured in the sporangial profile, making it possible to count the number of ranks or compartments. In the alga studied in this project, the number of locules in the large sporangia ranged from 9 to 11, with 18 to 22 sub-ranks, suggesting that the number of zoospores was not fixed but varied accordingly. There were fewer numbers of zoospores produced in the small sporangia located on secondary growth regions of erect filaments.

The mechanism of zoospore release is consistent with descriptions of other Ectocarpales (Toth 1976a & 1976b, La Claire & West 1979, Clayton 1984). There is general agreement that the motiles move passively out of the sporangia and this is supported by observations of the flagella trailing behind the motile as it moves through the pore at the sporangial tip. Experiments in which mature sporangia were mechanically ruptured resulting in the release of motiles, also indicates that internal pressure exists within the sporangium prior to release (Toth 1976a). The most likely explanation of the force driving motile release is put forward by Toth as discussed in the literature review. Staining of sporangia of Feldmannia sp. embedded in Spurr's resin with PAS, showed slight pink colouration of the matrix surrounding the developing motiles, indicating the presence of carbohydrate. Hydration of this carbohydrate matrix would certainly serve to create an internal pressure within the sporangium. This is based on the assumption that the walls of the sporangia are

semi-permeable. Observations of the flow of dyes into Feldmannia sp. sporangia supports this assumption.

The permeability of sporangia of different ages seems to suggest that water is able to pass into the sporangium throughout the development of the sporangium. The gradual production of mucilage throughout motile cell development, within the sporangium, would ensure that there was a gradual increase in the internal pressure as the matrix hydrates. Finally, the internal pressure would be so great that the motile cells are pushed out of the sporangium through the weakest part in the sporangial wall, namely the apical pore.

Toth (1976a) also shows the partial digestion of the cell wall in the sporangium tip of Pylaiella littoralis. Since the walls at the tips of sporangia were not found to be significantly thinner in sections through mature sporangia of Feldmannia sp., it is thought that if digestion does occur, it must be just prior to release. The organelles involved in the production of enzymes were not discussed by Toth (1976a) and in the Feldmannia sp. studied no additional vesicles that could be involved in enzyme production or transport were observed in the apical zoospore cells. Clayton (1984) noted erosion of the inner layer of the sporangial wall in Scytosiphon, which could contribute to the overall weakening of the sporangial wall.

The mucilage surrounding the motiles is exuded out of the sporangia along with the motiles. According to Toth (1976a), the mucilage is always dissolved on contact with the external medium, while Knight (1929) states that the motiles actively disentangle themselves from the mucilage before swimming free. In Feldmannia sp., it appeared that the mucilage usually dissolved, however, occasionally a mucilage plug formed at the sporangium tip.

The origins of the mucilage surrounding the motiles is unclear. Clayton (1984) suggested that the break-down products of the mature sporangial cell walls in Scytosiphon constitute the matrix. Since the alginic acid component was eroded more extensively than the cellulosic component, the matrix would be largely composed of alginic

acid. From observations of Feldmannia sp., the space between the motile and the locule wall contained a matrix and while the matrix was much more prevelent in the final stages of motile cell development, it did not alter in composition during zoosporogenesis. At all stages, the matrix appeared to contain fibrils and granules and was easily recognisable. Contrary to the suggestions of Clayton (1984), the matrix is present in the space between the motile and locule wall (long before the cell wall thins), just prior to motile cell release.

The walls of locules are not completely broken down during zoospore release, but rather, the motiles appear to stream in single file through a central region. A similar mechanism of release was observed in Scytosiphon (Clayton 1984). Cross sections through sporangia of E.parvus (Lofthouse & Capon 1975) revealed a central column through which the motiles would presumably move to be released from the sporangium. No such canal was evident in Feldmannia sp.. It is thought that a defined central canal does not exist in Feldmannia sp.. Rather, there is an unequal break down of the cell walls, occurring mostly in the central region.

Light microscope observations of released apical zoospores showed that they behaved no differently from the other zoospores. These zoospores were observed at the ultrastructural level to contain a full complement of organelles, including flagella and eyespots. Furthermore, on settlement of these zoospores, germination ensued. This is contrary to the observations of Lofthouse and Capon (1975), who reported that the apical cells contain spores which are probably non-viable due to their poorly defined membrane systems and organelles.

5.5 Ultrastructural observations

A number of changes in ultrastructure take place in the mature sporangia of Feldmannia sp.. This is consistent with the findings of Markey & Wilce 1976a & 1976b, La Claire & West 1979, and Clayton 1984. In general, these changes included the pulling away of the

cytoplasm from the cell wall as the zoospores began to round up; eyespots within the chloroplasts were now present and flagella were in evidence for the first time. The locule walls also became noticeably thinner. The last change listed occurred just before the release of motile cells.

