Studies on the growth of dicranella from a mine tip

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STUDIES ON THE GROWTH OF *DICRANELLA* FROM A MINE TIP

by

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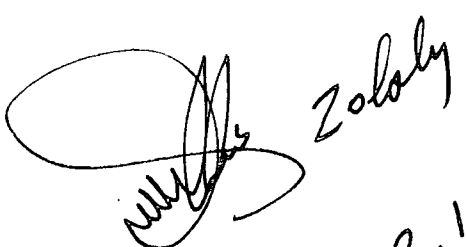
This thesis is entirely the result of my own work. It has not been accepted for any other degree, and is not being submitted for any other degree.



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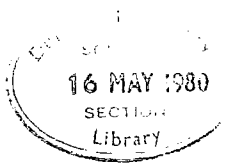

ABSTRACT

A study was made of a moss taken from a site (Elvins Tailing Pile, Missouri) heavily polluted by heavy metals, especially Zn, Cd and Pb. The dominant form of the moss at this site was protonemal. The moss possessed some features of three different species of Dicranella (D. varia, D. rufescens and D. staphylina).

This Dicranella sp. tolerated even higher levels of zinc in the laboratory than the environmental levels found in the field. The highest levels led to a relatively greater decrease in the production of gemmae and leafy shoots than of protonema. The production of gemmae and leafy shoots from filamentous protonema was more sensitive to zinc than from leafy shoots.

Increasing zinc levels led to an increase in the formation of intercellular spaces in the protonema; a decrease in phosphate levels also brought about the same response.

TO MY PARENTS

AND

MY WIFE

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CONTENTS

LIST OF ABBREVIATIONS

^{o}	degree for angles
^{o}C	degrees Celsius
EDTA	ethylenediaminetetra-acetic acid (disodium salt)
g	gram
HEPES	N-2 hydroxyethyl-1, piperazine-N'-ethanesulphonic acid
h	hour
ha	hectare
km	kilometre
l	litre
lx	lux
m	metre
µg	microgram
ml	millilitre
mm	millimetre
min	minute
M	molar
nm	nanometre
n	numbers of measurements
s.d.	standard deviation
tonne	metric ton
v	volume
w	weight
$\bar{x}$	mean value

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1. INTRODUCTION

1.1 General introduction

Zinc is an important micronutrient element for plants as it is an essential component of a variety of metalloenzymes (Price et al. 1972). If zinc is applied at high levels, however, it may be toxic to the same plants.

Among recent studies concerned with the effect of heavy metals in environmental systems, there has been an upsurge of interest in their effects on growth and developmental processes in plants. Most studies have concentrated on higher plants and algae, with relatively little attention being paid to bryophytes. Antonovics et al. (1971) and Whitton and Say (1975) have reviewed research on heavy metals and their toxicity to plants, and recently Dhruva et al. (1977) reviewed the influence of heavy metal pollution on lichens and bryophytes. Zinc is one of the heavy metals which may be toxic to plants if it occurs in the environment at high levels. It may affect organisms in various ways, such as a reduction in growth rate or inability to complete a particular stage in a life history (Whitton and Say, 1975). It seems from the literature that more studies are required in contaminated environments to find out the effects of heavy metals on bryophytes such as toxicity, growth rate, and developmental processes.

1.2 Chemistry of zinc

1.21 Factors influence solubility

Many factors play an important role in the solubility of zinc. In an account of factors which control the concentration of zinc in natural waters, Wedepohl (1972) noted that the influence of complexing by inorganic

ions in diluted solutions is not well understood, but that it may be responsible for preventing precipitation. He noted that inorganic anions control solubility by their abundance: only sulphide, phosphate and carbonate form zinc compounds of low solubility. Other compounds of comparatively low solubility are hydroxide and oxide.

1.211 pH

pH is a key factor affecting the solubility of zinc, either directly or indirectly (Zirino and Healy, 1970; Wedepohl, 1972; Zirino and Yamamoto, 1972; Hem, 1972; Wedow et al., 1973; Frost and Griffin, 1977). For instance, Zirino and Healy (1970) found when pH of sea water was lowered from pH 8.3 to 5.6 by the addition of CO_2 (20% CO_2 in N_2), the zinc and lead increased. A simple chemical equation shows how the zinc ion reacts with water under certain pH conditions (Bachmann, 1962):

$$Zn^{2} + H_2O \rightleftharpoons ZnOH + H^{+} \rightleftharpoons Zn(OH)_2 + H^{+}$$

and a reaction equation can be written as:

$$\frac{(ZnOH^{+})\quad(H^{+})}{(Zn^{2+}} = K$$

and allows the proportion of zinc as the different ion, to be determined for different pH values. In a review of zinc, Wedow et al. (1973) noted that previous studies showed that with increasing availability of hydroxyl ions, a solution containing zinc as the bivalent cation will precipitate $Zn(OH)_2$ at a pH of about 7-8. When the pH exceeds this value the zinc re-enters the solution, but as the zincate anion, ZnO_2^{2-}, or the hydrate zincate anion, $Zn(OH)_4^{2-}$. The reaction is reversible; as the pH of solution

decreases the hydroxide is precipitated once again (pH 7-8) and then redissolved as bivalent cation with further lowering of the pH.

At pH 8.3, zinc in sea water was calculated by Zirino and Healty (1970) to be 75% $Zn(OH)_2^o$, 8% Zn^{2+} and 4% $ZnCO_3^o$; at pH 5-6 59% of the total zinc was calculated to be present as Zn^{2+}, 25% as $ZnHCO_3^-$ and the balance was largely $ZnCl^+$ and $ZnCl_2^o$. However with decreasing hydroxide ion concentration, the abundance of ionic zinc relative to complexed zinc increases.
In a study of the calculation of the degree of interaction between each of the metal ions Cl^-, SO_4^{2-}, HCO_3^- and CO_3^{2-} as a function of pH, Zirino and Yamamoto (1972) found that all four metals were complexed to a considerable extent in sea water, hence only a small fraction of the total metal remains free. For example, the percentage of uncomplexed metal ions range from 17% for zinc to about 1% for copper.

Wedepohl (1972) noted that the precipitation of zinc hydroxide is restricted to the pH range, 5.5 to about 10.5; Hem (1972) commented that precipitation of hydroxide through pH adjustment in water treatment process ought to be a feasible means of lowering the zinc content to satisfactory levels in raw water excessive. Frost and Griffin (1977) found that the exchange - absorption plus precipitation of copper, zinc and cadmium from landfill leachate by clay minerals depended upon the pH and ionic strength of leachate. Precipitation contributed significantly to removal of copper, zinc and cadmium from leachate above 6.0. Recently Harding (1978) measured the extent of precipitation of zinc in an algal culture medium at four different pH values and three different concentrations of calcium. He found that an increase of pH values above 6.6 led to a decrease in the filtrability of zinc.

1.212 Alkalinity

Alkalinity is a measure of the quantity of the strong acid per litre required to attain a pH equal to that of a C_T - molar solution of H_2CO_3 (Stumm and Morgan, 1970). In a study of the effect of alkalinity on the solubility of zinc, Ernst *et al.* (1975) used three different values of alkalinity. The zinc curves with these three values were almost superimposed and were strongly pH dependent above pH 7.5. An increase in alkalinity led to a reduction in the solubility of zinc.

1.213 Silica

Zinc solubility may also be controlled by silicate in some waters (Hem, 1972). Hem showed that between pH 7.5 and 10.0 the silicate species were likely to be much less soluble than any of the other species.

1.214 Phosphate

In a study of the interaction between $ZnCl_2$ and NaOH in the presence of different concentrations of KH_2PO_4, Jurinak and Inouye (1962) found that at higher concentration of phosphate all zinc was precipitated as zinc phosphate. The data indicated that zinc formed the orthophosphate, $Zn_3(PO_4)_2 \cdot 4H_2O$.

1.215 Organic compounds

The influence of amino acids, together with naturally occurring amino acids present in a humic acid hydrolysate, on the solubility of some metals (including zinc) from their insoluble carbonates and sulphides,

has been studied by Rashid (1972). He found that up to 75 mg l^{-1} zinc was solubilized by the reagent grade amino acids, from its carbonates, but none from its sulphides. However the naturally occurring amino acids were able to solubilize 66 mg l^{-1} zinc from carbonate and 6.0 mg l^{-1} from the sulphide complex.

Zinc may be concentrated in natural environments by its incorporation into organic matter, by the formation of complexes of low solubility or by the uptake by biota (Hem, 1972). Pita and Hyne (1975) have shown that heavy metals such as zinc can be transported in the suspended load absorbed on the clay mineral surfaces. The organic component of sediments in reservoirs and streams has been shown by Pita and Hyne to be the most important fraction which absorbs zinc. Gadd and Griffiths (1978) reviewed the toxicity of heavy metals to micro-organisms. In soil, metals can be bound strongly by organic materials e.g. humic and fulvic acids and proteins. Humic acids are especially important and it has been stated that practically every aspect of the chemistry of heavy metals in soils, sediments and natural waters is related in some way to the formation of complexes with humic substances.

1.3 Occurrence of zinc

1.31 Zinc resources

The estimation of the reserves of zinc resources in the world which are identified is about 1.510×10^9 tonnes, and that of the undiscovered resources is about 3.575×10^9 tonnes; both identified and undiscovered zinc resources are estimated at over 5×10^9 tonnes (Wedow et al., 1973). Zinc has been estimated to represent 0.004% of the earth's

crust (Vallee, 1959).

Wedow et al. noted that zinc is produced from mining operations in more than 40 countries, on every continent except Antarctica; some potential resources of zinc are also present on the sea floor.

1.32 General occurrence of zinc in the environment

Antonovics et al. (1971) concluded from the literature that zinc and other heavy metals can occur under several circumstances. Soil may itself contain large quantities of the element and this contamination results either from the presence of undisturbed metal ore near the soil surface, or from the actual mining of ore bodies. Many waste products from mining activities are contaminated with metal at toxic levels and may produce large scale pollution. Areas below galvanized (zinc coated) fences and pylons may even have a zinc concentration high enough to be toxic.

Pearson et al. (1973) determined the average concentrations of zinc in rain water in the vicinity of Lake Windermere over a period of one year; it was 85 $\mu g\ l^{-1}$, and 80 $ng\ kg^{-1}$ in air. A report by Lazrus et al. (1970) gave an average concentration of 107 $\mu g\ l^{-1}$ zinc in rainfall collected at 32 points in United States for a period of 4 months. The zinc was deposited by rainfall at the amount of 2 - 496 g $hectare^{-1}\ month^{-1}$; the amount of zinc brought down in precipitation was distinctly greater in most of the eastern part of the United States. Determination of the concentrations of various elements including zinc in water samples at 726 locations, mostly rivers, throughout the U.S.A. was carried out by Durum et al. (1971). Most of the samples contained detectable amounts of

zinc ($\geq$ 10 mg l^{-1}). There was a noticeable regional pattern in the occurrence of zinc in the river water. Concentrations were above the detection limit in about 80% of samples from the states east and 65% from the states west of the Mississippi River. The median values for zinc and cadmium were 20 µg l^{-1} Zn and 1 µg l^{-1} cadmium, respectively. Hem (1972) reviewed the zinc concentrations recorded in both reports made by Lazrus et al. (1970) and Durum et al. He stated that the values reported by Durum et al. would seem to have rather limited significance, but the value of 107 µg l^{-1} Zn in rainfall (recorded by Lazrus et al.) implied that rainfall may be a major source of zinc in solution in river water. Because the major part of rain does not appear in runoff, Hem concluded that considerably more study is needed before these implications can be evaluated clearly.

1.33 Occurrence in mining environments

In a review of study of the effects of mining and milling for lead and zinc on the environment of Clark National Forest (Missouri, U.S.A.), Jennett et al. (1976) reported data on water quality in Crooked Creek. Zinc was present at 1.62 - 4.90 mg l^{-1} in unfiltered water and 1.60 - 6.80 mg l^{-1} in filtered water. The level of zinc in sediments was also shown to be relatively high, approaching 10 mg g^{-1}. Weatherley et al. (1967) studied the pollution of the Molonglo River in Australia. As a result of previous mining activity they found a marked tendency for high values of zinc in the river immediately below the pollution source, with a progressive lowering downstream through dilution and hydrolysis. The highest concentrations in this river were 34.5 mg l^{-1} at station 4, and 11.5 mg l^{-1} at station 5 while the lowest values of 3.0 and 0.9 mg l^{-1} zinc were at stations 6 and 10, respectively. Pita and Hyne (1975) made

a study on the depositional environment of zinc, lead and cadmium in reservoir sediments at Oklahoma. Despite the presence of low water retention dams, intervening reservoirs located downstream from a lead-zinc mining and milling area contained higher concentrations of zinc, lead and cadmium than reservoirs in other areas. The zinc content of the sediment of the third reservoir downstream from the mining area, the Fort Gibson Reservoir, ranged between 38 and 539 mg l^{-1} and was concentrated in the central, former river channel portion of the reservoir.

1.4 Zinc tolerance and toxicity

There have been many studies on zinc toxicity and tolerance; as mentioned above, most of these studies have been carried out on higher plants and algae, with relatively little work carried out on bryophytes. In a review of heavy metal tolerance in plants, Antonovics et al. (1971) concluded that it is impossible to define precisely what is implied by the description "metal tolerant" because the phrase is generally used in two main senses. Firstly it is used to describe any species which can be found in an area of toxicity, while another species can not occur there. It may also be used more precisely to refer to specific individuals of a species which are able to withstand greater amounts of toxicity than their immediate relatives on normal soil. Secondly it refers to a species normally non-tolerant but with an ability to evolve tolerant races. Antonovics et al. concluded that in the first case it is difficult to define what is happening unless further investigation is made to determine whether the species is already tolerant throughout its range including

uncontaminated sites, or whether it is an example of the second case and has evolved tolerant races. A further difficulty in assessing past studies is in most of the past studies metals have usually been added to laboratory media without making any measurement at the commencement or during the experiments of the true levels of metal in solutions (Whitton and Say, 1975). Even at the present time few studies give any clear indication of the extent to which any heavy metal is present in true solution or whether in a colloidal or larger particulate form.

Dhrava et al. (1977) reviewed the influence of heavy metal pollution on lichens and bryophytes; they concluded that on the basis of the difference in the physiology and tolerance of the plants with respect to zinc, some lichens can be divided into three groups: 1) species that display high accumulation of zinc with no apparent injury; 2) species whose zinc varied in successive samples; 3) species possessing high amounts of zinc but showing pathological changes. A list of lichens, mosses and liverworts tolerant of metal enriched environments was included in this review. Simola (1976) concluded from the literature that the moss Sphagnum fimbriatum seems to be more tolerant to copper and cadmium than some higher plants.

The distribution of bryophytes in streams with high zinc levels has been recorded by Say (1977), who found that three groups of species emerged from the rearrangement of species in relation to decreasing levels of zinc at the stream site. The first group was recognised as those which tended to occur at higher concentrations of zinc, species which occurred there were included Bryum pallens, Pohlia nutans and Dichodontium pellucidum. The second group of bryophytes occurred throughout the whole range of zinc values encountered and included Scapania undulata, Philonotis fontana and Dicranella varia. Finally bryophytes restricted to the lowest concentrations of the metal included Hygrohypnum ochraceum,

Brachythecium rivulare and Bryum pseudotriquetrum. The highest level of zinc at which the mosses Bryum pallens, Dichodontium pellucidum and Dicranella varia were found was at reach 0107/17 (Recording system held at Durham) with a mean of 8.2 mg l^{-1} Zn.

In experimental studies zinc was found to be less toxic than copper to both Funaria hygrometrica and Marchantia polymorpha. Toxic responses to zinc occur at much higher concentrations. Marchantia gemmalings appear to tolerate levels up to 100 mg l^{-1} Zn (Coombes and Lepp, 1974). Many different environmental factors have been shown to influence the toxicity of heavy metals. In laboratory culture media, zinc toxicity to the alga Hormidium rivulare was found to be decreased by increases in the levels of magnesium and calcium (Say and Whitton, 1977); it was increased by increases in pH and cadmium. Sodium, chloride and sulphate showed no detectable influence on zinc toxicity. The effect of all these were found to be sufficiently marked and it was concluded that they may be expected to have considerable importance in the field.

1.5 Accumulation of zinc

Whitton and Say (1975) noted that the internal concentration of heavy metals as a result of uptake is greater than the concentrations in the external environment, and that this appears to be widespread in aquatic organisms. They observed that data in the literature were presented in a variety of ways causing great difficulty when comparing results.

Brooks et al. (1973) reported that "Bryophytes have an extraordinary capacity to concentrate elements from substrate upon which they are growing and moreover will tolerate extremely severe ecological conditions".

In this respect the best known of the bryophytes are so called 'copper-mosses'". Skaar et al. (1973) chose mosses for their study because they accumulate heavy metals in concentrations which are toxic for other groups of plants. Czarnowska and Crochowska (1974) supported this when they found the contents of the iron, manganese, zinc and copperwere several times higher than analogous values reported for vascular plants. They concluded that mosses are an interesting material for research since they possess specific traits not noted in other plants. In a study of the tolerance of heavy metals in the rivers Ystwyth and Clarach, Wales, McLean and Jones (1975) found in general lower levels of iron, lead and manganese in the liverwort Scapania as compared with the less tolerant moss Fontinalis squamosa. During transplant experiments an increase in lead, copper and zinc occurred in this moss within 6 weeks whilst the moss began to decay after 18 weeks when transplanted into polluted sites.

In the review of heavy metal tolerance by Antonovics et al. (1971), they noted that plants may have an exclsuion mechanism to enable them to survive on contaminated soils, but that the tolerance mechanism of zinc must be internal. A study carried out on Dicranella varia occurring on old mine spoil heaps in the Pennines (Shimwell and Laurie, 1972) showed that most of the lead and zinc is excreted from the gametophytes and in periods of summer drought forms a powdery crust on the moss carpet. The analysis of this precipitation occurring on the moss produced abnormally high values for zinc (5250 and 6150 mg l^{-1}). It was suggested that theoretically, the absorption and precipitation of heavy metals by D. varia might prove a possible naural method of detoxification of spoil heap soil. This moss, which lacks a cuticle and absorbs water all over its gametophytic body, was shown to have a

higher heavy metal content than Philonotis fontana which has a more or less continuous cuticle. The absorption was shown to take place mostly towards the base of the gametophyte.

Ward et al. (1977) determined the range of heavy metal concentrations (expressed in $\mu g\ g^{-1}$ dry weight) in bryophytes from two mining areas in New Zealand. They found that near the dusty treatment plant, all bryophytes had higher metal concentrations when compared with the substrata. The range of zinc concentrations in the moss Hypnum cupressiforme found in this area, and other values for the same species from a background locality in Sweden are compared here:

Location	Zn ($\mu g\ g^{-1}$ dry weight)
New Zealand	
Mineralized areas (range)	112 - 156
Treatment Plants (range)	126 - 167
Background areas (mean)	17.2 ± 3.3
Sweden	
Lowest background (mean)*	82 ± 13

*data from Ruhling and Tyler (1971)

1.6 Morphological and physiological effects of zinc

The effects of zinc on the morphology and physiology of bryophytes are still understood poorly. Most research has been carried out on higher plants. Hampp et al. (1976) made a study on extracts of higher plants. They showed that CO_2 - fixation by isolated chloroplasts of spinach (Spinacia oleracea) was inhibited by zinc and cadmium at all concentrations investigated (10 to 1000 μM). De Filippis and Pallaghy (1976)

found that both photosynthesis and respiration were inhibited by zinc in Chlorella. Zinc led to a sharp reduction in pigment content, rates of cell divisions and metabolic activity. Coombes and Lepp (1974) were able to show that both copper and zinc produced deviations in the growth pattern of Funaria hygrometrica from those observed in control cultures. The presence of 10 mg l^{-1} produced numerous rounded cells containing reddish granular inclusions in the protonema. Protonemal growth was also poor and distorted. In Marchantia polymorpha the authors observed no noticeable changes in gemmalings grown in zinc-containing media.

1.7 Aim of project

It was decided to investigate the effects of zinc on the growth and development of strains of representative species from sites polluted by zinc. Initially the blue-green alga Plectonema gracillimum and the moss Dicranella were chosen. It did not prove possible to obtain either in bacteria-free culture. It was decided eventually to restrict the study to only one organism.

Dicranella was chosen for the following reasons:

(i) Contaminant bacteria seem likely to play a less important role for a moss than a blue-green alga

(ii) Rapid growth could be obtained in laboratory conditions

(iii) Morphological changes can be observed in the moss more easily than in Plectonema

(iv) Standard inocula of moss are easier to obtain than with Plectonema

2. MATERIALS AND METHODS

2.1 Collection and storage

The materials were collected from Elvins Tailing Pile (see 3.1) by B. A. Whitton on 21 June 1977, and stored at room temperature in a plastic bag. Soon after return to the Durham laboratory they were cultured into both liquid and solid Chu 10 D medium (Section 2.32) with 10 mg l^{-1} Zn. Incubation was carried out in a plant growth room at 25°C and 2200 lx. The remaining materials were stored in a polythene beaker at room temperature and in a dark place.

On 22 August 1979, similar materials were collected again from the same site by B. A. Whitton. They were stored by the above method.

2.2 Isolation and purification

2.21 Physical isolation

Enrichment cultures were examined carefully with a microscope to determine the organisms present (Section 3.3). The following method was used for isolation. Areas which appeared to contain an extensive growth of the organism were marked. With two fine needles, the organism was then moved to an area without any growth; such areas were cut and removed together with a small block of agar. This method was found suitable for isolating the moss from the associated Plectonema after two or more repeats. Initially attempts were made to remove heterotrophs with antibiotics (Section 2.221); when these failed,

physical methods were tried; the moss was eventually freed of fungi by dilution and repeated subculturing.

2.22 Purification with antibiotic

2.221 Fungi

Enrichment cultures were treated with cycloheximide and griseofulvin in an attempt to kill the fungi; both antibiotics were added (1g l^{-1}) to the agar medium, each as individual treatment. The cycloheximide was dissolved in distilled water and griseofulvin dissolved in alcohol. The solutions were sterilized by passing the solution through 0.22 μm Millipore filter. Each solution was added to the media before pouring the agar, at about 40°C. Both were found to be ineffective in suppressing the growth of fungi.

2.222 Bacteria

Several antibiotics were tried (penicillin, tetracycline, neomycin, polymixin, streptomycin, chlorotetracycline and oxytetracycline) in an attempt to obtain an axenic culture. One bacteria (rod) was killed by penicillin, but the moss still remained bacterized.

2.23 Purification with chemical

An attempt was made to grow the moss in pure culture by dipping the whole leafy shoots of Dicranella sp. in different concentrations of (up to 5%) sodium hypochorite solution for a few seconds, and then

placing them either on basal solid medium or in basal liquid medium, but the moss still remained bacterized.

2.3 Culturing

2.31 Culture vessels

The vessels used for all the tests carried out in liquid media were either 100 ml conical flasks or 50 ml boiling tubes, both of Pyrex glass. The conical flasks were used for long term experiments, and the boiling tubes used for shorter tests. The vessels used for solid media were petri dishes made either of Pyrex glass or pre-sterilized plastic.

All the glassware was cleaned and soaked in 10% HCl for 24 h and then rinsed directly in an automatic rinsing machine for 3 minutes with distilled water.

2.32 Culture medium

In preliminary tests different media were tested in order to obtain relatively rapid growth of the moss. A modification of the No. 10 medium of Chu (1942) proved suitable. The modifications included increased levels of calcium, nitrate, the addition of 0.05 mg l^{-1} Ni and the inclusion of EDTA (ethylenediaminetetra-acetic acid) as chelating agent. All media were prepared using "Analar" grade chemicals. The pH was buffered at 7.0 with HEPES (N-2 hydrozyethyl-1, piperazine-N'ethanesulphonic acid). This pH value lies near the field values found in 1977, being 0.1 pH unit above the mean. Nickel was added to the basal medium because preliminary

tests showed that some enhancement of growth occurred in media with this element. The composition of the media is shown in Tables 2.1 and 2.2.

Zinc was omitted from the microelement stock. Analysis by atomic absorption spectrophotometry showed that the level of zinc in the basal medium was less than 0.04 mg l^{-1}. Where further zinc was added a stock solution of $ZnSO_4 \cdot 7H_2O$ (1g l^{-1} Zn) was used. For all routine assays 50 ml medium was used with 100 ml conical flask or 10 ml of medium with 50 ml boiling tube. For solid media agar was used at a concentration of 1.8% (W/V). The technique for obtaining exactly 25 ml of solid medium was carried out by melting the agar in a beaker with continuous stirring by glass rod; it was then dispensed on to the plates using a 50 ml plastic syringe then left until solidified. Five plates were fixed together with aluminium foil and moved to the autoclave. Sterilization for all media and glassware used for culturing was done by autoclaving at $121^{o}C$ (= 10.35 KN m^{-2}) for 15 minutes. Subculturing and further inoculation were made under aseptic conditions.

salts	Chu 10	Chu 10 D	basal medium
$Ca(NO_3)_2.4H_2O$	-	57.59	172.77
$Ca(NO_3)_2$	40	-	-
KH_2PO_4	-	7.8	7.8
K_2HPO_4	10 or 5	-	-
$MgSO_4.7H_2O$	25	25	25
$NaHCO_3$	-	15.85	15.85
Na_2CO_3	20	-	-
$Na_2SiO_3.5H_2O$	25	10.9	10.9
$NiCl_2.6H_2O$	-	-	0.203
$ZnSO_4.7H_2O$	2	0.072	5.0
$FeCl_3$	0.8	-	-
Fe (as ferric iron ethylenediaminetetra-acetic acid chelate)	-	0.5	0.5
"C" stock of Kratz and Myers (1955)		0.25 ml (with zinc)	0.25 ml (omitting zinc)

Table 2.1 Composition of media (mg l^{-1}), based on salts added.

element	Chu 10	Chu 10 D	basal medium
B	0.35	0.125	0.125
Ca	9.77	9.77	29.31
Cd	-	-	-
Co	0.25	0.0025	0.0025
Cu	-	0.01	0.01
C	2.27	8.23	8.23
Cl	0.52	0.57	0.57
Fe	0.28	0.5	0.5
Pb	-	-	-
Mg	2.47	2.47	2.47
Mn	0.4	0.012	0.012
Mo		0.0025	0.0025
Ni	-	-	0.05
P	1.78 or 0.89	1.78	1.78
K	2.27 or 1.124	2.24	2.24
N	6.83	6.83	20.49
Si	3.31	1.44	1.44
Na	14.43	6.7	
S	3.25	5.71	5.71
Zn	0.45	0.072	-

Table 2.2 Composition of media (mg l^{-1}), based on total concentrations of elements present.

2.33 Inoculation

a) Liquid cultures

Two fine needles were used to help get as uniform inocula of the Dicranella as possible. A small inoculum was also preserved in iodine in a specimen bottle in order to compare and adjust the other inocula against its size.

b) Solid cultures

The method for obtaining a uniform inoculum from solid cultures is more accurate and easier than that from liquid cultures. The inoculum was made as uniform as possible by cutting small equal blocks from the solid culture; this was achieved with the aid of paper with black lines and an inoculation knife (flattened needle). The process of cutting was achieved by passing the knife on the lines which were crossed making small squares 0.09 cm^2. A source of light from beneath was used to help to identify the lines.

2.34 Incubation

a) Liquid cultures

All the boiling tubes were incubated in a tank which provided the cultures with constant temperature (25^{o}C) and moderate shaking, the tubes were placed in a rack at an angle, and illuminated from beneath with 2500 lx continuous cool white fluorescent light. Long term experiments were carried out in conical flasks without shaking, with continuous illumination from above with 2500 lx continuous cool

fluorescent tubes (2500 lx) at 25°C or 15°C.

b) Solid cultures

All experiments with solid media were carried out in the same plant growth rooms as those used for standing liquid cultures. Long-term experiments with solid medium were carried out in pre-sterilized plastic petri dishes and the short experiments carried out in Pyrex glass petri dishes. These experiments were all carried out at 15°C.

2.4 Measurement of growth

2.41 By area

Assessment of growth in solid cultures was attempted by measuring the area occupied by the moss. This was carried out by measuring the mean diameter of the area occupied by moss and, from this, calculating the surface area.

2.42 Chlorophyll a

Assessment of growth was also carried out by determination of the chlorophyll a in the cultures (section 2.52)

2.5 Analytical Method

2.51 Fractionation of zinc in medium

2.511 Apparatus

1) Filtration system (Millipore)
2) 0.22 μm Nuclepore filters
3) Specimen bottles
4) Pyrex glass 50 ml boiling tubes

All the glassware was cleaned as described in 2.31, with the exception that the Millipore filtration system was left to dry at room temperature.

2.512 Method

Zinc was added to the media either before or after autoclaving into the basal liquid media and the pH was buffered at 7.0 with HEPES (section 2.32). The zinc solution added after autoclaving was sterilized separately. An estimate of the extent of zinc precipitation in the media was carried out by filtering the solution through the Nuclepore filters and receiving the filtrate solution in the acid washed specimen bottles. Soon after filtration, zinc was measured using a Perkin-Elmer 403 atomic absorption spectrophotometer. The solutions were shaken vigorously before measurement. Measurement of total zinc in the original solution was made both before and after acidification. This was because most readings of the total zinc indicated lower values than were known to have been added to the solution.

initial Zn (mg l^{-1})	fraction	observed reading when 'initial' Zn added before autoclaving (n = 4)		observed reading when 'initial' Zn added after autoclaving (n = 4)	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.
0	T	0.01	0.002	0.01	0.002
	AT	0.01	0.002	0.01	0.003
	F	0.02	0.009	0.02	0.010
	AF	0.02	0.008	0.02	0.014
5	T	4.30	0.15	5.04	0.09
	AT	5.30	0.35	5.53	0.41
	F	3.73	0.18	3.85	0.26
	AF	3.93	0.20	3.95	0.21
10	T	7.08	0.40	8.68	0.11
	AT	9.23	1.25	10.35	0.21
	F	7.18	0.68	8.55	0.30
	AF	7.38	0.76	8.63	0.27
20	T	18.7	0.37	21.58	1.19
	AT	21.62	1.23	23.07	0.72
	F	12.20	0.10	14.13	0.54
	AF	12.46	0.17	14.23	0.54
30	T	28.03	0.80	30.18	1.20
	AT	30.80	1.39	31.33	0.41
	F	20.18	0.44	24.53	0.40
	AF	20.30	0.39	24.70	0.43
40	T	36.48	0.95	40.63	1.46
	AT	41.65	1.04	41.70	1.10
	F	26.63	0.61	36.38	0.72
	AF	26.75	0.60	36.50	0.74
50	T	43.9	0.24	47.88	0.29
	AT	50.05	0.94	50.00	0.70
	F	34.08	2.04	41.50	0.75
	AF	34.15	2.02	41.58	0.78
60	T	50.98	1.54	57.50	0.98
	AT	59.88	2.17	60.05	1.75
	F	40.40	0.95	50.85	1.10
	AF	40.40	1.06	51.02	1.11
70	T	57.85	0.08	64.58	2.48
	AT	66.45	2.10	69.98	1.77
	F	46.20	0.30	59.45	0.59
	AF	46.28	0.38	59.53	0.56
80	T	66.33	1.97	78.52	0.32
	AT	77.63	3.05	80.15	1.49
	F	55.10	3.29	67.88	1.35
	AF	55.18	3.31	68.00	1.31
90	T	72.78	1.45	88.45	0.45
	AT	82.85	5.00	90.38	0.41
	F	59.85	2.25	76.00	0.91
	AF	59.95	2.31	76.10	0.85
100	T	71.45	0.53	97.40	2.71
	AT	103.75	0.83	102.27	2.25
	F	62.33	2.30	83.63	1.13
	AF	62.38	2.31	83.73	1.18

Table No. 2.3 Influence of 3 different factors: filtration, acidification, and autoclaving on solubility of zinc (Zinc concentrations in mg l^{-1}; n = 4)

T = total; AT = acidified unfiltered solution; F = filtrate; AF = acidified filtrate solution

2.513 Result

Most of the zinc was in solution when added after autoclaving; the extent of precipitation increased when the zinc was added to the media before autoclaving (Table 2.3). Acidification showed that the "missing" zinc was really still present.

2.52 Chlorophyll a

2.521 Extraction

Extraction of chlorophyll a was carried out with methanol as an extracting agent. Marker (1972) suggested that methanol is much more effective in extracting pigments than acetone; moreover degradation of chlorophyll in methanol is less than in acetone. The method used for the extraction of chlorophyll a is similar to that given by Talling and Driver (1963). The details are given below.

a) Liquid cultures

The moss was harvested by vacuum filtration through Whatman GF/C glass fibre paper. The moss and solvent (95% methanol) were then placed in 30 ml McCartney bottles and incubated for 5 min. in a waterbath at 70°C, with occasional shaking, and filtered again through the glass fibre paper; this procedure was then repeated one or more times. The final filtrate was made up to a standard volume. The chlorophyll peaks were read immediately after extraction using a Perkin-Elmer 402 Ultraviolet - visible spectrophotometer. Absorption spectra were read at 665 nm. Extracts were then acidified by one drop of 1 N Analar HCl in the

optical cell and carefully mixed in with a pasteur pipette; the absorbance at 665 nm was then read again.

Neutralization of the extracts with magnesium carbonate was not used because preliminary tests to compare the effect of neutralization by magnesium carbonate on the absorption spectra showed little effect on the final peaks.

b) Solid cultures

The method of analyzing chlorophyll a in solid cultures was similar to that in liquid cultures, but here 100% methanol was added for extraction. The moss occurred both on and in the agar; the presence of agar led to a slight dilution of the methanol. A special glass pestle, which can be inserted into the McCartney bottle, was used for crushing the material. Five or more extractions were required for some samples.

2.522 Estimation

Chlorophyll a was calculated from formula given by Marker (1972), but here a different "acid factor" derived constant has been used. This formula has been written as follows:

$$\text{Chl } a \text{ } (\mu g/\text{sample}) = 2.56\ (A_b - A_a) \times {}^{V}/_{l} \times 13.1$$

A_b = absorbance at 665 nm before acidification

A_a = absorbance at 665 nm after acidification

V = volume of extract (ml)

l = light path of optical cell (cm)

13.1 = Constant, assuming a specific absorption coefficient of chl a in 95% methanol of 76.07 l g^{-1} cm^{-1}

2.56 = Constant derived from an acid factor of 1.621 (mean)

The acid factor was calculated from 75 samples of the moss and is 1.64 (s.d = $\pm$ 0.26). It was calculated according to the method of Marker (1972):

$$\frac{\text{absorbance at 665 nm before acidification}}{\text{absorbance at 665 nm after acidification}}$$

Using the mean of the acid factor, a constant of 2.56 was derived for use in the chlorophyll a equation. This constant was derived as follows:

$$\text{Constant} = \frac{\text{acid factor}}{\text{acid factor} - 1} = \frac{1.64}{1.64 - 1} = 2.56$$

2.6 Technique of Sectioning

2.61 Sectioning

Sectioning was carried out, using a technique modified from Peacock (1940).

The moss was fixed overnight in Rawling's Fluid (formal-acetic-alcohol). The moss was washed in 70% alcohol, then the following procedure was used:

1)	90% ethanol	2 min
2)	absolute ethanol	2 min
3)	absolute ethanol	2 min
4)	absolute ethanol/xylene 1:1	2 min
5)	xylene to clear	2 min

The moss was then placed in a fresh change of xylene and enough paraffin shavings were added to saturate the xylene.

The container was placed overnight in an oven at 57°C; more wax

was added gradually until the mixture was approximately 3/4 wax. The moss was next transferred to pure melted paraffin wax and kept at 57°C for 2 h. It was then placed in fresh paraffin wax kept molten in the oven (the melting point of the wax is 54°C). Fresh melted wax was poured into a mould and the moss placed in it and aligned for cutting on the microtome; after the wax block had solidified it was trimmed and attached to a wooden block to enable it to be sectioned. The sections were cut 7.5 µm thick.

The sections were mounted in the following manner. A thin layer of Mayers albumin was smeared on a clean slide. Section was floated on warm water at 35°C to stretch it and then picked up on the slide. The slide was then dried thoroughly.

2.62 Dewaxing and staining

Dewaxing and staining was carried out in the following sequence:

1)	dissolve wax in xylene	10 min
2)	fresh xylene	5 min
3)	xylene/ethanol 1 : 1	2 min
4)	absolute ethanol	2 min
5)	90% ethanol	2 min
6)	70% ethanol	2 min
7)	safranin, light green in Cellosolve	1 min
8)	wash in 70% ethanol	
9)	wash in 90% ethanol	

10) wash in absolute ethanol

11) wash in xylene

12) mount DPX Mountin Medium, (Raymond A. Lamb, London)

2.7 Measurement of cross-wall angles

The angles of cross-walls of the filamentous protonema were estimated from photographs. Large prints were used and the angles measured with a protractor. The line of the cross-wall was extended by pencil in order to help the measurement.

3. ELVINS TAILING

3.1 Location and description of site

Elvins Tailing Pile lies in the Old Lead Belt, Missouri, U.S.A. It is located about 113 km south of St Louis, entirely within the confines of St Francois County. Bordered between latitude 38° 00' and 37° 49'15" and longitudes 90° 37'30" and 90° 28'45", this century old mining region covers a land area of approximately 285 square km and comprises about 10% of the Big River Basin (Kramer, 1976).

Elvins Tailing Pile (Fig. 3.1A), bordering the northern side of Elvins City, is located in the northeast quarter of section 12, Township 36 North, Range 4 East. The deposit covers a land area of approximately 0.6 square km and is generally level with an extremely steep southeastern face. Grassy-type vegetation grows at its extreme north and northeastern edges. A shallow lake of approximately 1.2 ha borders the northern tip of this tailing accumulation. Another lake of about 1.0 ha borders the southwestern corner of this deposit. At the base of the southeastern face of Elvins Tailing Pile, there is a seepage (Fig. 3.1 B) which flows through a series of three small dams before eventually reaching Flat River Creek.

3.2 Composition of water

Water samples were collected from Elvins Tailing Pile both on 21 June 1977 and 22 August 1979. Results of the analysis of these water samples are presented in Tables 3.1 and 3.2. The water was about neutral when collected in 1977, but at a slightly lower pH when collected in 1979.

Fig. 3.1 Elvins Tailings Pile, Missouri on 22 August 1979:
(A) general view (B) source of stream 9014

A

B

element	unfiltered		filtered	
	21 June 77	22 August 79	21 June 77	22 August 79
Al	0.10	0.13	0.10	0.10
As			0.02	
Ca	368	309	360	271
Cd	0.107	0.078	0.10	0.081
Cl		5.1		
Co	0.46	0.46	0.48	0.44
Cu	0.021	0.027	0.23	0.008
F		0.72		
Fe	0.09	1.06	0.07	0.15
Pb	0.196	0.28	0.204	0.14
Mg	80	67	80	55
Mn	0.020	0.215	0.019	0.010
Si		0.65		
Ag	0.02	0.02	0.02	0.02
SO_4-S		260		
Ni	0.42	0.45	0.42	0.43
K	14.6	11.8	14.1	11.8
Na	6.8	5.2	7.4	5.8
Zn	19.1	22.8	19.1	21.0

Table 3.1 Element composition of Elvins Tailings Pile water in 1977 and 1979

	30 July 75*	21 June 77	22 August 79
temperature ^{o}C		24.5	21.5
pH	7.35	6.9	6.1
total alkalinity (mg l^{-1} $CaCO_3$)	119	11.0	

Table No. 3.2 Properties of Elvins Tailings Pile water.

(* from Kramer, 1976)

Kramer (1976) recorded the pH of the water at this site during July and August 1975 to be slightly alkaline. Kramer showed that hardness of this water consisted primarily of the non-carbonate forms.

3.3 Biology of site

a) Algae

When the Dicranella used for the present study was collected in 1977 from the site shown in Fig. 3.1B, only two species of algae were found. These were Plectonema gracillimum (Zopf) Hansg. and Stichococcus sp. Growth of these algae was studied briefly in the laboratory. The growth of Plectonema was very poor when subcultured into Chu D, AD, AC (see Sinclair, 1977), and Bold's medium (see Bold 1942), both in liquid and solid media. The basal medium (Table 2.1) developed was found to permit much better growth for culture of Dicranella (Section 2.32). The growth of Stichococcus was faster than Plectonema when grown in Chu 10 D medium. Other algae were recorded in 1979, but they were not studied further.

b) Bryophyte

One bryophyte, provisionally identified as Dicranella varia (Hedw.) Schimp. was the dominant photosynthetic organisms in the material collected.

c) Bacteria

Four different growth media (yeast extract, beef, malt and nutrient media) were used for partial characterization of the bacteria. Three species grew in all media; two formed white and one formed yellow colonies

on agar. Microscopic inspection showed that two were motile and one non-motile. All were gram-negative. They were very resistant to antibiotics (Section 2.22), although the yellow colony was killed by penicillin.

d) Fungi

Three species of fungi were present with the materials: Alternaria sp., Fusarium sp. and one not identified: (The generic identifications were made G.H. Banbury.) Growth of all three fungi was rapid, a whole plate being colonized within a few days.

e) Animals

Protozoa and nematodes were found with the materials collected from the site. These organisms did not rersist repeated subculture of the plant.

4. MORPHOLOGY AND GROWTH

4.1 Morphology of field materials

Materials collected from the field were inspected carefully, both in order to aid identification and also as a basis for later experiments in the laboratory.

a) Materials collected in June 1977 (see 2.1)

Shoots 8 - 13 mm tall, branched at the base but rare, leaves lanceolate, tapering to acute apex, those of upper part of shoot longer (0.5 - 1.0 mm long, 0.18 - 0.21 mm wide at the broadest part just above the base) than those of lower part (0.e - 0.4 mm long, 0.12 mm - 0.17 mm wide) (Fig. 4.1); margin plane, with a few obscure teeth near apex; nerve ending in apex, occupying 1/6 to 1/5 of width of base, defined in section with two rows of cells (Fig. 4.3C); cells more or less rectangular, but some walls oblique (Fig. 4.2 B-C), 8 - 12 μm in mid-leaf. Perichaetial leaves with sheathing base, longer than stem leaves (2.0 - 3.5 mm long) recurved to squarrose (Fig. 4.10E). Rhizoidal gemmae variable, typically 40 - 50 x 25 - 31 μm, pale brown (Fig. 4.8A-G); more or less spherical gemmae also present, both rhizoidal and protonemal, dark brown (Fig. 4.9 F-G). Sporophyte unknown. Old stems and rhizoids become dark brown.

b) Materials collected in August 1979 (See 2.1)

These materials were similar to those collected in 1977, but differed in height of shoots, arrangement and length of leaves. The shoots were usually longer (10 - 25 mm); the leaves were usually

erecto-patent (whereas those of 1977 were spreading) and longer; upper leaves 1.3 mm long, 0.35 mm wide; lower leaves up to 0.44 mm long, 0.18 - 0.22 mm wide.

4.2 Comparison with various descriptions of Dicranella species

The Elvins moss was named provisionally as Dicranella varia (Hedw.) Schimp., but differs in various respects from the descriptions of this moss given in Watson (1968) and Smith (1978). According to the descriptions the leaves of D. varia are longer than those of the Elvins moss (Table 4.1); they are usually erecto-patent to slightly secund in D. varia, whereas in the Elvins moss they are patent to spreading; the cells are smaller in D. varia. The perichaetial leaves of D. varia are not differentiated from the stem leaves. Irregular rhizoidal gemmae occur in D. varia, but the dark brown more or less spherical rhizoidal and protonemal gemmae of the Elvins moss have not been noted.

The Elvins moss also resembles Dicranella rufescens (With.) Schimp. in some respects such in shape of stem leaves, dimensions of cells, but D. rufescens differs from the Elvins moss in the following ways. The shoots are much shorter than those of Elvins moss; perichaetial leaves in D. rufescens are similar to the uper stem leaves, whereas in the Elvins moss they are clearly differentiated from them (Fig. 4.10E). D. rufescens has red pigment in the old rhizoids and stems, but these parts are brown in the Elvins moss. Both mosses have rhizoidal gemmae, but these are not alike.

The moss seems to be more closely to Dicranella staphylina Whitehouse than any other species of Dicranella, with a close resemblance in features such as shoot branching, leaf shape, perichaetial leaf shape, cell dimensions, and colour of the old stems and rhizoids. There are however some differences between D. staphylina and the Elvins moss. The shoots are much shorter in D. staphylina. The nerve ends below the apex in D. staphylina, whereas in the Elvins moss it ends at the apex. The perichaetial leaves of D. staphylina are shorter than those of the Elvins moss. The irregular rhizoidal gemmae of the Elvins moss are much more similar to those of D. staphylina than those of D. varia, although they are smaller (40 - 50 x 25 - 31 μm); the dark brown more or less spherical rhizoidal and protonemal gemmae have not been noted in D. staphylina.