5.6. The Golgi and associated organelles

Small membrane-bound inclusions, adjacent to the nucleus are thought to be the precursors of the dictyosome in Feldmannia sp.. It is likely that these precursors coalesce to form the larger dictyosomal vesicles. Similar observations were made in Giffordia granulosa (Loiseaux 1973), where these inclusions were thought to be derived from the blebbing of the outer nuclear envelope. Observations of blebs on the outer nuclear envelope of Feldmannia sp. suggests that this may also be the site of dictyosome formation in this species.

Consistent with a number of observations (Gibb 1962 in Bouck 1965, Bouck 1965), the space on one side of the chloroplast formed by the chloroplast envelope and chloroplast endoplasmic reticulum in the Feldmannia sp. studied, contains numerous membrane profiles. These were seen to form a complex network of profiles in sections Ectocarpus sp. (Oliviera and Bisulputra 1973). Bouck (1965) noted that these profiles occasionally arose as projections from the chloroplast endoplasmic and in turn, the chloroplast endoplasmic reticulum was attached to the nucleus by a ramifying strand. The latter observations were not evident in Feldmannia sp..

Bouck (1965) went on to suggest that the pyrenoid, nucleus, chloroplast envelopes and the Golgi were involved in the transport of food reserves and secretory precursors produced by the chloroplast. The close association of these same membranes in Feldmannia sp. could facilitate a similar process of distribution of carbon compounds produced in the chloroplast. The close association of these membranes and organelles may also explain why the membrane profiles are found within the space formed from the chloroplast envelope and why chloroplast endoplasmic reticulum are only located on the side

nearest the dictyosome. This positioning would ensure that this space is close to other organelles involved in the transport process. The compounds synthesised in the chloroplasts may be temporarily stored within the space and utilized when required. These compounds may then be more readily available than those stored in the pyrenoids.

Evans and Holligan (1972) explained a mechanism whereby waste-products could be expelled from cells. According to these authors by-products of photosynthesis originate as osmiophilic bodies in the chloroplasts. These bodies are then expelled from the chloroplast where they coalesce in the cytoplasm to form larger osmiophilic bodies. These larger osmiophilic bodies are then extruded out of the cell wall (Baker & Evans 1973). As far as the sporangial cells of the Feldmannia sp. are concerned, a similar process does not appear to take place:

- While small osmiophilic bodies were detected in the chloroplasts of the alga studied, these were not as electron dense as the larger osmiophilic bodies in the cytoplasm. This may well be due to the smaller size of the osmiophilic bodies in the cell.
- The numbers of osmiophilic bodies increased with increasing age of the sporangium. This suggests that accumulation and not extrusion of by-products was taking place.
- It seems unlikely that within the confines of a sporangium one particular cell would expel its wastes into an adjoining cell.
- No osmiophilic material was evident in any of the cell walls of Feldmannia sp..

Insufficient detail of the vegetative cells was collected to make speculations as to whether a similar pattern of waste extrusion as outlined for sporangia takes place in vegetative cells.

5.7. Cell wall formation

A number of stages in the formation of the cell wall were evident in the Feldmannia sp. and based on this, some speculations can be made about the processes involved. The first stage in cell wall formation

is the laying down of a thin layer of cell wall material. Evidence of the origins of this layer were not apparent. However, it is possible that it was transported to the region of cell wall formation by Golgi-derived vesicles.

Fibrous-like material is also transported in vesicles to the site of cell wall formation, where on release from the vesicles, is laid down on the first formed layer. The vesicles are suggested to be Golgi derived, since smaller, yet similar vesicles were detected blebbing off from the Golgi. In Feldmannia sp. sections, vacuoles releasing their contents into the region of the cell wall were apparent and it appeared that in some instances the vacuoles on either side of the developing cell wall were empty. This could be related to the plane of sectioning, where the region containing fibres was not sectioned. Alternatively, the contents could have already been released.

Another suggestion is that the vacuoles are not involved in cell wall formation, but are the precursors to the formation of permanent vacuoles within each cell. However, at no other stage of development of the cells had as many vacuoles been observed as during cell wall formation. The vacuoles on either side of the developing cell wall eventually decreased in size, possibly once they had released their contents.

Clayton (1984) suggested that the main components of the cell wall are cellulose and alginic acid. When the cell walls are broken down just prior to release, it is the cellulosic component that remains intact while the alginic acid component is broken down. It is possible then, that the first formed layer may well be the cellulosic component, while the fibrillar layer laid down later is alginic acid in composition. To prove this it would be necessary to perform staining techniques at different stages of cell wall formation.

Large lysosomes or plasmalemmasomes containing electron dense globules were in evidence in the cytoplasm and alongside the cell wall. To determine whether or not, these structures were lysosomes would require performing the Gomori reaction (Gomori 1952 in Gahan 1984). This test confirms the presence of acid phosphatase enzymes,

commonly associated with lysosomes. The role of lysosomes and plasmalemmasomes in Feldmannia sp. is unclear, particularly in view of the fact that the globules were apparent alongside the cell walls of mature sporangial cells. A number of workers (Cole & Lin 1970 in Baker & Evans 1973) have suggested that plasmalemmasomes may play a role in cell wall formation, but have no evidence to support this statement.