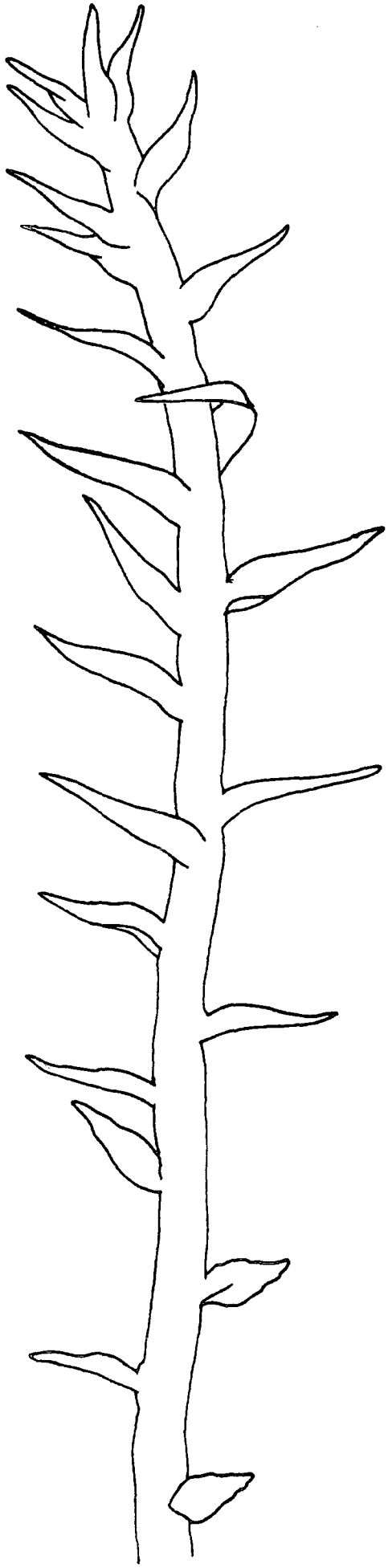
plant	sex and features of leafy shoots	leaf					sporophyte	gemmae	author
		arrangement	shape	margin	cells	nerve			
collected in 1977	dioecious 8 - 13 mm tall	erecto-patent to patent when dry, spreading when moist	lanceolate, tapering to acute apex base not sheathing (Fig. 42A), upper leaves (0.5 - 1.1 mm long), lower leaves (0.3 - 0.4 mm long) (Fig. 4.1) perichaetial leaves with sheathing base, longer than stem leaves 2 - 3.5 mm long, (Fig. 4.10E)	plane, with a few obscure teeth towards apex (Fig. 4.2 A,B)	± rectangular, but some end-walls oblique 8 - 12 µm wide (Fig. 4.2B-C)	thin, ending at the apex, occupies 1/6 to 1/5 width of the base (Fig. 4.2A)	unknown	irregular or regular outline; cell rhizoidal (Fig. 4.8A-D and Fig. 4.9)	
collected in 1979	dioecious 10 - 25 mm tall	erecto-patent when moist	lanceolate, tapering to acute apex, base not shedding upper leaves up to 1.3 mm long; lower leaves shorter up to 0.4 mm long	plane, with a few obscure teeth towards apex	± rectangular, but some end-walls oblique 8 - 12 µm wide	thin, ending at apex, usually 1/6 to 1/5 width of base, rarely 2/3 width	unknown	irregular or regular outline; rhizoidal and rarely also protonemal	
D. varia	dioecious 2 - 10 (-30) mm tall	straight to slightly secund when dry, credo-patent to slightly secund when moist	upper leaves linear - lanceolate gradually tapering from insertion to acuminate apex, base not sheathing; lower leaves shorter, wider; perichaetial leaves similar in shape to stem leaves	usually narrowly recurved, entire or minutely toothed near apex, sharply defined in section with two rows of guide cells with scattered stereids above	basal cells ± rectangular, incrassate, smaller near margin; above narrowly rectangular to linear, unistratose throughout 4 - 9 µm wide in mid-leaf	ending at apex or excurrent, about 55 - 85 (-100 µm) wide at leaf base, occupying 1/5 of width of base	seta deep reddish-brown; capsule inclined ovoid, gibbous smooth when dry	irregular, pale brown, rhizoidal 100 - 140 (250) x 60 - 95 µm often present	Smith (1978)
	dioecious shoots are much less curved to one side	not noted	lanceolate, curved and tapering and up to 3 mm long; perichaetial leaves not noted	lack the sharply saw-edged tip; narrowly revolute for much of their length	even in the upper part of the leaf, are long and narrowly rectangular	occupies about 1/6 of the breadth of leaf base	red seta and capsule; smooth (not furrowed) curved capsule	not noted	Watson (1968)
	dioecious	secund	lanceolate, not sheathing; perichaetial leaves not differentiated from upper leaves	recurved margin a regular feature of the large leaves	lamina cells 5 - 9 µm wide	strong	not noted	variable in size and shape, pale brown 100 - 140 (-250) µm; rare to frequent	Whitehouse (1966, 1969)
D. rufescens	dioecious up to 10 mm tall, often reddish tinge	erect to flexuose - secund when dry, secund when moist	narrowly lanceolate; base not sheating, gradually tapering to acuminate to subulate apex; perichaetial leaves similar in shape to upper stem leaves	plane; denticulate, at least above	basal cells irregular, rectangular; 8 - 14 µm wide in mid-leaf	nerve thin ending at apex or excurrent	seta deep red; capsule erect, ellipsoid, symmetrical, smooth; lid with oblique beak	rhizoidal, composed of 1 - 3 large cells; occasional, especially in senescent plant	Smith (1978)
	smallest species of the genus (in U.K.); recognized by reddish organs; colour accentuated on drying	not noted	not noted	plane	not noted	not noted	not noted	not noted	Watson (1968)
	red pigment in old stems and rhizoids	usually secund (up to 2 mm long)	narrow outline; perichaetial leaves similar to upper stem leaves in shape	plane throughout and with more conspicuous teeth near apex	not noted	not noted	not noted	consist of two much enlarged cells, each 70 - 100 µm in diameter and red in colour; occasionally 3 - 4 cells in the gemma arranged in a row	Whitehouse (1969)
D. staphylina	apparently dioecious plant up to 5 mm tall	erect when dry, erecto-patent to spreading, rarely secund	lanceolate, tapering to acute apex; perichaetial leaves with sheathing base, ± abruptly narrowed to long flexuose or squarrose limb	plane or recurved below, with a few obscure teeth towards apex	± rectangular 10 - 14 µm wide in mid-leaf	thin, ending below apex	unknown	brownish rhizoidal gemmae, 80 - 100 x 50 - 80 µm always present	Smith (1978)
	normally dioecious, but plant pseudo-dioecious in cultivation, about 5 mm tall, branched at base; old stem and rhizoid become brown	erecto-spreading to spreading, not secund or only slightly so	lanceolate 0.6 - 1.0 mm long and about 0.25 mm wide at the widest part just above the base; base not sheathing the stem; perichaetial leaves 1.25 - 2.5 mm long, with a wide sheathing base; narrowed	often plane, but sometimes recurved below in large leaves with a few obscure teeth near apex	± rectangular, but some end-walls oblique; 30 - 70 x 10 - 14 µm with walls 2 µm thick	rather weak, about 40 40 µm wide at base, ending just below apex	unknown	rhizoidal, irregular in shape, but often more or less isodiametric, 80 - 100 x 50 - 80 µm	Whitehouse (1969)

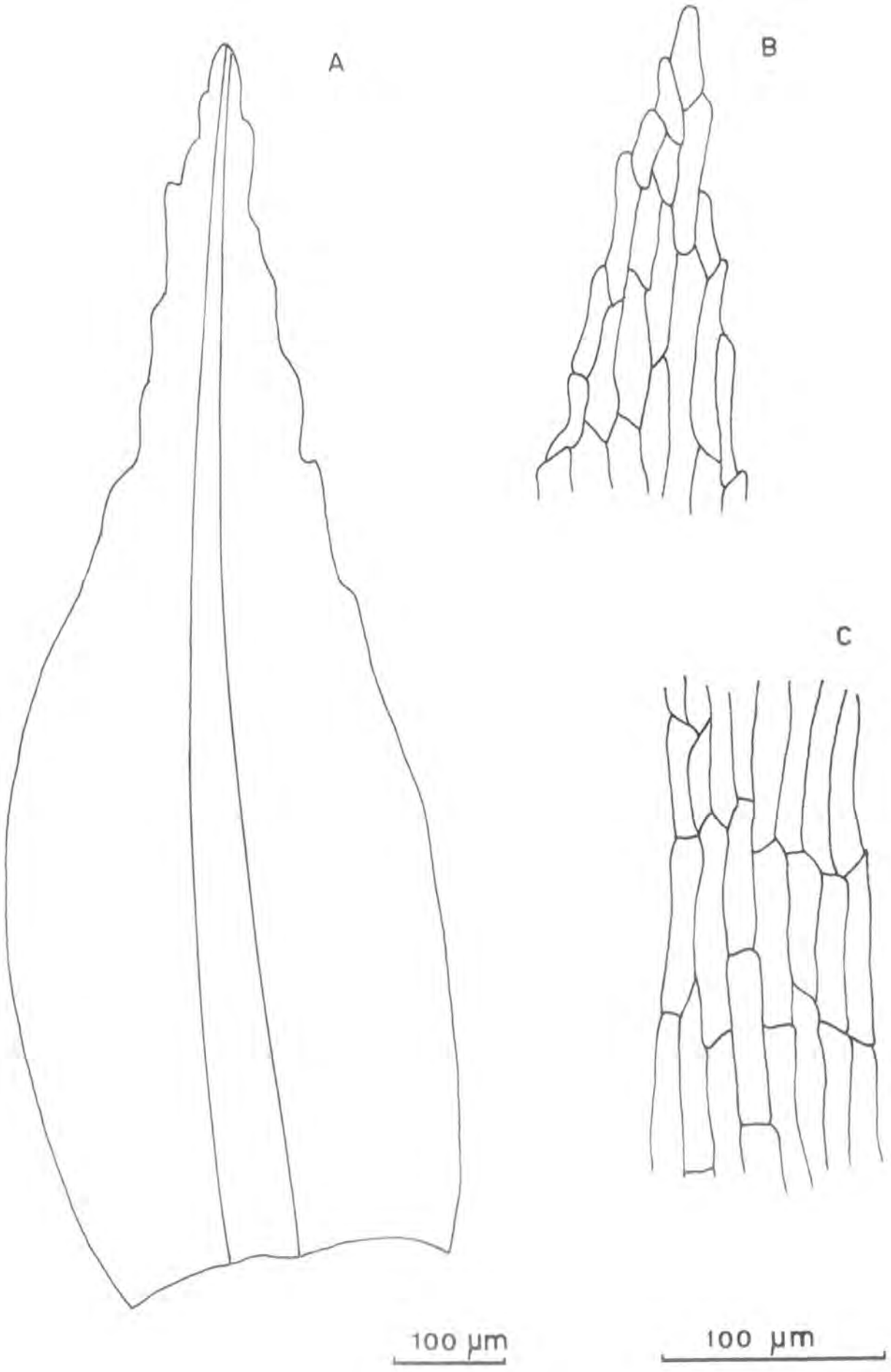
Table 4.1 Comparison of the studied moss with descriptions of three species, D. varia, D. rufescens and D. staphylina from the literature.

Fig. 4.1 Leafy shoot grown in laboratory, showing the arrangement of leaves

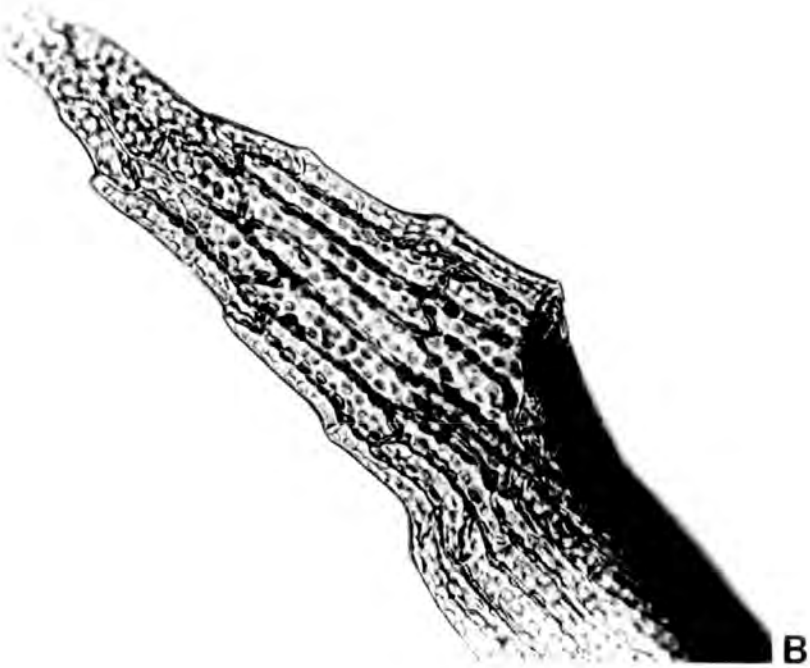
Fig. 4.2 Leaf structures: (A) leaf showing vein; (B) apex; (C) cells in mid-leaf

Fig. 4.3 Leaf shape: (A) young leaf; (B) adult leaf; (C) cross-section in adult leaf.


1 mm


A
B
C
100 µm
100 µm

A



B

C

4.3 Morphological features

4.31 Filamentous protonema

a) Shape and dimensions

The filamentous protonema collected from the field in August 1979 were quite similar in width (Fig. 4.4B) to those collected in June 1977, but the cells were shorter than those of the latter. However when the filaments were grown in agar, they became similar in shape and dimensions of those collected in 1977 (Fig. 4.4A). In the laboratory there were slight differences in shape and dimensions of filaments when grown in liquid and when grown in agar. The filamentous protonema grown in liquid were thinner (Fig. 5.4 A) than those grown on agar (Table 4.2). The protonema grown on agar became slightly thicker and the cells, especially those of the branches, became shorter (Fig. 4.5A) Young filamentous protonema grown either in liquid or solid media were much-branched (Fig. 4.6A), with abundant chloroplasts; older protonema had fewer chloroplasts and the cross-wall was generally oblique.

Table 4.2 Dimensions of field and laboratory protonemal filaments

environment	width of filament	length of cells
field materials collected in 1977	24 - 28 μm	42 - 104 μm
field materials collected in 1979	24 - 28 μm	32 - 56 μm
culture on agar	24 - 28 μm	42 - 104 μm
" " "	16 - 20 μm	79 - 145 μm
culture in liquid	16 - 20 μm	79 - 145 μm

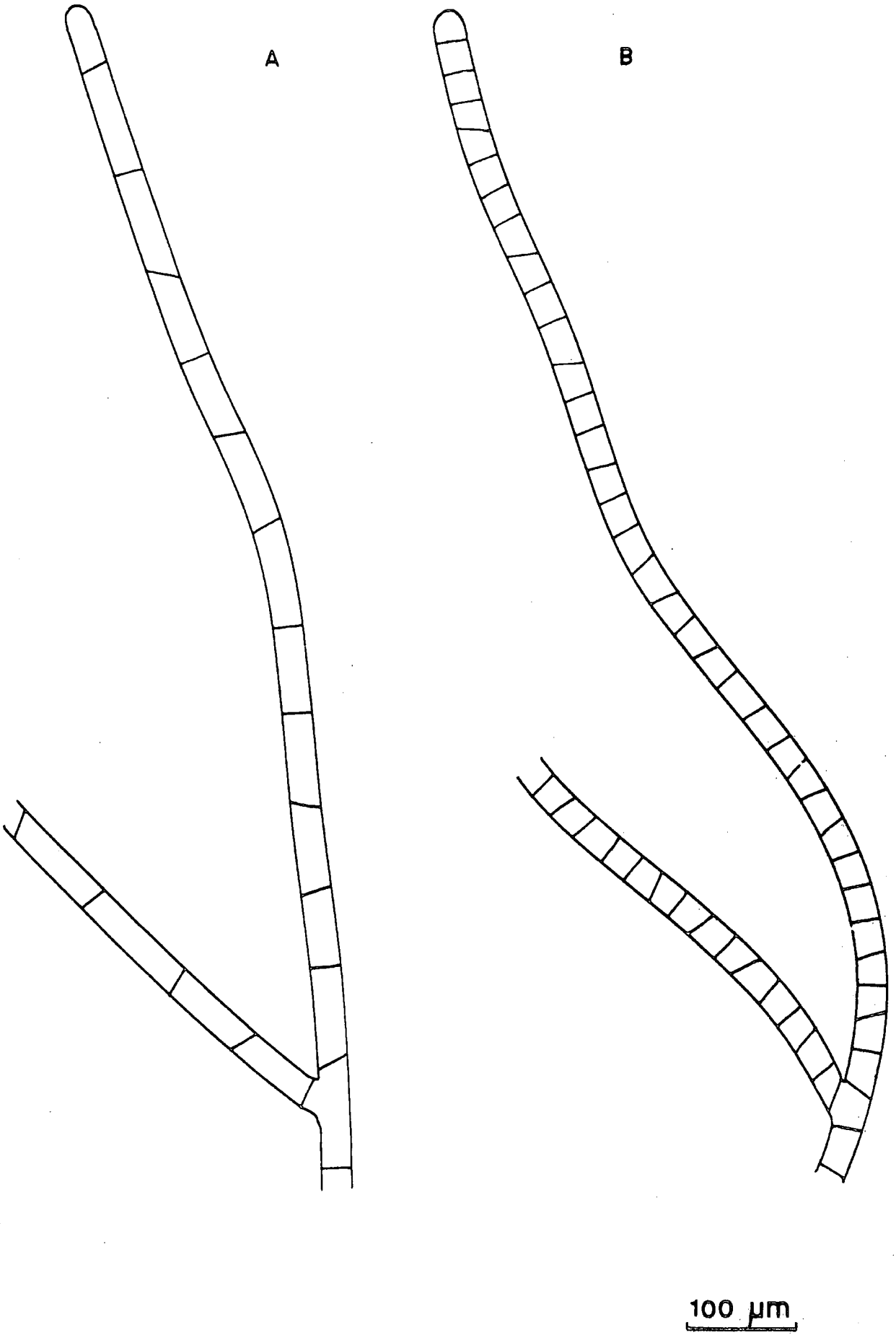


Fig. 4.4 Filamentous protonema from field : (A) 1977; (B) 1979

b) Origin

The filamentous protonema usually originated as a result of growth and branching of the main filament. In addition the gemmae also germinated as filamentous protonema (Fig. 4.9 B-C). Stout protonemal filaments may grow out from the stem of the leafy shoots, usually above the insertion of a leaf.

4.32 Intercellular spaces

Spaces between the cells of the protonemal filaments were seen only in cultures grown in the laboratory. The frequency in a filament varies markedly and some filaments have no intercellular spaces. Typically they occurred every 5 to 12 cells (Fig. 4.6B) but sometimes they occurred beside adjacent cells. They occurred especially in filamentous protonema grown in liquid media, being rare on solid media until the agar started to dry out. The intercellular spaces resulted from a separation of the two opposite cross-walls of two adjacent cells, with a space gradually developing. Filaments with these spaces tended to break easily at this position (Fig. 4.7).

4.33 Gemmae

a) Occurrence and shape

Gemmae were rarely present in plants taken from the field; examples were however seen both on rhizoids and filamentous protonema Only rhizoidal gemmae were observed in the materials of 1977. Two kinds of gemmae were observed on rhizoids: pale brown with an irregular outline (Fig. 4.8A-C) or dark brown gemmae with a more

regular outline (Fig. 4.9A-C). The outline of the protonemal gemmae was always regular. In the laboratory, gemmae were always formed profusely on filamentous protonema, but the rhizoidal gemmae were formed only in old cultures. These gemmae with an irregular outline were small and always occurred in the final filaments of rhizoids (40 - 50 x 25 - 31 μm). Gemmae with a more regular outline were larger (96 - 140 x 70 - 88 μm) and were quite similar to those occurring on filamentous protonema; both form as solitary gemmae.

b) Germination

The gemmae usually germinated (in a lateral or terminal position) to produce a protonemal filament, (4.9B) but rarely another gemmae formed at the terminal end.

4.34 Rhizoids

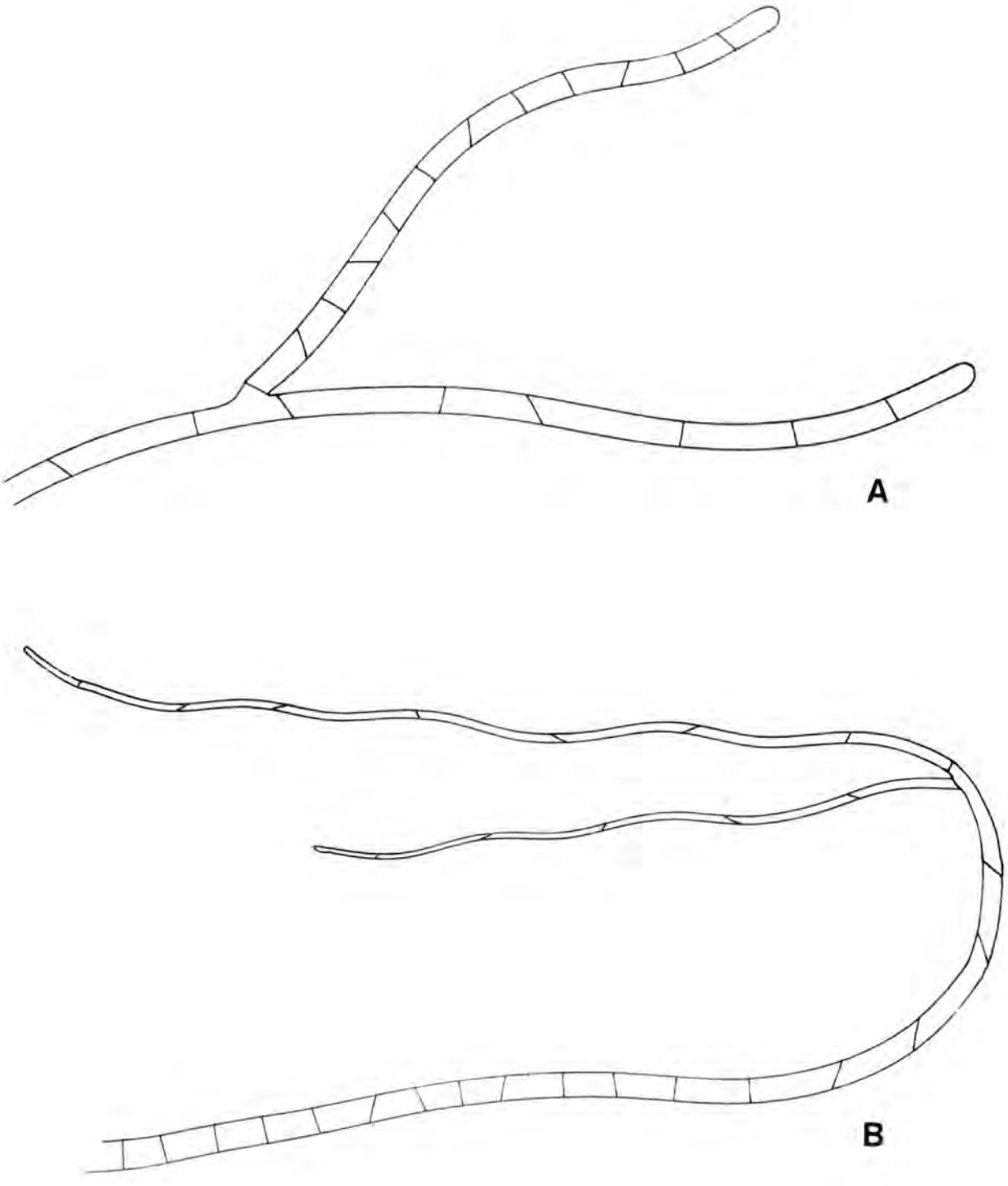
a) Development from leafy shoots

Rhizoids were usually developed from the lower part of the leafy shoot and grew towards the substratum. Two kinds of rhizoids had been observed on the leafy shoots in both field and laboratory. All leafy shoots collected from the field in both 1977 and 1979 produced quite similar rhizoids from the basal portion of stem and along the shoot. Those rhizoids originating from the basal portion were stout and dark brown, while rhizoids from the upper part of shoot were usually pale brown, short and less branching. Shoots grown in the laboratory produced both these kinds of rhizoids (Fig. 4.10 E), but they differed slightly in dimensions and shape (Table 4.3). In liquid

moss grown in	substrate of rhizoid	main filament of rhizoid			
		origin	width	length	colour
field in 1977 and 1979	soil	basal part of 1. sh.	20 - 28	120 - 608	dark brown
		stem of 1. sh.	20 - 24	120 - 608	" "
agar medium	agar	basal part of 1. sh.	20 - 28	120 - 256	" "
	air	stem of 1. sh	16 - 24	120 - 608	pale or dark brown
	agar	protonema	12 - 16	80 - 200	dark brown or colourless
liquid medium	liquid	basal part of 1. sh.	20 - 28	120 - 608	dark brown
	glass	stem of 1. sh.	20 - 24	120 - 608	pale or dark brown
	liquid	protonema	12 - 16	80 - 200	colourless
	"	"	16 - 20	20 - 88	dark brown

Table 4.3 Dimensions of the rhizoids (µm) of populations from different sources 1. sh = leafy shoot

Fig. 4.5 Protonema in laboratory : (A) filamentous protonema grown in solid medium; (B) rhizoid originated from filamentous protonema


A
B
100 µm

media, leafy shoots developed both in and on the liquid. The shoots developed on the liquid produced rhizoids along the stem and very rarely grew out from the apex of the shoot in the air zone. They were stout, with a brown colour; when these rhizoids were attached to the wall of the conical flask, they became longer and more branched. On agar the rhizoids were similar to those produced in the field and on the surface of the liquid medium; they were however formed sparingly on the stem.

b) Development of rhizoids from filamentous protonema

Filamentous protonema produced branching rhizoids, colourless or brown, with oblique cross walls (Fig. 4.5B). The frequency of rhizoid production was variable in liquid medium, but relatively constant in agar medium.