Plasmodesmata occur between the subtending vegetative cell and sporangial cells, as well as between loculi. The exact function of these structures is unknown, however, they are thought to enable 'communication' or interaction between cells. In mature sporangia, as the locule cell walls are broken down, the plasmodesmata are no longer visible. This is consistent with the observations in mature sporangia of Scytosiphon (Clayton 1984).

5.8. The structure and formation of the flagella

The process of flagellar formation was studied in Pylaiella littoralis (Markey & Wilce 1976b) and a number of these stages can be recognised in Feldmannia sp.. In both species the first indication of flagellar formation is the occurrence of a pair of centrioles, close to the nucleus and perinuclear dictyosomes. Basal bodies at the distal end of each centriole give rise to a basal plate. Centrioles, with microtubules extending from them, were without basal bodies or basal plates in Feldmannia sp.. Consistent with flagellar development in P.littoralis, the smooth flagellum of Feldmannia sp. developed appressed to the edge of the flagellar vesicle for part of its length, while the tinsel flagellum developed away from the vesicle wall. In P.littoralis, the region of the smooth flagellum appressed to the vesicle periphery had a swollen flagellar membrane and was thus thicker than the tinsel flagellum. However, both flagella in Feldmannia sp. had the same width.

In some sections, the flagella occurred inside of the cytoplasm while in others, the vesicle appeared to extend into the surrounding matrix, external to the cytoplasm. It seems likely that, due to

their length, the flagella coil around the motile either in their vesicles or in the surrounding matrix outside the motile cell. In Feldmannia sp., the flagella occur in vesicles for most of their length. These vesicles appeared to contain the same ground substance as the matrix, and thus the vesicles were confluent with the surrounding matrix even though they traversed the motile cytoplasm at intervals. Similar observations were made in P.littoralis by Markey and Wilce (1976b).

However, these workers do not explain how the flagella coil within and around the developing motile cell. In Feldmannia sp., flagellar shafts in flagellar vesicles occurred, spatially separated in the cell, in groups of threes. Three flagellar shafts were often visible since the tinsel flagellum is longer than the smooth flagellum and coils twice round the motile as opposed to once in the case of the smooth flagellum.

In these sections of flagella in groups of three, no mastigonemes were evident. Since mastigonemes have been observed to form proximally (Markey & Wilce 1976b, Berkaloff & Rousseau 1979 in Moestrup 1982), it is possible that the sections of Feldmannia sp. were taken higher up along the flagellum, where mastigonemes had not formed yet. These same authors recorded distal ends of flagella, both free of hairs, and bearing hairs, suggesting that eventually mastigonemes distribute themselves along the entire length of the flagellum.

The mastigonemes are thought to be transported to the flagella bases in vesicles of endoplasmic reticulum, where they are released into the flagellar vesicle (Moestrup 1982). No evidence of mastigoneme formation was apparent in sections of the alga in this study.

Loss of mastigonemes from flagella of negatively stained zoospores meant that details of their overall shape were not revealed. An acronema at the tip of the flagellum was in evidence, however. It is possible that the acronema aided initial attachment to the substrate as was the case in Chorda tomentosa (Toth 1976b). This is merely speculation, since attachment by the acronema was not actually

observed.

The insertion of mastigonemes on the flagellar surface of developing motiles of Feldmannia sp., were observed on one side of the flagellum only. This was also the case in P.littoralis (Markey & Wilce 1976b) and could represent an early stage of development. Later on in the development of the flagella, it is likely that the mastigonemes become arranged along the entire length of the flagellum. This was the case in antherozooids of Ascophyllum Stockhouse and Fucus (Bouck 1965), where the mastigonemes were inserted helically along the flagellum.

In Feldmannia sp., the eyespots were located close to the flagella. The close proximity of these structures in related species has led to theories on the relationship of the flagella and eyespot in phototactic responses (Bouck 1965, Clayton 1984). More specifically, the plasmamembrane, chloroplast endoplasmic reticulum and chloroplast membrane lie close to the eyespot, which in turn lies close to the microtubules of the flagellar root system (Clayton 1984). A similar situation of association was evident in the motiles of Feldmannia sp., (however, no root system was observed) but not in the developing zoospores still within the sporangium. It is possible that the plane of sectioning of the sporangia may have obscured this detail, or that the cytoplasm does become appressed to the chloroplast and eyespot with continued shrinking of the cytoplasm. It would be difficult to prove the action of these structures in phototactic responses. Their consistent association in phototactic algae seems to suggest that their distribution within the cell is not coincidental, but functionally significant, and most likely related to phototactic responses.

Autofluorescence of the posterior flagellum did not occur in motiles of the Feldmannia sp. studied. This is in contrast to a large number of other brown algal zoids possessing eyespots, as observed by Kawai (1988) and Muller (1988). Closer examination of the filters used revealed that while the wavelengths of the exciter filters overlapped for part of their range, there was a discrepancy of 20nm in the visible spectrum. More specifically, the spectral range of the