4.35 Leafy shoot plants

4.351 Development on protonema

Leafy shoots were developed on the filamentous protonema (Fig. 4.8 H and 4.9 E), both in liquid and solid media (Section 4.51). These shoots produced perichaetial leaves which differed in shape to stem leaves (4.2) and developed rhizoids along the stem.

4.352 Development on stem

Secondary leafy shoots often developed along the stems (Fig. 4.9D) similar in shape to the original shoot. These shoots usually occurred at

Fig. 4.6 Features of protonema in laboratory: (A) branches arising from the main filament; (B) two adjacent intercellular spaces in protonemal filament grown in liquid; (C) intercellular spaces formed in protonemal filaments grown in solid medium allowed to dry outl

Fig. 4.7 Formation of intercellular space : (A-C) early stages; (D) filament breaking at position of space; (E-F) stages showing the enlargement of the space; (G) last stage showing position at which filament break when the intercellular space has ready formed.

Fig. 4.8 Reproductive structures : (A-E) irregular outline rhizoidal gemmae formed in rhizoids of moss grown in laboratory; (F-G) irregular outline rhizoidal gemmae formed in rhizoids of moss grown in field; (H) bud of leafy shoot originating from green filamentous protonema (grown in liquid)

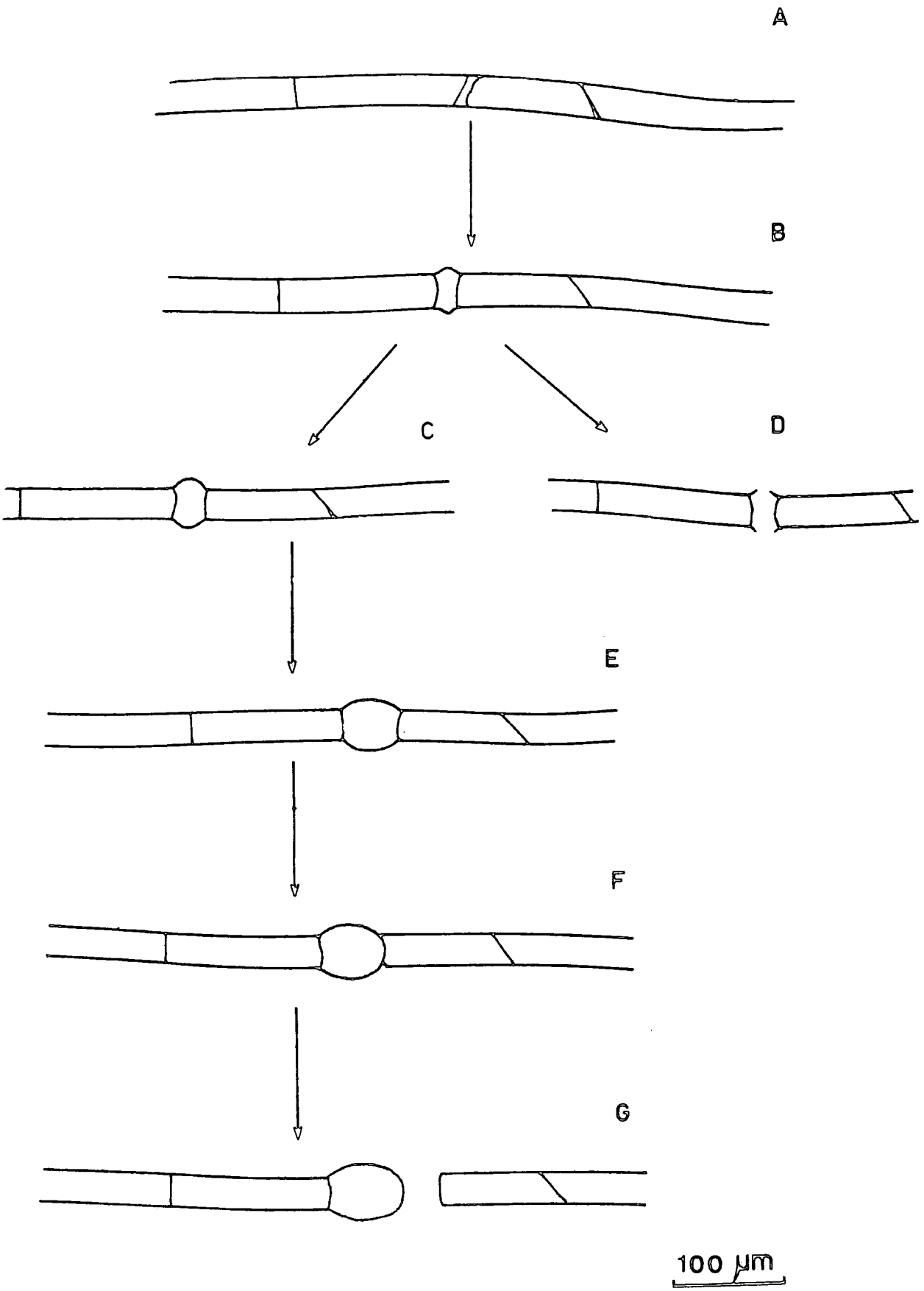
Fig. 4.9 Development of vegetation structures : (A) brown protonemal gemma; (B) germination of filamentous protonema from the apex of protonemal gemma; (C) filamentous protonema developed from protonemal gemma; (D) bud of leafy shoot originating from stem of an adult leafy shoot; (E) bud of leafy shoot originating from thick brown filamentous protonema (grown in solid medium)

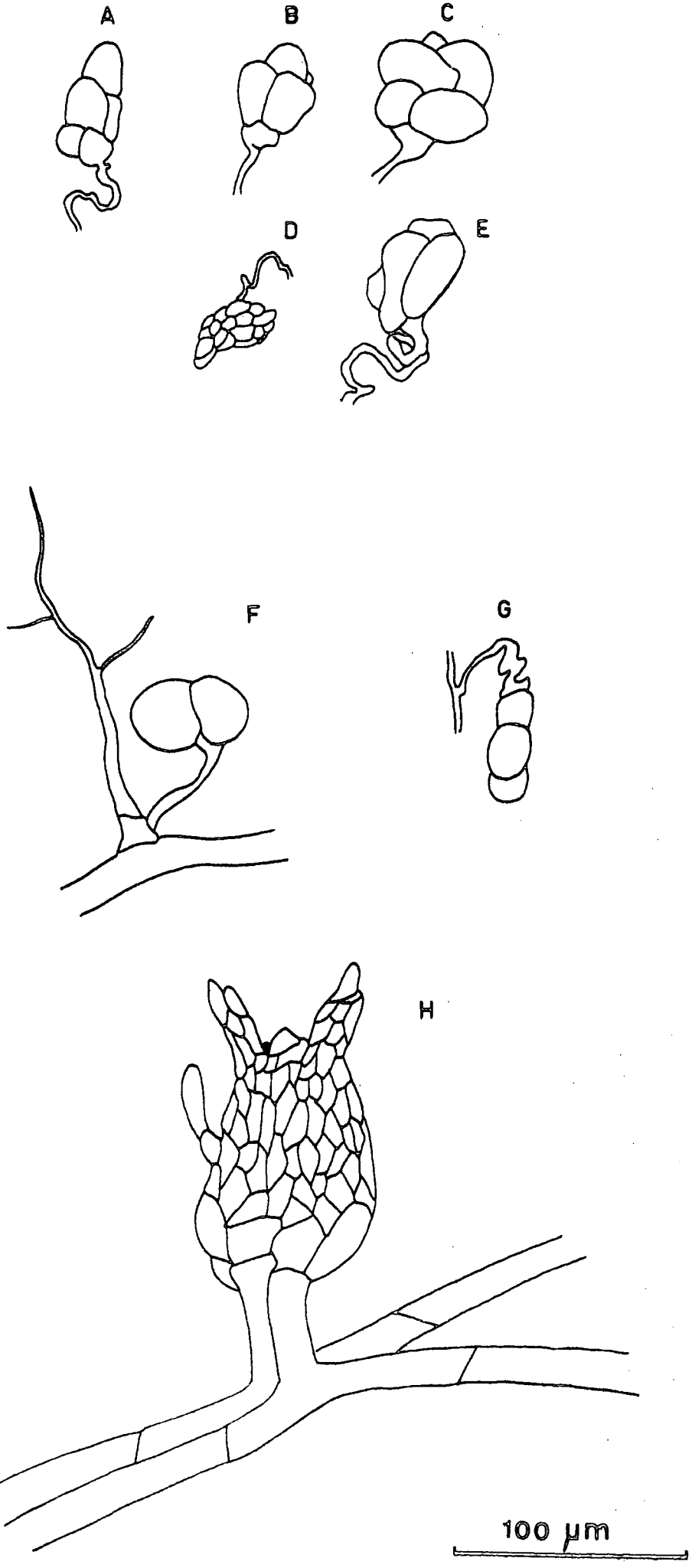
Fig. 4.10 Growth of moss : (A) solid medium showing most of the formation of leafy shoots near the center of the plate (diameter of plate is 8.5 cm); (B) liquid medium, showing the brown mat; (C) leafy shoot from field; (D) leafy shoot grown in field and laboratory; (E) leafy shoot grown in laboratory.

A

B

C


A
B
C
D
E
F
G
100 µm

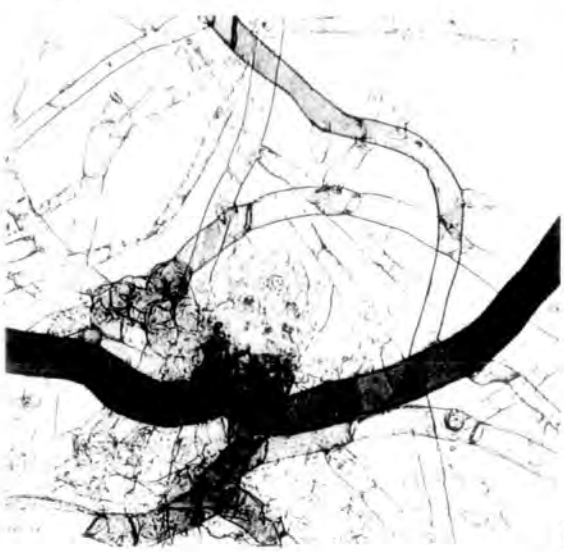

A
B
C
D
E
F
G
H
100 µm

A

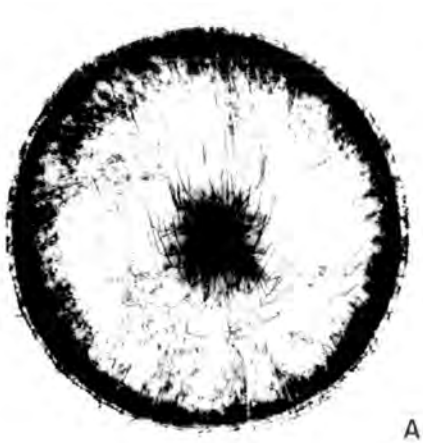
B

C

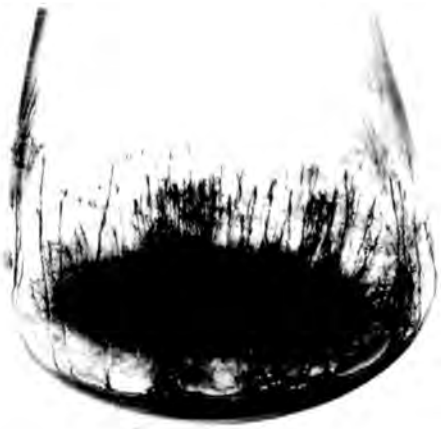
D



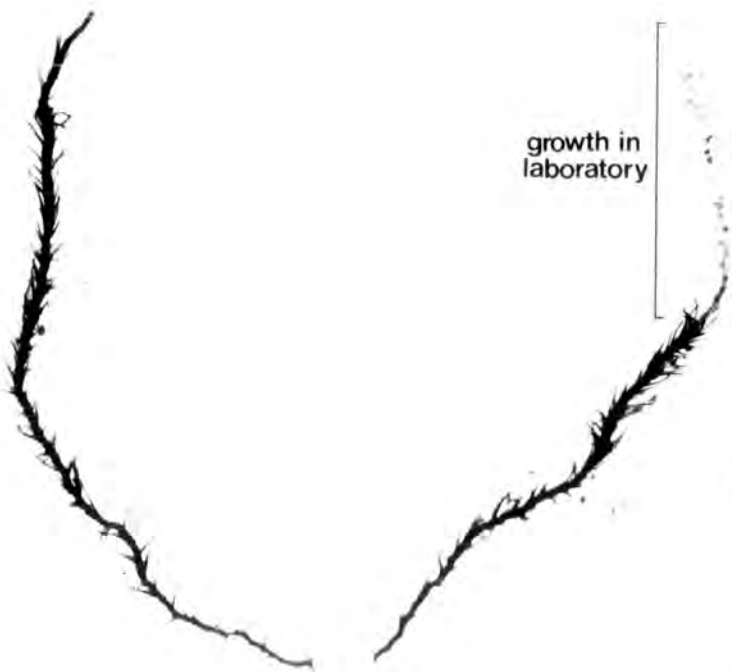
E



A

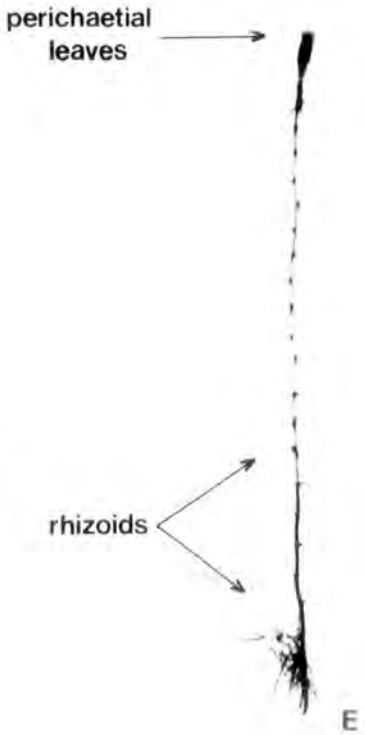


B


growth in laboratory
growth in field

C

D


perichaetial leaves
rhizoids

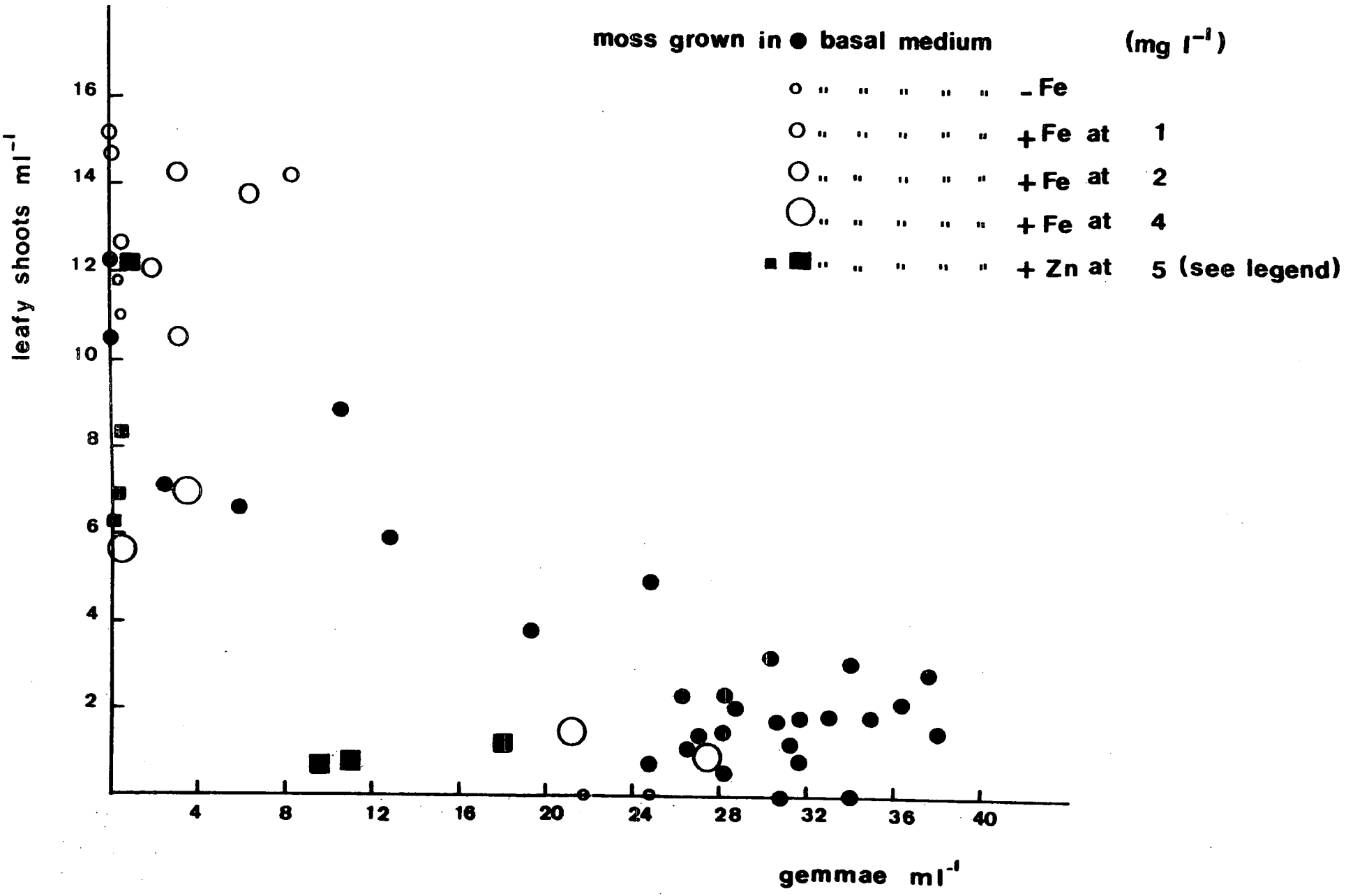
E

the insertion of the leaf. Rhizoids developed on the basal portion of these shoots when they became older.

4.4 Production of gemmae and leafy shoots

The production of each of these structures was variable from one culture to another. Gemmae and leafy shoots were often found to be present in the same culture, but either could be dominant. Sometimes only gemmae or leafy shoots were found in a particular culture. The number of gemmae usually exceeded the numbers of leafy shoots (Fig. 4.11).

Fig. 4.11 Production of gemmae and leafy shoots after four weeks. all originated from filamentous protonema except ■ which originated from leafy shoot : shaken, 25°C, 2600 lx. (Total volume used for experiment was 10 ml, but data expressed per ml)


moss grown in ● basal medium (mg l⁻¹)
○ " " " " " - Fe
○ " " " " " + Fe at 1
○ " " " " " + Fe at 2
◯ " " " " " + Fe at 4
■ ■ " " " " " + Zn at 5 (see legend)
leafy shoots ml⁻¹
16
14
12
10
8
6
4
2
4
8
12
16
20
24
28
32
36
40
gemmae ml⁻¹

4.5 Influence of environmental factors

4.51 Liquid v. Solid

In liquid media, the growth and development of gemmae and leafy shoots not only occurred inside the liquid, but often also formed a mat on the surface of the liquid (Fig. 4.10B). With solid (agar) media leafy shoots always occurred both in and on the agar (Fig. 4.10A); gemmae were not found with solid media. The production of leafy shoots was much more abundant in liquid than solid media. The shoots inside the agar were usually curved or slightly contorted, with much reduced leaves. Secondary leafy shoots developed along the stems in liquid media more than those developed on solid media. In the latter case they occurred only on the surface.

4.52 Shaking v. Static

A slight difference in the morphology of the moss was observed between shaken and static cultures. The growth of shaken cultures was faster than that of static liquid cultures. Static cultures survived for much longer periods than shaken cultures.

4.53 Light v. Dark

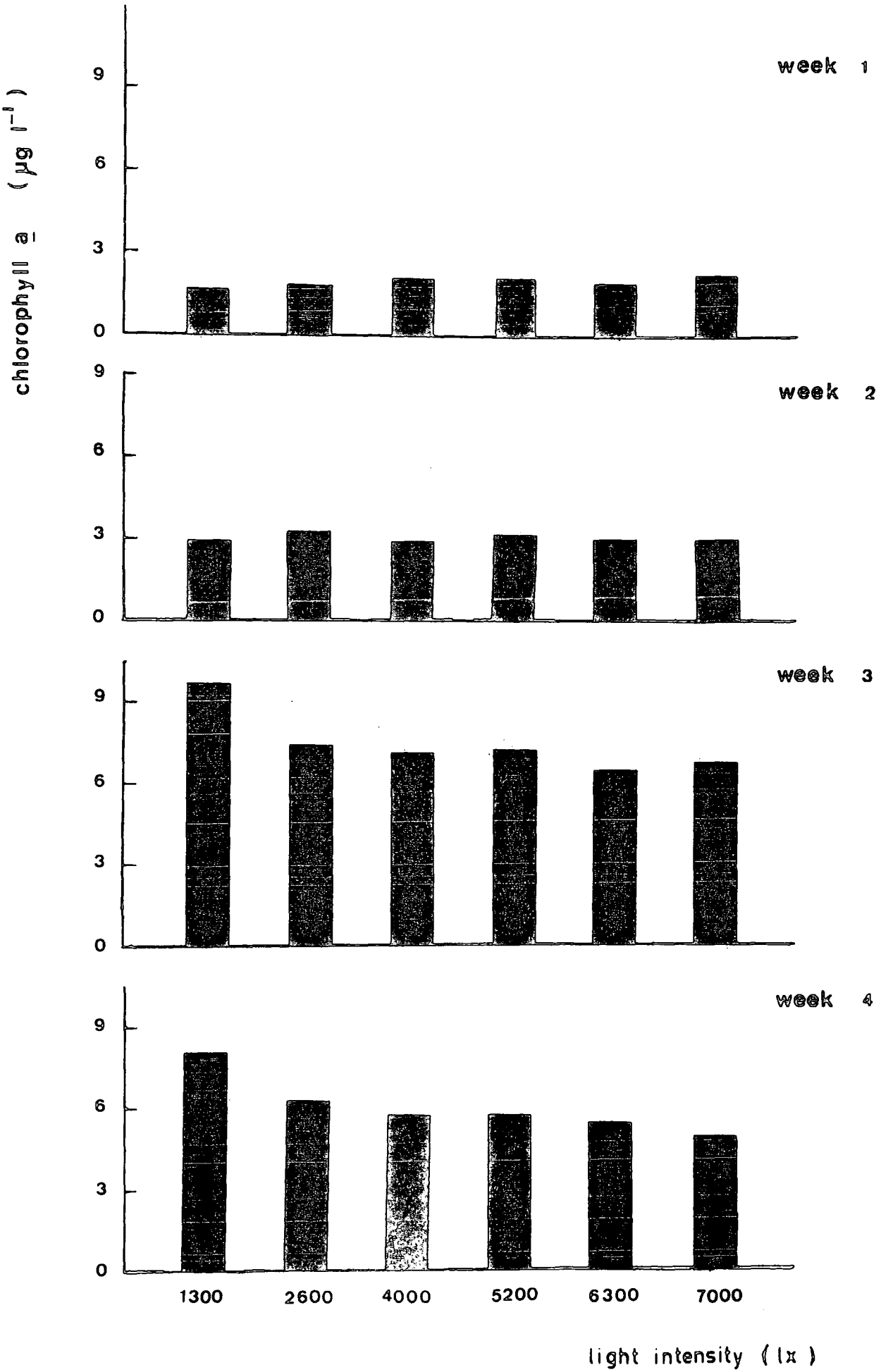
Although growth and production of gemmae were inhibited completely in the dark the moss remained alive (in shaken liquid cultures at 25°C) for at least 4 weeks. Such cultures were able to grow when transferred to the light (25°C, 2600 lx, with shaking).

Those cultures incubated directly in the light showed the first appearance of gemmae at the lower light intensities (1300 and 2600 lx) in the third week, while at higher light intensities (4000 - 7000 lx), gemmae were formed in the second week (Table 4.4). High light intensity enhanced the subsequent production of gemmae and leafy shoots. Chlorophyll a reached a maximum in the third week (Fig. 4.13).

time (weeks)	1			2			3			4		
light intensity (lx)	gemmae	l.sh.	int.s.	ge.	l.sh.	int. s.	ge	l.sh.	int. s.	ge.	l.sh.	int.s.
1300	0	0	0	1.4	0	0	4.8	0	0	21.8	0	3.4
2600	0.03	0	0	1.4	0	0	8.7	0	0	30.0	0.4	3.4
4000	0	0	0	1.9	0	0	9.1	0	0	35.8	0.1	3.5
5200	0	0	0	1.5	0.1	0	10.1	0	0	41.2	0	3.5
6300	0	0	0	2.3	0	0	10.8	0.2	0	45.6	0	3.6
7000	0	0	0	2.7	0	0	12.8	0	0	37.8	0	3.5

Table 4.4 Influence of light intensity on the production with time of gemmae (ge) leafy shoots (l.sh) and intercellular spaces (int s) in shaken liquid medium at 25°C, n=2 except for 2600 lx where n = 4; details given in Table 4.7 (Total volume = 10 ml, but data expressed per ml).

Fig. 4.12 Influence of light intensity on production of chlorophyll *a* with time : shaken, 25°C, 2600 lx; n = 2, except at 2600 lx where n = 4. (Total volume used for experiment was 10 ml, but data expressed per l)


chlorophyll a (µg l⁻¹)
week 1
week 2
week 3
week 4
0
3
6
9
1300
2600
4000
5200
6300
7000
light intensity (lx)

4.6 Influence of chemical factors

4.61 Iron

4.611 Materials and methods

Materials were moved from the liquid basal medium and incubated in iron-free liquid medium for four days prior to the experiment. Inoculation was made as the method described in 2.33. Tubes were incubated with shaking for four weeks at 25°C and 2600 lx. All media were buffered as described in Section 2.32.

4.612 Results

Gemmae and leafy shoots were still produced abundantly at the lower concentrations (Table 4.5). Gemmae and leafy shoots occurred in the same culture, but the ratio varied markedly. The total combined number of gemmae and leafy shoots is expressed graphically in Fig. 4.13. The frequency of intercellular spaces was greater in these lower concentrations of iron.

Fe (mg l^{-1})	gemmae ml^{-1}		leafy shoots ml^{-1}		intercellular spaces ml^{-1}	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
0	12.2	10.8	5.7	5.7	6.5	1.0
0.5	11.0	11.1	7.6	3.8	3.0	0.6
1.0	2.8	3.3	14.3	1.0	2.9	0.5
2.0	3.8	16.7	12.7	1.5	2.6	0.5
4.0	12.8	10.7	3.8	2.6	2.3	0.6
8.0	12.5	12.3	3.6	3.6	2.2	0.6

Table 4.5 Influence of iron on the production after four weeks of gemmae, leafy shoots and intercellular spaces in shaken liquid medium at 25°C, 2600 lx. n = 4 (Total volume = 10 ml, but data expressed per ml)

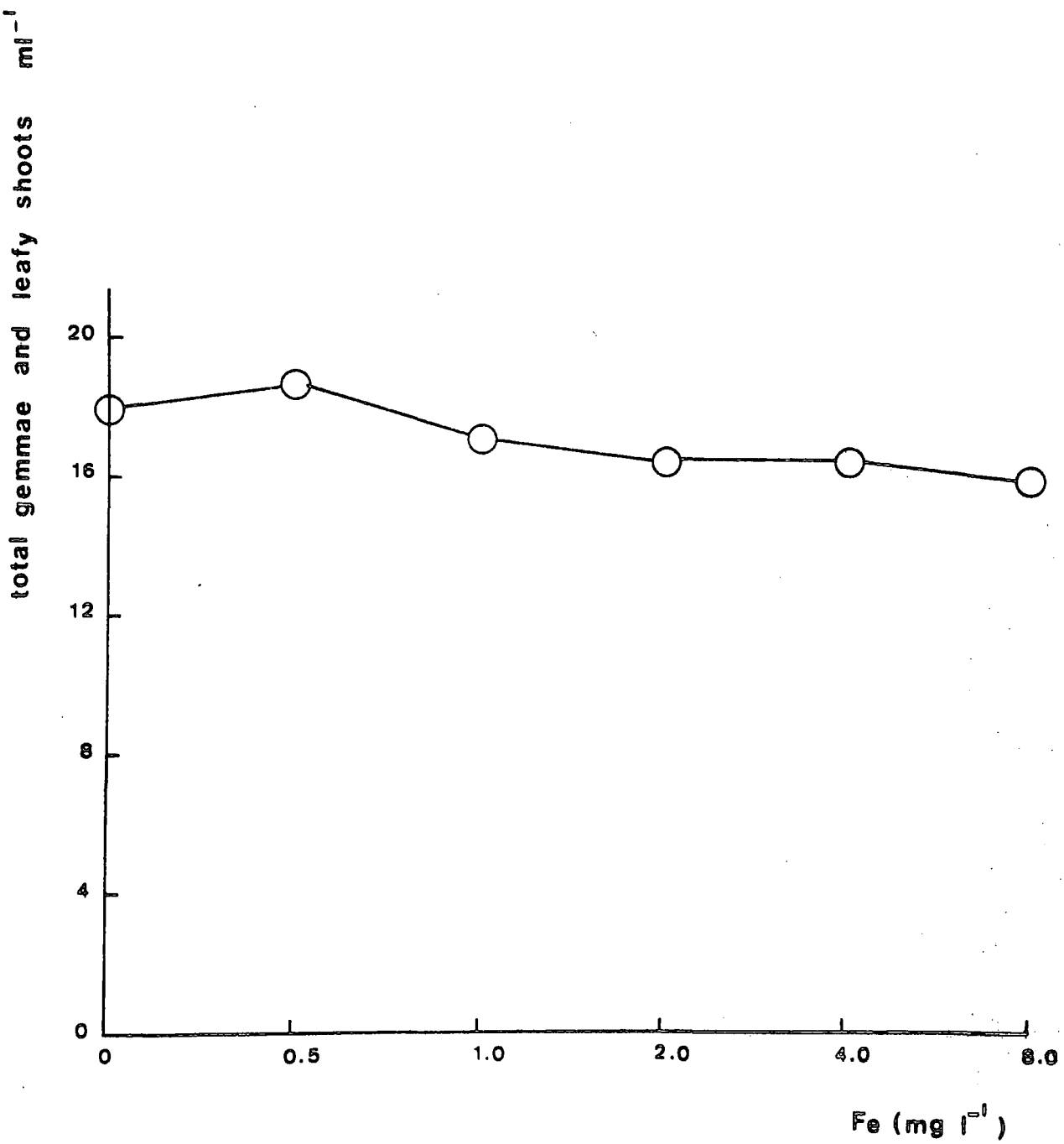


Fig. 4.13 Influence of Fe on total gemmae and leafy shoots after four weeks : shaken, 25°C, 2600 lx; n = 4. (Total volume used for experiment was 10 ml, but data expressed per ml).

4.62 Phosphate

4.621 Materials and methods

Materials were moved from the liquid basal medium and incubated in phosphate-free liquid medium for four days prior to the experiment. KCl was used to maintain the level of K in the medium and it was buffered as described in 2.32. Incubation was made for four weeks at 25°C and 2600 lx with moderate shaking.

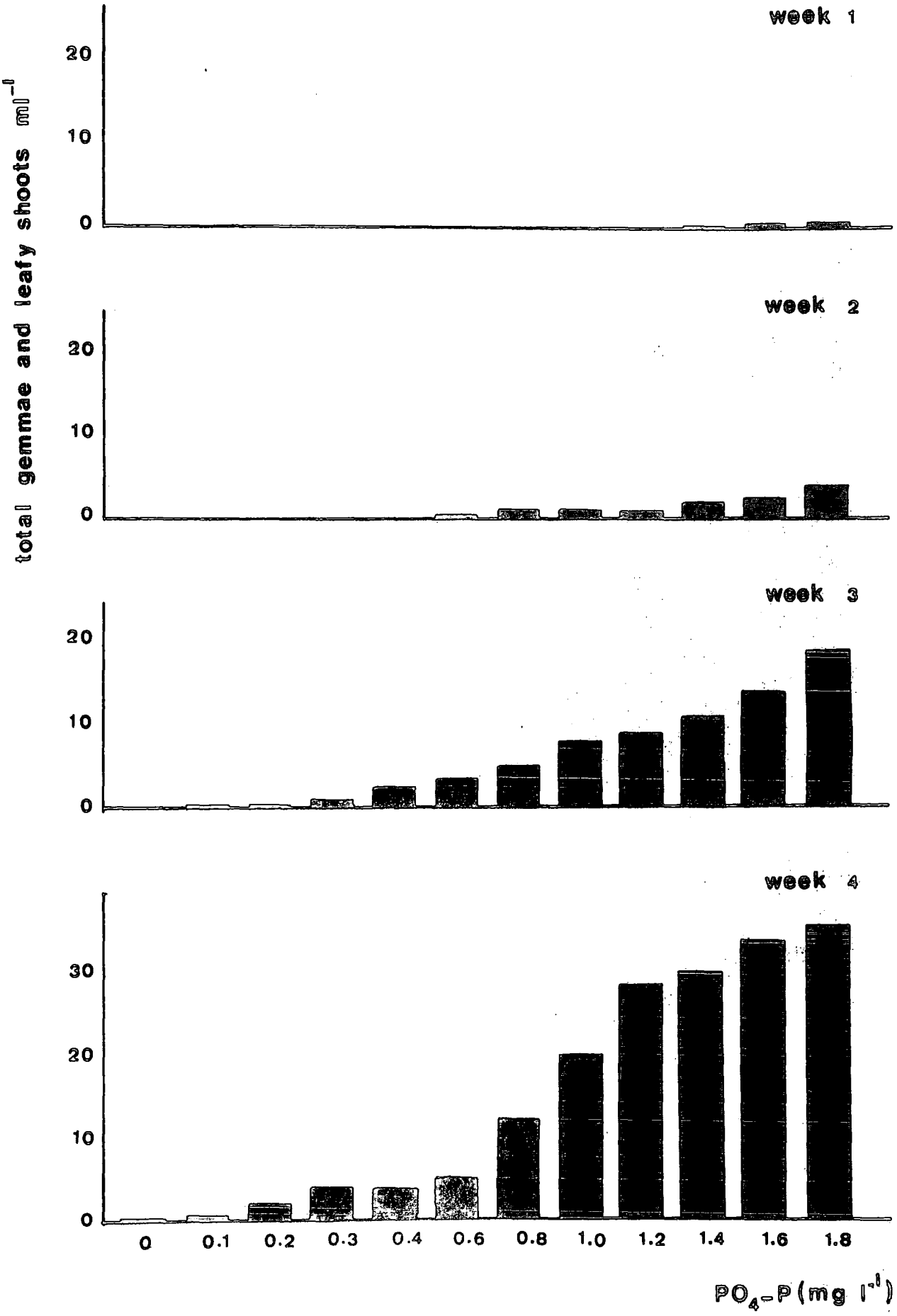
4.622 Results

Deficiency of phosphate led to a marked reduction of gemmae and leafy shoots production, (Table 4.6). The total combined number of gemmae and leafy shoots is expressed graphically in Fig. 4.14. The formation of intercellular spaces was stimulated.

PO_4-P ($mg\ l^{-1}$)	gemmae ml^{-1}								leafy shoots ml^{-1}								intercellular spaces ml^{-1}							
	one week		two weeks		three weeks		four weeks		one week		two weeks		three weeks		four weeks		one weeks		two weeks		three weeks		four weeks	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
0							0.2	0.02											3.1	0.9	10.6	2.1	25.4	4.9
0.1					0.2	0.1	1.0	0.3											2.3	1.0	9.6	1.7	24.3	4.5
0.2					0.3	0.1	2.1	0.5											2.0	1.0	7.5	2.5	20.3	4.3
0.3					0.9	0.2	3.6	0.6											1.3	0.5	6.8	1.5	18.9	4.1
0.4					1.0	0.2	3.5	0.7													5.3	1.3	15.8	3.2
0.6			0.5	0.2	2.0	0.6	4.8	0.9													2.3	1.0	9.5	2.0
0.8			0.7	0.2	5.2	0.8	12.2	1.8							0.2	0.1							6.3	1.7
1.0			0.7	0.2	7.9	1.2	19.3	3.0							0.5	2.2							5.8	1.0
1.2			1.1	0.3	8.7	0.9	28.0	3.6					0.4	0.3	0.4	0.3							4.2	0.5
1.4	0.03	0.04	1.6	0.4	10.7	1.2	29.0	3.1					0.5	0.2	0.7	0.5							3.7	0.9
1.6	0.1	0.1	2.5	0.5	13.3	1.9	32.6	3.1					0.4	0.2	1.7	0.6							3.5	0.8
1.8	0.13	0.08	3.7	0.5	18.4	1.8	35.6	2.3					0.5	0.3	1.6	0.6							3.2	1.1

Table 4.6 Influence of phosphate on the production with time of gemmae, leafy shoots and intercellular spaces in shaking liquid medium at 25°C and 2600 lx. n = 4 (Total volume = 10 ml, but data expressed per ml)

Fig. 4.14 Influence of PO_4-P on total gemmae and leafy shoots with time: shaken, 25°C, 2600 lx; n = 4. (Total volume used for experiment was 10 ml, but data expressed per ml)


week 1
week 2
week 3
week 4
total gemmae and leafy shoots ml⁻¹
0
10
20
30
0
0.1
0.2
0.3
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
PO4-P (mg l⁻¹)

4.7 Growth

4.71 Growth form

4.711 Liquid cultures

a) Static

Soon after subculture into liquid medium, an inoculum grew and produced a new protonemal branch. After about 5 weeks, profuse growth was apparent; protonemal gemmae and leafy shoots appeared in the liquid, and when the cultures became still older rhizoidal gemmae were formed. Numerous filamentous protonema developed at the surface of the liquid. Soon after their appearance there, they usually turned brown; they continued to produce new brown branches which formed a thick brown mat. Both the filaments of the protonema in the liquid and those of the brown mat eventually produced more leafy shoots. The brown mat expanded until it touched the glass wall of the conical flask (Fig. 4.10B); then some filaments of the protonema and leafy shoots grew upward into the air, although sticking to the glass wall. Leafy shoots growing in liquid produced numerous green filaments of protonema along their stems, whereas those of the brown mat produced only brown rhizoids.

b) Shaking

The growth form was similar to that in static liquid cultures, but all growth was restricted to the liquid. Some filaments of the protonema developed on the surface of the liquid, forming a thin brown mat; they did not produce any gemmae or leafy shoots.

4.712 Solid cultures

The inoculum produced new branches of filamentous protonema soon after inoculation. Most initial protonemal growth was restricted to the agar block of the inoculum and the subsurface region of the plates. After about three weeks, growth occurred profusely at the surface of the agar; after about four weeks, numerous leafy shoots appeared on the agar surface and also a few inside the agar (Fig. 4.10A). These leafy shoots often became longer than those on the surface (4.51). Finally the cultures appeared to become dormant and remained so until the cultures died.

4.72 Growth rate

a) Liquid cultures

Assessment of growth was carried out by determination of chlorophyll a (See Fig. 4.12). Estimates were also made of gemmae and leafy shoots (Table 4.7); changes during growth of the combined totals of these two structures are shown in Fig. 4.15.

b) Solid cultures

Assessment of growth on solid cultures was attempted, both by estimates of the area occupied by the moss and by determination of chlorophyll a (Fig. 4.16). The numbers of leafy shoots per culture is shown in Table 4.8.

time (weeks)	1		2		3		4	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
gemmae	0.1	0.08	1.4	0.4	8.7	1.6	29.9	2.9
leafy shoots	0		0		0.1	0.08	0.4	0.5
intercellular spaces	0		0		0		3.4	0.7

Table 4.7 Influence of time on the production of gemmae, leafy shoots and intercellular spaces in shaken liquid medium at 25°C, 2600 lx. n = 4 (Total volume = 10 ml, but data expressed per ml)

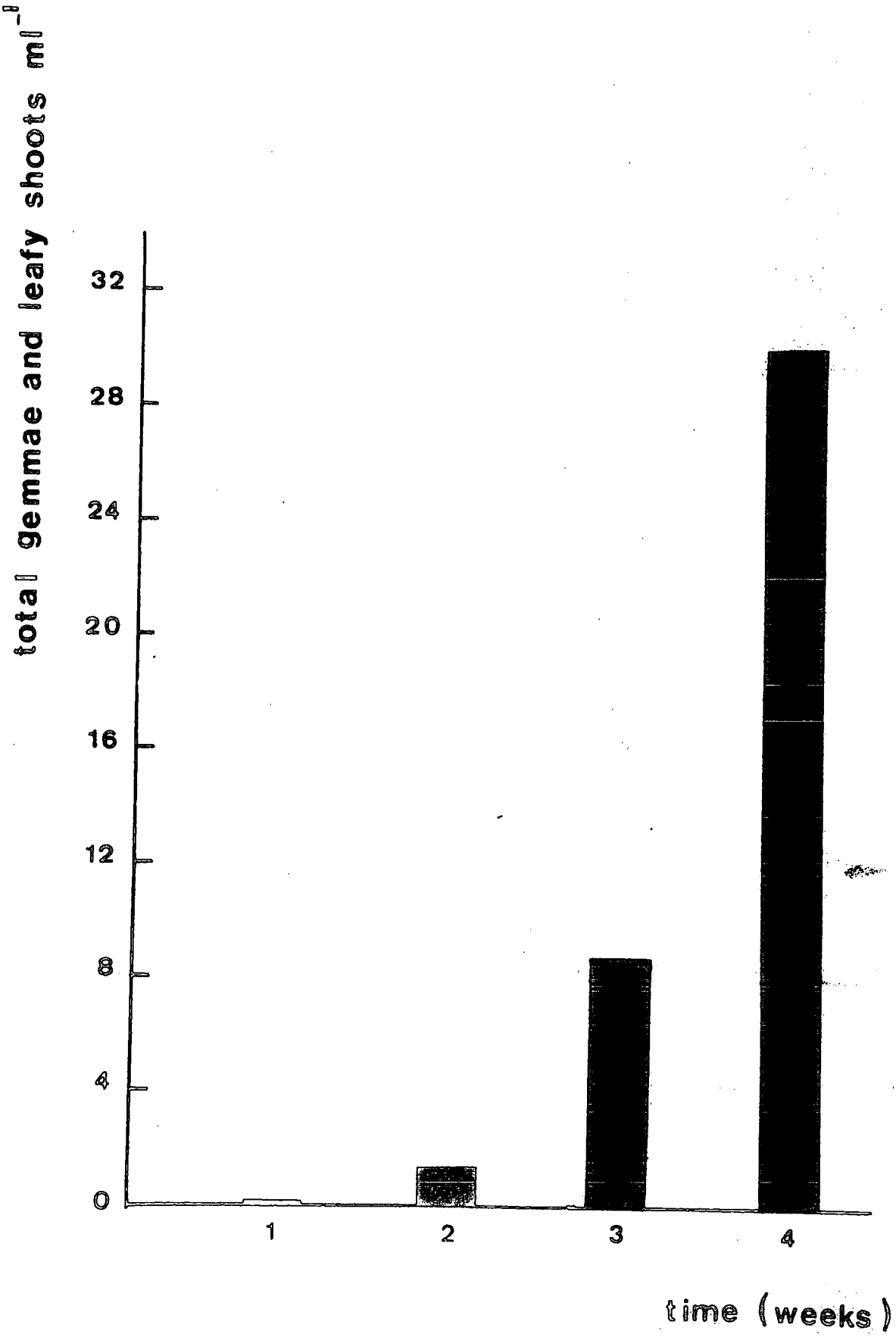


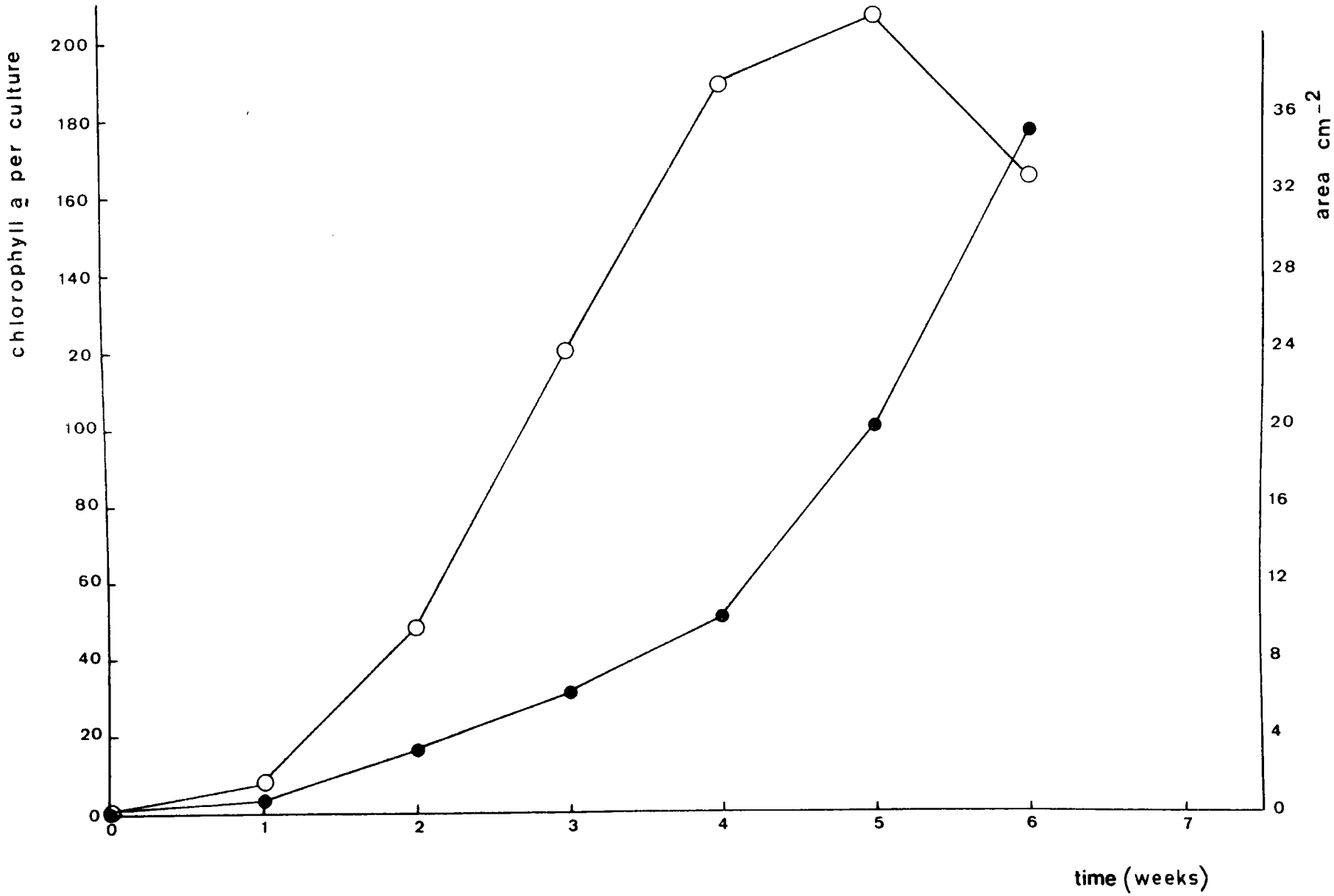
Fig. 4.15 Formation of total gemmae and leafy shoots with time: shaken, 25°C, 2600 lx; n = 4. (Total volume used for experiment was 10 ml, but data expressed per ml)

time (weeks)	µg chl a cm^{-2}	leafy shoots cm^{-2}		leafy shoots per culture	
		$\bar{x}$	s.d.	$\bar{x}$	s.d.
1	13.2	0		0	
2	15.1	0		0	
3	19.6	0		0	
4	16.3	0.1	0.06	1	0.7
5	10.0	0.1	0.05	2	1.1
6	4.6	0.3	0.1	9	4.5
7*	2.9	1.0	0.1	25	4.5

Table 4.8 Influence of time on the production of leafy shoots and chl a in solid medium at 25°C and 2600 lx. No gemmae or intercellular spaces present. n = 4.

* growth of the moss colonized the whole agar plate, but the agar became contracted i.e. total agar surface was reduced.

Fig. 4.16 Comparison of chlorophyll a and area occupied by moss during growth: solid medium, 25°C, 2600 lx; n = 4.


chlorophyll a per culture
200
180
160
140
20
100
80
60
40
20
0
area cm^{-2}
36
32
28
24
20
16
12
8
4
0
0
1
2
3
4
5
6
7
time (weeks)

5. INFLUENCE OF ZINC

5.1 Toxicity

5.11 Toxicity test

In preliminary tests for zinc toxicity, a wide range of zinc concentrations (0, 5, 10, 20 .... 100 mg l^{-1})in liquid media were used. The method for culturing carried out in this assay has been described in 2.3, but a brief description is included here. Inocula as protonema or leafy shoots were added to media to which any zinc has already been added, and tubes were then incubated in a tank shaker at 25°C, 2600 lx, with moderate shaking for the remaining 24 h . The initial pH of the medium in the tubes did not drift any more than 0.05 pH unitsduring the period of the experiments.

Growth in the tubes was compared visually at weeks 1, 2, 3 and 4, both against preserved replicates of the original inocula, and also against other tubes. Observations were recorded on each occasion according to the procedure of Whitton (1970):

1. maximum concentration causing no inhibition
2. minimum concentration causing slight inhibition
3. maximum concentration at which moss is alive
4. minimum concentration at which moss is killed.

At the end of the fourth week the moss was moved for counting gemmae and leafy shoots and other microscopic inspection. Table 5.1 shows the effect of zinc on these structures. There was a slight difference in the production of protonema between the values observed for 1 and 2 when they were produced by either the protonema themself or by the

Observations	Production of filamentous protonema by		Production of gemmae and leafy shoots by	
	filamentous protonema	leafy shoot	filamentous protonema	leafy shoot
maximum concentration causing no inhibition	20	15	0.04	2
minimum concentration causing slight inhibition	25	20	1	5
maximum concentration at which moss is alive or showing marked inhibition in producing gemmae and leafy shoots	70	70	10	25
minimum concentration at which moss is killed or stopped producing gemmae or leafy shoots	80	80	15	30

Table 5.1 Influence of zinc (mg l^{-1}) on the growth and the production of gemmae and leafy shoot of the moss in liquid medium at 25°C and 2600 lx

leafy shoot plants, and there was no differences in the protonemal growth between the values observed for 3 and 4 by the same former mentioned structures; but a great difference has been shown in values of the production of gemmae and leafy shoots between those which were produced by the protonema and the others which were produced by leafy shoots. Only very poor protonemal growth developed from the surface of the leafy shoot added to media containing 70 mg l^{-1} Zn.

5.12 Observations

Several processes were investigated with regard to possible effects of zinc: production of protonema, gemmae and leafy shoots and intercellular spaces (Tables 5.2, 5.3, 5.4 and 5.5). It is apparent that zinc at higher concentrations inhibited the growth of Dicranella. The moss was killed in the highest concentration of zinc (100 mg l^{-1}) after about 5 days, while it remained alive in media containing 80 mg l^{-1} Zn for about one week; both protonemal filaments produced either by the growth and branching, or springing from the stem of the leafy shoot which occurred in the high levels of zinc, were very poor and short; the production of gemmae and leafy shoots was also inhibited, but at much lower concentrations of zinc. Variable results for the production of the intercellular spaces was observed (5.22 and 5.23). No other noticeable morphological changes occurred at the higher zinc concentrations.

5.13 Assays

From the results of the toxicity tests, it soon became apparent that higher levels of zinc were needed to inhibit protonemal growth and

much lower concentrations of zinc affected the production of gemmae and leafy shoots as is mentioned in 5.12.

5.2 Morphological effects

5.21 Production of gemmae and leafy shoots

a) Liquid cultures

In these studies, the effect of zinc on the production of both gemmae and leafy shoots formed either by the filamentous protonema or by the leafy shoots was investigated (Tables 5.2 & 5.3). Zinc inhibited considerably the production of gemmae by protonema more than that by leafy shoots; 10 mg l^{-1} Zn inhibited the production of these structures produced by the filamentous protonema, but much higher levels of zinc (25 mg l^{-1}) were needed to inhibit their production by leafy shoots (Table 5.3). The effect of zinc with filamentous protonema as the inoculum is shown in Fig. 5.1. In the case of the production of gemmae "which were the dominant" it was observed that even the lowest concentration of zinc (1.0 mg l^{-1}) had an inhibitory effect on their production (Table 5.4). The effects of zinc became more marked with time (Table 5.5). Thus 5 mg l^{-1} Zn reduced the production of gemmae approximately 17% in 2 weeks compared with the control, but by 4 weeks the number of gemmae in 5.0 mg l^{-1} Zn was about 11% of the control.

b) Solid cultures

The effect of zinc on the production of gemmae and leafy shoots in agar media is shown in Table 4.6. The highest concentration of zinc (10 mg l^{-1}) had a slight inhibitory effect on the production of leafy

Zn (mg l^{-1})	gemmae ml^{-1}		leafy shoots ml^{-1}		intercellular spaces ml^{-1}	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
0	30.2	2.8	2.2	0.6	3.3	0.5
5	2.9	0.8	0.03	0.04	4.9	0.6
10	0.1	0.08	0		7.4	1.5
15	0		0		9.6	1.3
20	0		0		20.5	3.4
25	0		0		19.2	3.8
30	0		0		5.4	1.1
35	0		0		0	

Table 5.2 Influence of zinc on the production after four weeks of gemmae, leafy shoots and intercellular spaces by filamentous protonema in shaken liquid medium at 25°C and 2600 lx; n = 4
(Total volume = 10 ml, but data expressed per ml)

Zn (mg l^{-1})	gemmae ml^{-1}		leafy shoots ml^{-1}			intercellular spaces ml^{-1}	
	$\bar{x}$	s.d.	initial	$\bar{x}$	s.d.	$\bar{x}$	s.d.
0	0.3	0.2	0.1	8.0	1.3	2.8	0.7
5	0.1	0.1	0.1	6.8	1.0	4.1	0.7
10	0.2	0.4	0.1	3.1	0.6	7.1	1.3
15	0.8	0.6	0.1	1.7	0.4	9.1	0.8
20	0.6	0.6	0.1	1.2	0.4	14.3	2.1
25			0.1	0.5	0.3	9.9	1.4
30			0.1			3.4	0.9
35			0.1				

Table 5.3. Influence of zinc on the production after four weeks of gemmae, leafy shoots and intercellular spaces by leafy shoots in shaken liquid medium at 25°C and 2600 lx. n = 4 (Total volume = 10 ml, but data expressed per ml)

Zn (mgl^{-1})	gemmae ml^{-1}		leafy shoots ml^{-1}		intercellular spaces ml^{-1}	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
0	26.0	5.1	4.6	3.5	3.3	0.7
1	19.7	2.1	1.6	0.6	3.5	0.7
2	8.3	4.3	3.6	4.8	3.6	0.7
3	6.5	0.8	0.6	0.2	3.9	0.7
4	4.1	0.7	0.2	0.08	4.2	1.1
5	2.7	0.7	0.1	0.07	4.8	1.2
6	1.7	0.3			5.1	1.1
7	0.9	0.3			5.6	1.2
8	0.4	0.1			5.9	0.9
9	0.2	0.08			6.9	1.6
10	0.1	0.08			8.3	1.8

Table 5.4 Influence of zinc on the production after four weeks of gemmae, leafy shoots and intercellular spaces produced by filamentous protonema in shaken liquid medium at 25°C and 2600 lx ; n = 4 (Total volume = 10 ml, but data expressed per ml)

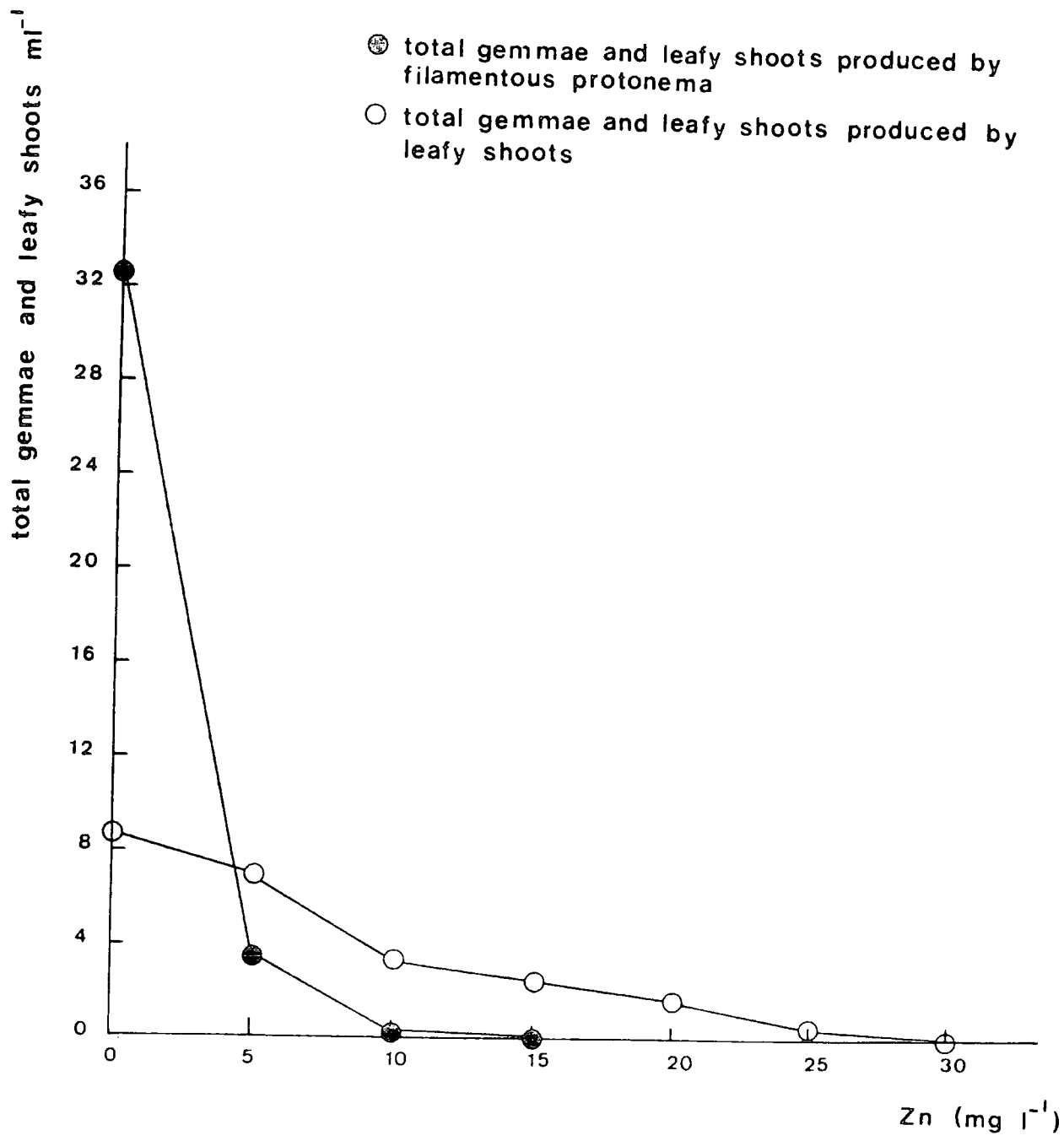


Fig. 5.1 Influence of zinc on total gemmae and leafy shoots produced after four weeks by filamentous protonema: shaken, 25°C, 2600 lx; n = 4. (Total volume used for experiment was 10 ml, but data expressed per ml)

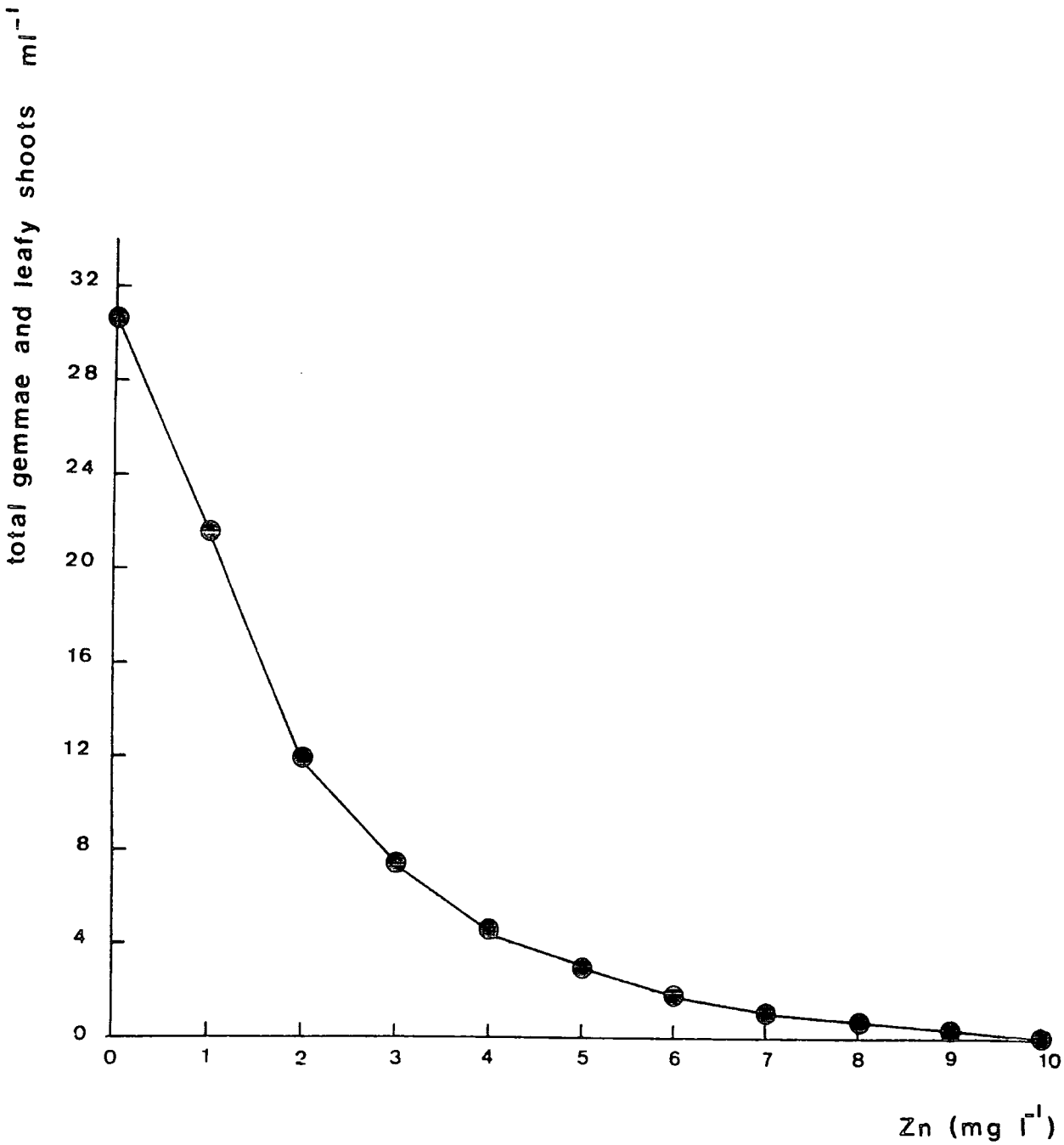


Fig. 5.3 Influence of zinc on total gemmae and leafy shoots produced in successive weeks by filamentous protonema: shaken, 25°C, 2600 lx; n = 4. (Total volume used for experiment was 10 ml, but data expressed per ml)

Zn (mg l^{-1})	gemmae ml^{-1}								leafy shoots ml^{-1}								intercellular spaces ml^{-1}							
	week 1		week 2		week 3		week 4		week 1		week 2		week 3		week 4		week 1		week 2		week 3		week 4	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
0	0.1	0.08	1.8	0.3	10.7	1.4	32.4	3.5	0		0.1	0.03	0.2	0.2	2.1	0.6	0		0		0		3.0	0.8
1	0		1.2	0.3	6.1	0.8	21.2	3.6	0		0.03	0.04	0.03	0.04	2.1	1.4	0		0		0		3.2	0.8
2	0		0.9	0.2	3.4	0.6	11.9	2.4	0		0		0		1.0	0.3	0		0		0		3.5	0.7
3	0		0.7	0.2	2.3	0.5	7.9	1.6	0		0		0		0.5	0.2	0		0		0		4.1	0.9
4	0		0.4	0.3	1.7	0.5	4.6	1.2	0		0		0		0		0		0		0		4.3	1.0
5	0		0.3	0.1	1.1	0.4	3.5	1.0	0		0		0		0		0		0		0		4.9	1.2
6	0		0.1	0.07	0.8	0.3	2.3	0.8	0		0		0		0		0		0		0		5.2	1.1
7	0		0.03	0.04	0.5	0.2	1.1	0.4	0		0		0		0		0		0		0		5.9	1.2
8	0		0		0.1	0.04	0.8	0.3	0		0		0		0		0		0		0		6.0	0.8
9	0		0		0		0.3	0.02	0		0		0		0		0		0		0		6.7	1.3
10	0		0		0		0.2	0.05			0		0		0		0		0		0		7.9	1.3

Table 5.5 Influence of zinc on the production (with time) of gemmae, leafy shoots and intercellular spaces produced by filamentous protonema in shaken liquid medium at 25°C and 2600 lx. n = 4. (Total volume = 10 ml, but data expressed per ml).

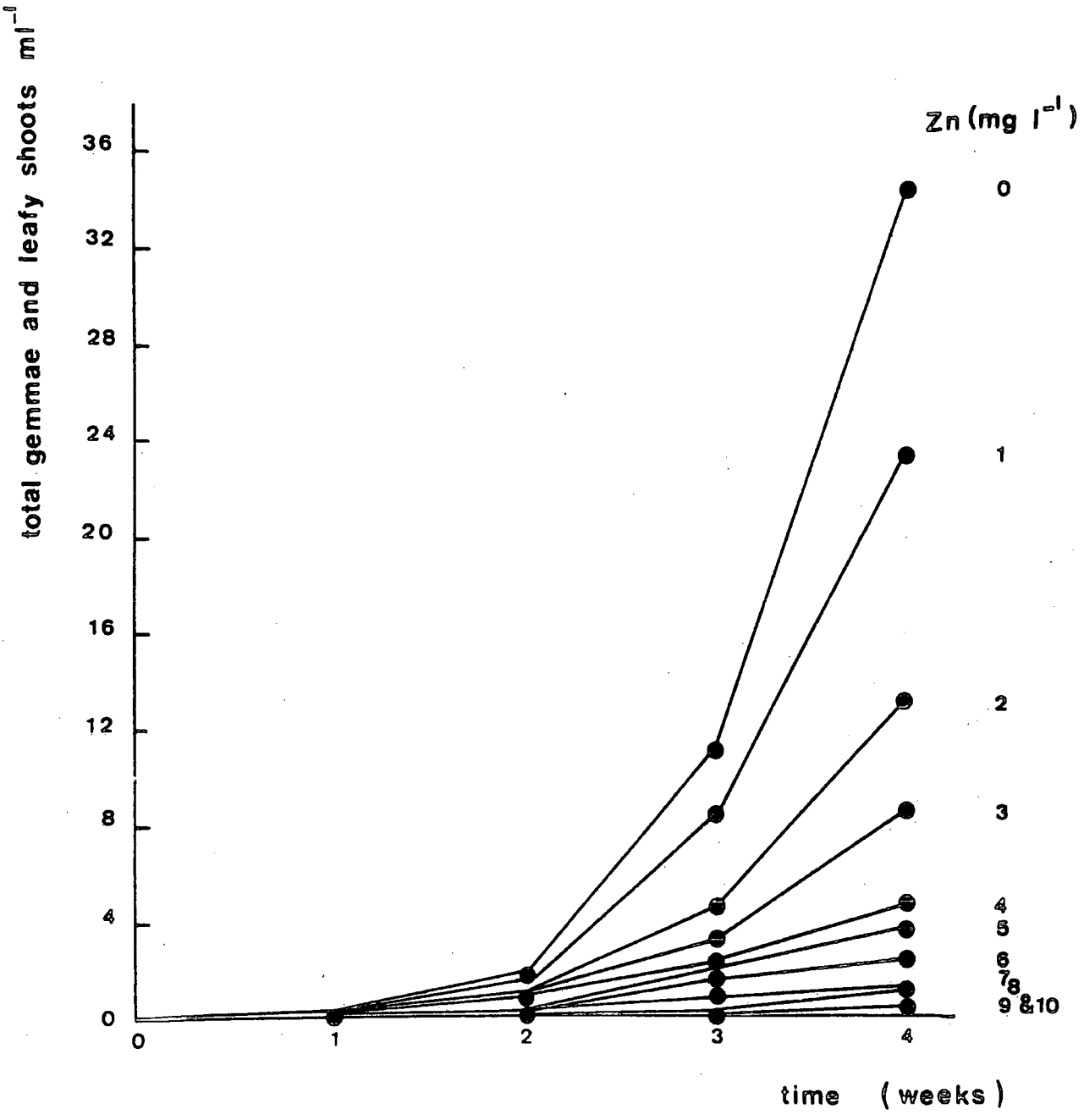


Fig. 5.2 Comparison of influence of zinc on total gemmae and leafy shoots produced after four weeks by filamentous protonema and by leafy shoots: shaken, 25°C, 2600 lx; n = 4. (Total volume used for experiment was 10 ml, but data expressed per ml)

Zn (mg l^{-1})	time (weeks)													
	1		2		3		4		5		6		7 *	
	area	l.sh.	area	l.sh	area	l.sh.	area	l.sh.	area	l.sh.	area	l.sh.	area	l.sh.
0	0.7	0	3.3	0	6.5	0	12.8	2	22.2	4	34.8	9	26.7	30
1	0.6	0	3.2	0	6.8	0	12.2	2	23.2	4	35.0	11	26.0	29
2	0.7	0	3.2	0	6.8	0	12.1	1	22.5	3	33.2	9	26.0	22
3	0.7	0	3.2	0	6.3	0	12.4	2	21.3	3	27.8	6	26.0	30
4	0.7	0	3.2	0	6.3	0	12.1	1	22.3	2	32.2	9	25.6	27
5	0.6	0	3.2	0	6.2	0	12.3	1	22.1	3	31.9	9	26.0	30
6	0.6	0	3.1	0	6.5	0	12.4	0	22.3	2	32.2	9	25.8	19
7	0.7	0	3.1	0	6.4	0	11.7	1	21.5	2	32.2	9	26.4	15
8	0.6	0	3.0	0	6.4	0	11.5	1	20.6	1	32.2	9	25.6	19
9	0.6	0	3.1	0	6.3	0	11.9	0	20.4	1	31.2	6	26.0	21
10	0.6		3	0	6.0	0	11.1	0	20.6	2	31.3	8	26.0	18

Table 5.6 Influence of zinc on the production (with time) of leafy shoots in solid medium at 25°C, 2600 lx. No gemmae or intercellular spaces present.

* growth of the moss colonized the whole agar plate, but the agar contracted i.e. total agar surface was reduced.

shoots. No gemmae could be observed among zinc concentration. The effect of zinc on the production of these structures did not become less marked with time.

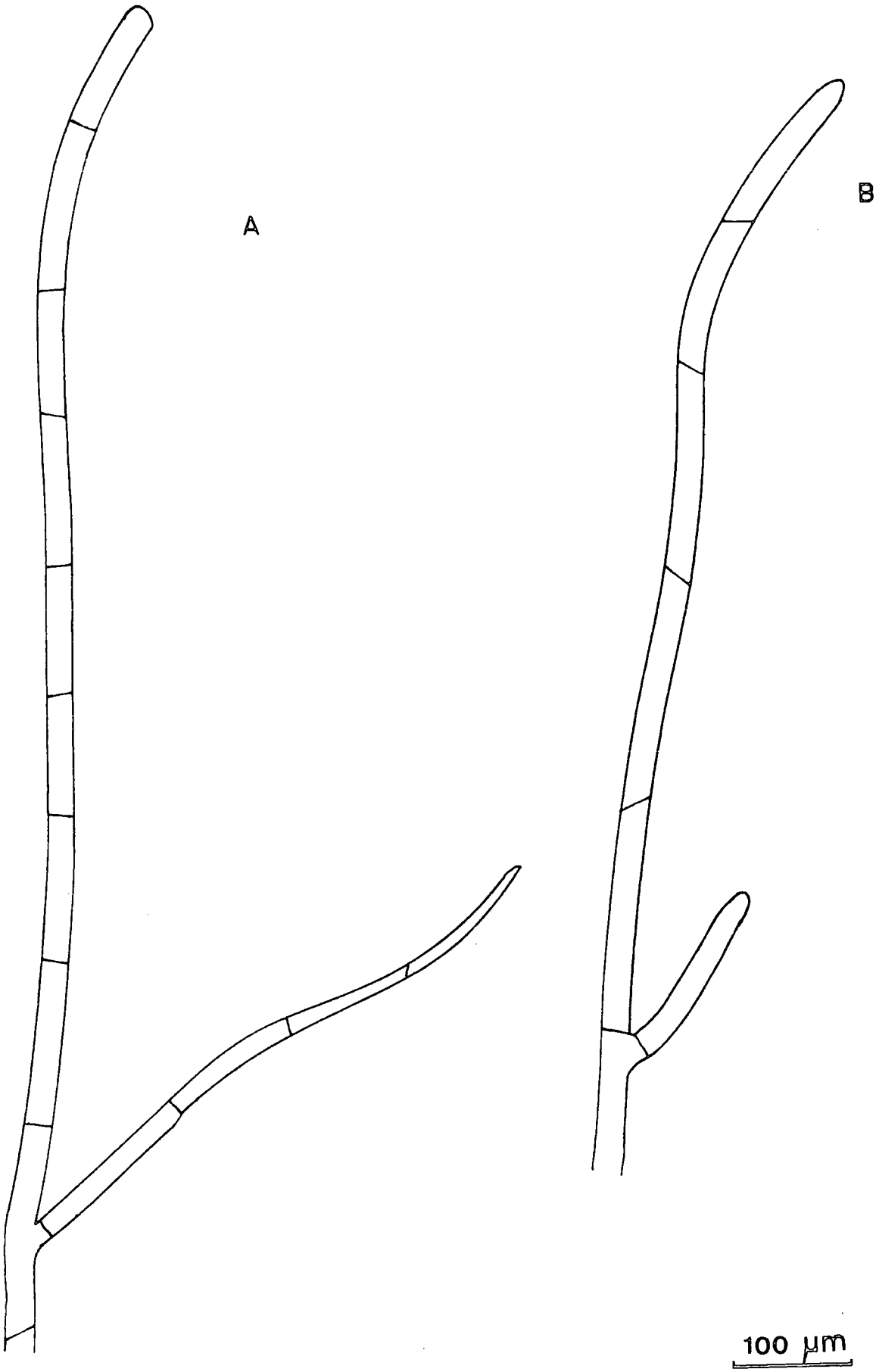
5.22 Cross-wall of protonemal filament

A comparison was made of the angle of the cross-wall in materials both without and with zinc enrichment of the medium. Details of the cultures used for these observations are as follows. The cultures were taken from liquid basal medium with shaking at 25°C and 2600 lx when they were 4 weeks old; the medium was buffered at pH 7.0. The results are summarized in Table 5.7. 65% of the walls were oblique (80°) in the presence of the high level of zinc as opposed to 47% in the absence of 'extra' zinc.

Zn ($mg\ l^{-1}$)	angle of cross-wall						
	20 - 30	31 - 40	41 - 50	51 - 60	61 - 70	71 - 80	81 - 90
0	1	2	10	6	11	17	53
70	5	2	8	15	14	21	35

Table 5.7 Influence of zinc on angle of cross wall of filamentous prontomema; measurement made in each case on 100 cells. (90^{o} is a typical cell-wall at right angles to outer surface)

Fig. 5.4 Cross-walls in filamentous protonema grown in : (A) liquid medium without zinc; (B) liquid medium at high levels of zinc.


A
B
100 µm

6. DISCUSSION

6.1 Zinc toxicity and its effects

6.11 Field observations

The moss used for the study reported here, Dicranella sp., was taken from a heavily polluted site, Elvins Tailing Pile (Section 3.1). The water near the source of the main flow showed zinc was at very high levels (19.1 - 22.8 mg l^{-1} Zn). Dicranella sp. was widespread and sometimes abundant at this site, with the protonema especially conspicuous. It was the only moss present at this site, but a few algae such as Plectonema gracillimum and Stichococcus sp. were found in association with it (Section 3.3)

As mentioned in Section 1.4, Say (1977) recorded that Dicranella varia, together with Philonotis fontana and Scapania undulata, were found to occur throughout the whole range of zinc values tolerated by bryophytes in the stream studied by him. The concentrations of zinc in the present study area were however higher than the upper limit (8.2 mg l^{-1} Zn) reported for these bryophytes by Say. From the present study it is clear that Dicranella sp. tolerates even higher concentrations of zinc than recorded by Say and that the upper limit shown in his stream may have been influenced by other factors.

6.12 Comparison between laboratory and field observations

When the Dicranella sp. from Elvins was brought to the laboratory, it was again resistant to zinc (Section 5.1). It tolerated in fact much higher levels of zinc than recorded in the field. The higher levels did however bring about different responses in different structures. There was selective

inhibition of the formation of gemmae and leafy shoots. From the results of the effects of zinc on the production of both structures, there was evidence that leafy shoots were more resistant to zinc in producing gemmae and leafy shoots than those produced by filamentous protonema. A level of 10 mg l^{-1} Zn caused a slight inhibition of the production of gemmae and leafy shoots when they were formed from other leafy shoots. The same level caused a much greater inhibition, when they were formed from filamentous protonema. The inhibition of filamentous protonema from either source nevertheless needed much higher concentrations of zinc than the formation of gemmae and leafy shoots (Table 5.1). However there was a problem which needs to be solved in order to explain the effect of zinc on the formation of gemmae and leafy shoots. This was the variability of the ratios of the two structures; usually both structures were formed in the same culture, but either could be dominant (see Fig. 4.11). Some other factors seems to alter the pattern of the development, such that a protuberance formed on the protonema can develop either into gemmae or a leafy shoot bud; these possible factors have not been discovered yet. Chopra and Rawat (1973) found that Bryum klinggraefii was not able to produce gametophytic buds, but always produced gemmae. The authors suggested that the cultural conditions were apparently unfavourable for the initiation of gametophytic buds and a short circuit of perennating structures is therefore established in which development takes place from gemmae to gemmae. They were not able to offer the factors which were responsible for the alternative types of development, but they reported that experiments were under way to study various physical and chemical factors which will alter the pattern of the development; these are apparently still unpublished. Lewis (1974) has studied the effect of heavy metals, including zinc, on 36 samples of Eurhynchium riparioides collected from various parts of an unpolluted stream (Nant Beg, an upland stream running

through Forestry Commission land). The highest level of zinc in the waters was 0.06 mg l^{-1}. She found that zinc and certain heavy metals did not seem to inhibit lateral shoot production, for levels ranging from 0.01 - 10 mg l^{-1} Zn no significant difference in the results. Other studies have been carried out by Coombes and Lepp (1974) on the effect of copper and zinc on the growth of Marchantia polymorpha and Funaria hygrometrica in solid medium. Their results showed that toxic responses to zinc occurred at even higher concentrations than those used in the present studies. Marchantia gemmalings tolerated quite well levels of zinc up to 100 mg l^{-1}. 10 mg l^{-1} Zn reduced only slightly both germ tubes of Funaria hygrometrica. Much higher concentrations of zinc were required before inhibition became apparent.

The present results were compared with those of Lewis (1974). In Dicranella sp., 20 mg l^{-1} Zn inhibited the production of gemmae and leafy shoots developed by leafy shoots, while this level caused a slight inhibition in the production of lateral shoots of Eurhynchium. However Lewis did not study higher concentrations of zinc which might inhibit this production of lateral shoots. The dissimilarity between the present results and those of Lewis may perhaps be explained by Eurhynchium being more tolerant to heavy metals than the Dicranella sp. Eurhynchium showed no inhibition at 1 mg l^{-1} Cu; this may be compared with the results of Coombes and Lepp (1974) who found with the same level of copper that buds of Funaria were only formed sparingly. Higher copper concentrations inhibited completely bud formation. This suggests that Eurhynchium is very resistant to the heavy metals studied, particularly zinc, lead and iron. Bazzaz et al. (1977) studied some effects of cadmium on the growth of bryophytes and found that the spores of the same strain of Funaria used by Coombes and Lepp (1974) were able to germinate in excess of 82% in 5 mg l^{-1} Cd. On the basis of their results, it would appear that for the

strain investigated, zinc is somewhat less toxic to propagules than either copper or cadmium.

Intercellular spaces were formed abundantly in Dicranella sp. with increasing the levels of zinc up to 30 mg l^{-1}; higher concentrations inhibited completely the formation of these structures. This may have been due simply to the fact that increasing zinc levels in the solution led to an inhibition in growth. On other hand increasing zinc levels in solution led to an increase in precipitation of phosphate as zinc phosphate (Jurinak and Inauge, 1962) making a deficiency of this ion in solution. This may be an important factor in the formation of these structures (see Section 6.222). It can be concluded that these structures can be formed under unfavourable conditions.

6.2 Comment on influence of environmental factors in laboratory

6.21 Physical factors

6.211 Light

From the results of Section 4.53 it can be seen that the production of gemmae and leafy shoots was increased by increasing the light intensity, but it would appear that the highest light intensity of 7000 lx inhibited the production of both structures slightly. These results may be compared with studies on Funaria hygrometrica and Phascum cuspidatum made by several previous authors noted by Szweykowska (1963), Funaria hygrometrica and Ceratodon purpureus made by Szweykowska and Mackowiak (1962) and by Szweykowska (1963), Marchantia nepalensis made by Chopra and Sood (1970) and Eurhynchium riparioides made by Lewis (1974). Their results confirm that generally an increase in light intensity leads to an increase in the production of vegetative reproductive structures. Szweykowska and Mackowiak (1962) observed that liquid cultures of

Ceratodon purpureus produced buds only occasionally and after prolonged periods of cultivation when the cultures were grown under only two fluorescent tubes (low light intensity). Chopra and Sood (1970) found that at light intensities of 500 - 1000 lx there was no production of gemma cups in Marchantia nepalensis, but at 2000 lx gemma cups and gemmae were formed; production was increased by increasing the light intensity. The influence of different light intensities was very pronounced upon the production of lateral shoots of Eurhynchium as the number of laterals was very poor at the lowest light intensity (350 lx) and none developed in the dark. Although the reduction in light intensity decreased the production of gemmae and leafy shoots of the Elvins Dicranella sp., but they were still formed in relatively large numbers in the lowest light intensity tested (1300 lx). From all these studies it can be seen that vegetative reproductive structures may occur in the low light intensity only sparingly or may be relatively abundant. It can however be concluded that bryophytes generally increase the production of vegetative reproductive structures with an increase in the light intensity.

The effect of light in the reproduction of chlorophyll a by the Elvins Dicranella sp. showed that no significant differences between the all cultures grown in different light intensities (1300 - 7000 lx) in week 1 and week 2, but in week 3 and week 4 the production of chlorophyll a was increased relatively at the lowest light intensity (1300 lx). The Eurhynchium studied by Lewis (1974) showed that the production of chlorophyll a was increased by increasing the light intensity. The present results are thus dissimilar to those given by Lewis.

There was no significant difference between the production of intercellular spaces in the lowest and highest light intensity. This seems to suggest that the factor of light intensity has no fundamental role to the formation of these structures.

6.22 Chemical factors

6.221 Iron

From the results on Table 4.5 it seems that there is no significant difference between the effects of the low and high concentrations of iron; the variability of the production of gemmae and leafy shoots however causes a muddled picture. (This problem has been discussed in Section 6.12.) However the total combined number of gemmae and leafy shoots gives a more clear picture of the influence of iron on the production of both structures (Fig. 4.13). Similar studies by Lewis (1974) showed that iron had no influence on the production of lateral shoots. It seems that iron does not play a special role in the production of gemmae and leafy shoots, other than the presumed requirements for ordinary growth.

More intercellular spaces were formed in the absence of iron than in its presence. This phenomenon confirms that intercellular space formation occurs under unfavourable conditions

6.222 Phosphate

From the results in Table 4.6, it can be seen that sufficiency of phosphate led to an increase of the production of gemmae and leafy shoots. Deficiency of this ion led to a reduction of the production of gemmae and leafy shoots and none were formed in the absence of phosphate. Lewis (1974) showed for Eurhynchium riparioides a marked reduction in shoot length and the absence of lateral shoot production at the lowest concentration of phosphate (1 mg l^{-1}P). The concentrations which she used were much higher

than those used in the present study, but an impression can nevertheless be gained. Sufficiency of phosphate can lead to an increase in the production of these structures and the levels of phosphate which influence this production depends on the organism tested. Lewis (1974) suggested that the absence of lateral shoot production in the lowest level of phosphate which she used may have been due to the fact that the phosphate ion became depleted during the course of the experiment. Phosphate is an important nutrient for growth; buds cannot be developed to leafy shoot if the synthesis of an appropriate store of nutrient in the protonema cannot take place (Bopp, 1961).

Deficiency of phosphate led to a considerable increase in intercellular spaces. As the results of other experiments showed that the intercellular spaces only formed when the cultures got older, this may indicate the phosphate had been exhausted from the medium.

6.3 Comment on experimental studies

It was evident during the experiment studies that the materials gave variable results. Further, while all the experiments on effects of zinc were repeated four times, those on iron and light intensity were carried out only once. For instance the numbers of gemmae found in 0.5 mg l^{-1} Fe in the medium differ in the experiments summarized in Tables 4.4 and 4.5. Future research requires intensive study of the factors giving rise to the variable results. In particular more studies are needed on the patterns of development leading either to gemmae or leafy shoot buds.

6.4 Taxonomy

From the description of the Elvins moss in Section 4.2 and Table 4.1, it can be seen that it possessed some features of *Dicranella varia* and some of *D. rufescens* and *D. staphylina*. This made it difficult to give a specific name. The Elvins moss was identified provisionally as *D. varia* as it was similar to it in many respects. The descriptions obtained from the literature (Table 4.1) however reveal some differences, particularly the differentiation of the perichaetial leaves and the stem leaves and the enlargement of the leaf cells. Those features of the Elvins moss which differed from those of *D. varia* and *D. rufescens* were similar to those of *D. staphylina*. The resemblance of such features particularly the differentiation of the perichaetial leaves with stem leaves, branching of shoots, leaf cells and colour of the old stem and rhizoids make the Elvins moss close to *D. staphylina*. These differences of the Elvins moss from any of the three species of *Dicranella* made it difficult to give it a specific name and it is perhaps better to refer to it simply as *Dicranella* sp. It seems possible that some of these differences may be due simply to the influence of the environment. Further investigations and comparisons should be carried out to make firm conclusions.

SUMMARY

(i) A study was made of a moss which had been taken (by B.A. Whitton) from Elvins Tailing Pile, Missouri, U.S.A., a site heavily polluted with heavy metals (especially Zn, Cd, Pb). In spite of the high levels of zinc (19.1 - 22.8 mg l^{-1}) the moss formed a conspicuous cover at this site though mainly as protonema.

(ii) It proved difficult to give the moss a specific name, because it possessed features of three different species of Dicranella (D. varia, D. rufescens and D. staphylina). It is called simply Dicranella sp. in the present account.

(iii) Experiments in the laboratory showed that Dicranella sp. could tolerate even higher concentrations of zinc than found in the field.

(iv) There were differences in the sensitivity of different structures. The production of gemmae and leafy shoots was more sensitive than the production of filamentous protonema. The production of gemmae and leafy shoots formed from filamentous protonema was more sensitive than that formed from other leafy shoots. 10 mg l^{-1} Zn inhibited markedly this production when produced by filamentous protonema, while this inhibition occurred at 25 mg l^{-1} Zn.

(v) The formation of intercellular spaces in the protonema increased with increasing zinc levels.

(vi) The influence of light intensity (1300 - 7000 lx) on the production of gemmae, leafy shoots, intercellular spaces and chlorophyll a was investigated. An increase in light intensity

increased the production of gemmae and leafy shoots and not increased the intercellular spaces

(vii) The influence of iron (0 - 8 mg l^{-1}) on the production of gemmae, leafy shoots and intercellular spaces was investigated. An increase in iron concentrations did not increase the production of gemmae and leafy shoots and decreased slightly the production of intercellular spaces.

(viii) The influence of phosphate (0 - 1.8 mg l^{-1} P) on the production of gemmae, leafy shoots and intercellular spaces was investigated. An increase in phosphate increased the production of gemmae and leafy shoots. Decrease of phosphate increased the production of intercellular spaces.

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APPENDIX

Appendix I Individual readings of Tables and Figures of Chapter 4

I.1 Production of gemmae, leafy shoots and intercellular spaces

reference	age (weeks)	factor studied	individual readings of gemmae ml $^{-1}$				leafy shoots ml $^{-1}$				intercellular spaces ml $^{-1}$			
			1	2	3	4	1	2	3	4	1	2	3	4
Table 4.4		light intensity (lx)												
	1	2600	0.1	0	0	0.2	0	0	0	0	0	0	0	0
	2	1300	1.6	1.2			0	0			0	0		
		2600	0.9	1.9	1.5	1.1	0	0			0	0		
		4000	2.3	1.5			0	0			0	0		
		5200	2.2	0.8			0	0			0	0		
		6300	2.8	1.8			0	0			0	0		
		7000	3.1	2.3			0	0			0	0		
	3	1300	6.1	3.5			0	0			0	0		
		2600	7.8	11.1	8.9	6.9	0	0	0	0	0	0	0	0

I.1. contd.

reference	age (weeks)	factor studied	individual readings of gemmae ml $^{-1}$				leafy shoots ml $^{-1}$				intercellular spaces ml $^{-1}$			
			1	2	3	4	1	2	3	4	1	2	3	4
		4000	9.4	8.8			0	0			0	0		
		5200	12.0	8.2			0	0			0	0		
		6300	12.3	9.3			4	0			0	0		
		7000	13.1	12.5			0	0			0	0		
	4	1300	22.9	20.7			0	0			3.6	3.2		
		2600	34	28.2	26.5	30.9	0	0.5	1.1	0	4.2	3.2	2.3	3.9
		4000	41.1	30.5			0.2	0			3.5	3.5		
		5200	43.9	38.5			0	0			3.9	3.1		
		6300	51.3	39.9			0	0			4.1	3.1		
		7000	44.1	31.5			0	0			3.3	3.7		
Table 4.5	4	Fe(mgl^{-1})												
		0	1.5	0.9	2.9	1.9					7.8	6.1	5.2	6.9
		0.5	10.3	12.7	24.5	30.5	8.9	5.5	0.8	1.7	3.9	3.1	2.3	2.8

I.1 cont.

reference	age (weeks)	factor studied	individual readings of gemmae ml^{-1}				leafy shoots ml^{-1}				intercellular spaces ml^{-1}			
			1	2	3	4	1	2	3	4	1	2	3	4
		1.0	2.7	3.1	19.3	28.9	13.5	11.4	5.1	2.1	2.7	3.2	3.6	2.2
		2.0	2.5	4.6	7.8	1.1	12.3	14.2	9.5	10.7	3.1	2.9	2.5	1.8
		4.0	27.1	29.7	19.6	32.2	2.6	1.4	3.7	1.3	2.8	3.0	1.9	1.6
		8.0	31.5	29.6	27.1	31.7	-	-	1	1	3.0	2.5	1.4	1.9
Table 4.6	1	PO_4-P mg l^{-1}												
		0 - 1.2	No structure has been formed at this time											
		1.4	-	-	0.1	-								
		1.6	0.2	-	-	0.2								
		1.8	0.2	-	0.2	0.1								
	2	0									3.6	2.7	1.8	4.1
		0.1									1.8	0.9	3.6	2.7
		0.2									1.8	3.6	1.0	1.8

I.1 contd.

reference	age (weeks)	factor studied	individual readings of gemmae ml^{-1}				leafy shoots ml^{-1}				intercellular spaces ml^{-1}			
			1	2	3	4	1	2	3	4	1	2	3	4
		0.3									0.9	0.9	1.8	1.8
		0.4												
		0.6	0.4	0.3	0.9	0.4								
		0.8	0.8	0.5	1.0	0.6								
		1.0	0.4	0.7	1.0	0.7								
		1.2	0.8	1.1	0.9	1.5								
		1.4	1.2	1.5	1.9	1.8								
		1.6	2.4	1.7	2.7	3.2								
		1.8	3.2	4.5	3.9	3.3								
	3	0									10.8	7.2	12.6	11.7
		0.1	0.1	0.1	0.3	0.2					10.8	7.2	11.6	9.0
		0.2	0.2	0.3	0.5	0.1					5.4	10.7	4.8	9.0
		0.3	1.2	0.8	0.7	1.0					9.0	5.4	7.2	6.4
		0.4	1.1	1.3	0.7	0.9					4.8	3.6	7.2	5.4

I.1 contd.

reference	age (weeks)	factor studied	individual readings of gemmae ml $^{-1}$				leafy shoots ml $^{-1}$				intercellular spaces ml $^{-1}$			
			1	2	3	4	1	2	3	4	1	2	3	4
		0.6	2.4	1.6	1.2	2.7					0.9	1.8	3.6	2.7
		0.8	5.2	4.8	6.4	4.3								
		1.0	7.6	8.1	9.6	6.4								
		1.2	9.3	7.6	9.9	8.0	0.8	-	0.4	0.4				
		1.4	12.1	10.3	11.2	9.2	0.4	0.9	0.3	0.4				
		1.6	11.6	12.1	13.1	16.4	0.8	0.2	0.4	0.3				
		1.8	15.5	16.4	14.6	12.5	0.9	0.7	0.3	0.2				
	4	0	0.4	-	0.2	-					30.6	18.2	23.4	28.8
		0.1	0.8	0.5	1.2	1.4					18.0	30.6	25.2	23.4
		0.2	1.9	1.5	2.8	2.3					18.0	25.2	14.4	23.4
		0.3	2.9	3.3	4.6	3.7					19.8	16.2	25.2	14.4
		0.4	3.6	3.1	2.7	4.5					17.6	18.0	19.8	12.6
		0.6	5.9	4.4	3.6	5.2					12.6	9.0	7.2	9.0
		0.8	11.8	14.7	12.6	9.7	-	0.4	0.2	0.1	9.0	5.4	6.3	4.5

I.1 contd.

reference	age (weeks)	factor studied	individual readings of gemmae ml $^{-1}$				leafy shoots ml $^{-1}$				intercellular spaces ml $^{-1}$			
			1	2	3	4	1	2	3	4	1	2	3	4
		1.0	24.1	28.8	28.4	16.0	0.6	0.8	0.4	0.2	4.5	5.4	7.2	6.3
		1.2	26.9	25.2	31.6	32.4	0.4	0.8	0.3	-	4.4	3.6	3.9	4.9
		1.4	30.1	27.6	22.3	31.9	0.9	0.6	1.4	-	3.6	4.8	2.4	4.0
		1.6	30.8	37.6	29.6	32.4	2.7	0.9	1.8	1.5	4.6	3.2	2.4	3.8
		1.8	34.8	36.3	31.6	38.0	1.0	2.3	0.8	1.5	3.2	1.6	4.8	3.2

I.2 Total combined number of gemmae and leafy shoots

reference	age (weeks)	factor studied	individual readings of total gemmae and leafy shoots ml $^{-1}$			
			1	2	3	4
Fig. 4.13	4	Fe(mgl^{-1})				
		0	1.5	0.9	2.9	1.9
		0.5	19.2	18.2	29.6	32.2
		1	16.4	14.5	24.2	32.6
		2	14.8	18.8	17.3	11.8
		4	28.5	33.4	23.3	33.5
		8	31.5	29.6	28.1	32.7
Fig. 4.14	1	PO_4-P(mgl^{-1})				
		0-1.2	No structure formed at this time			
		1.4	0	0	0.1	0
		1.6	0.2	0	0	0.2
		1.8	0.2	0	0.2	0.1
	2	0-0.4	No structure formed at this time			
		0.6	0.4	0.3	0.9	0.4
		0.8	0.8	0.5	1.0	0.6
		1.0	0.4	0.7	1.0	0.7
		1.2	0.8	1.1	0.9	1.5
		1.4	1.2	1.5	1.9	1.8
		1.6	2.4	1.7	2.7	3.2
		1.8	3.2	4.5	3.9	3.3

I.2 continued

reference	age (weeks)	factor studied	individual readings of total gemmae and leafy shoots ml $^{-1}$			
			1	2	3	4
	3	0	0	0	0	0
		0.1	0.1	0.1	0.3	0.2
		0.2	0.2	0.3	0.5	0.1
		0.3	1.2	0.8	0.7	1.0
		0.4	1.1	1.3	0.7	0.9
		0.6	2.4	1.6	1.2	2.7
		0.8	5.2	4.8	6.4	4.3
		1.0	7.6	8.1	9.6	6.4
		1.2	10.1	7.6	10.3	8.4
		1.4	12.5	11.2	11.5	9.6
		1.6	12.4	12.3	13.5	16.7
		1.8	16.4	17.1	14.9	12.7
	4	0	0.4	0	0.2	0
		0.1	0.8	0.5	1.2	1.4
		0.2	1.9	1.5	2.8	2.3
		0.3	2.9	3.3	4.6	3.7
		0.4	6.3	3.1	2.7	4.5
		0.6	5.9	4.4	3.6	5.3
		0.8	11.8	15.1	12.8	9.8
		1.0	24.7	29.6	28.8	16.2
		1.2	27.1	26.0	31.9	32.4
		1.4	31.0	28.2	23.7	31.9
		1.6	31.7	38.5	31.4	33.9
		1.8	35.8	38.6	32.4	39.5

reference	age (weeks)	factor studied	individual readings of total gemmae and leafy shoots ml^{-1}			
			1	2	3	4
Fig. 4.15		influence of time				
	1		0.1	0	0	0.2
	2		0.9	1.9	1.5	1.1
	3		7.8	11.1	8.9	6.9
	4		34.0	28.7	27.6	30.9

1.3 Production of chlorophyll a and area occupied by moss

a) Chlorophyll a

reference	age (weeks)	factor studied	individual readings of chlorophyll a ml^{-1}			
			1	2	3	4
Fig. 4.12	1	light intensity (lx)				
		1300	1.6	1.8		
		2600	1.6	2.0	2.1	1.9
		4000	2.1	2.3		
		5200	1.8	2.4		
		6300	2.3	1.5		
		7000	2.7	1.9		
	2	1300	3.4	2.4		
		2600	3.4	3.3	3.2	3.2
		4000	3.2	2.2		
		5200	3.4	3.0		
		6300	3.2	2.8		
		7000	3.0	3.0		

I.3 contd.

reference	age (weeks)	factor stuided	individual readings of chlorophyll a ml^{-1}			
			1	2	3	4
	3	1300	11.7	7.7		
		2600	7.9	7.3	7.2	7.2
		4000	7.1	7.3		
		5200	7.9	6.7		
		6300	7.3	5.7		
		7000	5.9	7.5		
	4	1300	9.3	6.9		
		2600	5.8	6.4	6.2	6.2
		4000	5.9	5.5		
		5200	6.2	5.2		
		6300	6.4	4.6		
		7000	5.5	4.5		

I.3 contd.

b) Area and Chlorophyll *a*

reference	factor studied	age (weeks)	individual readings of chlorophyll *a* ml^{-1}											
			area cm^{-2}				chlorophyll *a* per culture				leafy shoots per culture			
			1	2	3	4	1	2	3	4	1	2	3	4
Fig. 4.16	influence of time on area occupied by moss during growth and production of chlorophyll *a*	1	0.5	0.8	0.6	0.6	29.3	31.9	34.4	32.9	0	0	0	0
			0.6	0.7	0.4	0.4					0	0	0	0
			0.5	0.6	0.6	0.6					0	0	0	0
			0.5	0.6	0.6	0.7					0	0	0	0
		2	2.4	3.6	4.2	2.7	40.2	53.7	57.0	41.9	0	0	0	0
		3	5.9	6.6	6.9	5.5	117.4	134.1	125.8	100.6	0	0	0	0
		4	12.3	11.1	12.0	11.1	209.6	185.0	185.0	176.0	1	1	2	0
		5	23.8	16.6	20.4	22.1	209.0	185.0	230.6	201.2	3	0	2	2
		6	35.8	35.8	34.8	36.3	167.7	150.9	146.72	188.6	7	15	5	10
		7*	26.4	24.6	26.0	25.1	83.5	62.9	79.7	67.1	31	22	26	19

Appendix II Individual readings of Tables and Figures of Chapter 5.

II.1 Production of gemmae, leafy shoots, and intercellular spaces

reference	age (weeks)	factor studied	individual readings of gemmae ml^{-1}				leafy shoots ml^{-1}				intercellular spaces ml^{-1}			
			1	2	3	4	1	2	3	4	1	2	3	4
Table 5.2	4	Zn(mgl^{-1})												
		0	27.2	28.0	31.6	34.0	2.4	1.5	2.9	3.1	3.5	3.8	3.1	2.6
		5	3.2	4.1	2.4	2.0	0	0.1	0	0	4.1	5.8	4.7	5.1
		10	0.1	0.2	0	0.2	0	0	0	0	9.7	6.8	7.3	5.6
		15	0	0	0	0	0	0	0	0	10.5	7.8	11.2	8.9
		20	0	0	0	0	0	0	0	0	18.0	24.1	23.5	16.4
		25	0	0	0	0	0	0	0	0	19.0	16.2	25.2	15.8
		30	0	0	0	0	0	0	0	0	5.8	6.9	4.1	4.8
		35	0	0	0	0	0	0	0	0	0	0	0	0
Table 5.3	4	0	4.5	0	1.4	0	6.6	8.9	7.1	10.3	1.9	3.8	2.9	2.7
		5	0	0	0.9	0	8.3	6.4	5.8	6.9	4.3	5.1	3.7	3.3
		10	0.6	0	1.5	1.0	2.8	3.9	2.3	3.3	9.3	6.3	7.1	5.8
		15	0.9	0	0	1.3	1.6	1.9	1.1	2.1	7.8	9.4	10.1	8.9

II.1 contd.

reference	age (weeks)	factor studied	individual readings of gemmae ml^{-1}				leafy shoots ml^{-1}				intercellular spaces ml^{-1}			
			1	2	3	4	1	2	3	4	1	2	3	4
		20	0	0	0	0	1.3	1.0	1.8	1.7	15.4	11.3	13.9	16.9
		25	0	0	0	0	0.6	0.3	0.9	0.2	9.1	11.8	8.1	10.7
		30	0	0	0	0	0	0	0	0	3.7	4.8	2.3	2.9
		35	0	0	0	0	0	0	0	0	0	0	0	0
Table 5.4	4	0	28.5	31.1	7.4	27.0	2.1	3.3	10.7	2.4	4.1	3.3	3.6	2.1
		1	18.9	20.2	16.8	22.8	1.6	0.9	2.5	1.3	4.1	3.9	2.3	3.5
		2	11.7	9.8	10.9	0.9	1.2	0.6	0.7	11.9	4.4	3.2	2.6	4.2
		3	6.0	7.6	5.6	6.9	0.5	0.5	0.9	0.3	4.8	3.6	4.1	2.9
		4	3.8	3.0	4.5	4.9	0.2	0.3	0.1	0.1	4.1	5.8	3.9	2.8
		5	3.0	2.3	3.6	1.8	0.1	0.2	0.1	0	4.1	6.1	5.8	3.1
		6	1.7	1.2	2.1	1.9	0	0	0	0	4.2	3.9	6.3	5.8
		7	0.8	0.6	0.8	1.3	0	0	0	0	4.8	4.2	7.2	6.3
		8	0.3	0.7	0.3	0.5	0	0	0	0	7.2	5.2	4.8	6.3
		9	0.1	0.3	0.2	0.1	0	0	0	0	7.2	6.3	9.0	4.8
		10	0	0.2	0.2	0	0	0	0	0	9.0	7.2	6.2	10.8
Table 5.5	1	0	0	0.2	0.1	0	0	0	0	0	0	0	0	0
		1-10	No structure formed at this time											

II.1 contd.

reference	age (weeks)	factor	individual readings of gemmae ml^{-1}				leafy shoots ml^{-1}				intercellular spaces ml^{-1}			
			1	2	3	4	1	2	3	4	1	2	3	4
	2	0	1.3	1.9	2.1	1.7	0	0.2	0	0				
		1	1.6	1.1	0.7	1.2	0.1	0	0	0	0	0	0	0
		2	1.1	0.5	1.0	0.8	0	0	0	0	0	0	0	0
		3	0.5	0.9	0.7	0.5	0	0	0	0	0	0	0	0
		4	0.2	0.6	0.4	0.2	0	0	0	0	0	0	0	0
		5	0.2	0.1	0.4	0.3	0	0	0	0	0	0	0	0
		6	0.1	0.2	0.1	0	0	0	0	0	0	0	0	0
		7	0.1	0	0	0	0	0	0	0	0	0	0	0
		8	0	0	0	0	0	0	0	0	0	0	0	0
		9	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	11.7	9.2	8.3	13.4	0.3	0.1	0	0.4	0	0	0	0
		1	5.6	7.0	4.8	6.4	0.4	0	0.1	0.2	0	0	0	0
		2	3.4	2.4	4.1	3.8	0	0	0	0.1	0	0	0	0
		3	2.1	2.9	1.8	2.4	0	0	0	0	0	0	0	0
		4	1.8	1.4	2.3	1.1	0	0	0	0	0	0	0	0

II.1 contd.

reference	age (weeks)	factor studied	individual readings of gemmae ml^{-1}				leafy shoots ml^{-1}				intercellular spaces ml^{-1}			
			1	2	3	4	1	2	3	4	1	2	3	4
		5	0.8	1.2	1.7	0.7	0	0	0	0	0	0	0	0
		6	0.9	0.5	1.1	0.5	0	0	0	0	0	0	0	0
		7	0.8	0.4	0.3	0.6	0	0	0	0	0	0	0	0
		8	0.1	0.1	0.1	0.2	0	0	0	0	0	0	0	0
		9	0	0.2	0.1	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	28.1	33.1	37.6	30.9	2.3	1.9	2.9	1.3	3.9	3.0	3.3	1.8
		1	21.2	27.6	30.7	29.2	3.1	3.7	0.9	0.5	4.0	3.6	2.0	3.2
		2	15.8	10.1	9.6	12.1	0.9	1.5	1.1	0.6	4.4	3.2	2.8	4.1
		3	10.3	7.2	5.8	8.1	0.8	0.3	0.6	0.4	4.6	5.1	4.3	2.7
		4	6.1	3.1	5.7	5.4	0	0	0	0	4.1	5.8	2.9	4.4
		5	3.8	4.9	2.2	2.9	0	0	0	0	4.3	6.2	5.8	3.3
		6	1.1	2.1	3.3	2.8	0	0	0	0	4.5	3.9	6.6	5.9
		7	0.7	1.2	0.9	1.5	0	0	0	0	5.4	4.2	7.3	6.7

II.2 contd.

reference	age (weeks)	factor studied	individual readings of gemmae ml^{-1}				leafy shoots ml^{-1}				intercellular spaces ml^{-1}			
			1	2	3	4	1	2	3	4	1	2	3	4
		8	1.1	0.8	0.4	0.7	0	0	0	0	7.2	5.8	5.7	6.0
		9	0.3	0.1	0.2	0.3	0	0	0	0	7.4	6.3	4.8	8.1
		10	0.1	0.2	0.1	0.2	0	0	0	0	8.4	9.5	7.6	5.9

II.2 Total combined number of gemmae and leafy shoots

reference	age (weeks)	factor studied	total combined number of gemmae and leafy shoots ml^{-1} 1	2	3	4
		$Zn(mgl^{-1})$				
Fig. 5.1	4	0	31.0	34.4	28.1	29.4
		1	20.5	21.1	19.3	24.1
		2	12.9	10.4	11.6	12.8
		3	6.5	8.5	5.9	7.2
		4	4.0	3.3	4.6	5.0
		5	3.1	2.5	3.7	1.8
		6	1.7	1.2	2.1	1.9
		7	0.8	0.6	0.8	1.3
		8	0.3	0.7	0.3	0.5
		9	0.1	0.3	0.2	0.1
		10	0	0.2	0.2	0.1
			Production from filamentous protonema			
Fig. 5.2	4	0	29.6	29.5	33.3	37.1
		5	3.2	4.2	2.4	2.0
		10	0.1	0.2	0	0.2
		15	0	0	0	0
		20	0	0	0	0
		25	0	0	0	0
		30	0	0	0	0
			Production from leafy shoots			
		0	11.1	8.9	8.5	10.3
		5	8.3	6.4	6.7	6.9
		10	3.4	3.9	3.8	4.3

II.2 continued

reference	age (weeks)	factor studied	total gemmae and leafy shoots ml^{-1}			
			1	2	3	4
		$Zn(mgl^{-1})$				
5.2		15	2.5	1.9	1.1	3.4
		20	1.3	1.0	1.8	1.7
		25	0.6	0.3	0.9	0.2
		30	0	0	0	0
5.3	1	0	0	0.2	0.1	0
		1-10	no structure formed at this time			
	2	0	1.3	2.1	2.1	1.7
		1	1.7	1.1	0.7	1.2
		2	1.1	0.5	1.0	0.8
		3	0.5	0.9	0.7	0.5
		4	0.2	0.6	0.4	0.2
		5	0.2	0.1	0.4	0.3
		6	0.1	0.2	0.1	0
		7	0.1	0	0	0
		8	0	0	0	0
		9	0	0	0	0
		10	0	0	0	0
	3	0	12.0	9.3	8.3	13.8
		1	6.0	7.0	4.9	6.6
		2	3.4	2.4	4.1	3.9
		3	2.1	2.9	1.8	2.4
		4	1.8	1.4	2.3	1.1
		5	0.8	1.2	1.7	0.7
		6	0.9	0.5	1.1	0.5
		7	0.8	0.4	0.3	0.6

II.2 continued

reference	age (weeks)	factor studied	total gemmae and leafy shoots ml^{-1}			
			1	2	3	4
		$Zn(mgl^{-1})$				
5.3		8	0.1	0.1	0.1	0.2
		9	0	0.2	0.1	0
		10	0	0	0	0
	4	0	30.4	35	40.5	32.2
		1	24.3	31.3	31.6	29.7
		2	16.7	11.6	10.7	12.7
		3	11.1	7.5	6.4	8.5
		4	6.1	3.1	5.7	5.4
		5	3.8	4.9	2.2	2.9
		6	1.1	2.1	3.3	2.8
		7	0.7	1.2	0.9	1.5
		8	1.1	0.8	0.4	0.7
		9	0.3	0.1	0.2	0.3
		10	0.1	0.2	0.1	0.2

II.3 Area occupied by moss

reference	age (weeks)	factor studied	individual readings of area cm^{-2} 1	2	3	4	leafy shoots
Table No. 5.6	1	Zn (mg l^{-1})					
		0	0.7	0.6	0.7	0.6	
		1	0.6	0.6	0.7	0.6	
		2	0.7	0.6	0.9	0.6	
		3	0.6	0.6	0.6	0.8	
		4	0.6	0.6	0.7	0.8	
		5	0.6	0.6	0.7	0.8	
		6	0.7	0.6	0.9	0.6	
		7	0.6	0.6	0.9	0.7	
		8	0.6	0.5	0.7	0.8	
		9	0.6	0.5	0.9	0.5	
		10	0.6	0.5	0.7	0.6	
	2	0	3.5	3.2	3.4	3.4	
		1	2.2	2.9	3.3	3.4	
		2	2.9	3.2	3.4	3.4	
		3	3.1	2.8	3.3	3.4	
		4	3.3	2.9	3.4	3.2	
		5	3.2	2.8	3.4	3.4	
		6	2.8	3.2	3.5	2.9	
		7	3.5	3.8	2.9	3.2	
		8	3.4	2.9	2.6	3.2	
		9	3.5	2.9	2.8	3.3	
		10	3.8	2.6	2.5	3.0	

No leafy shoot formed at this time

No leafy shoot formed at this time

II.3 continued

reference	age (weeks)	factor studied	individual readings of area cm^{-2}				leafy shoots / sample			
			1	2	3	4	1	2	3	4
	3	Zn ($mg\ l^{-1}$)								
		0	6.6	5.8	7.0		0	0	0	
		1	7.0	7.6	7.1		0	0	0	
		2	6.6	7.1	7.0		0	0	0	
		3	5.4	6.6	7.1		0	0	0	
		4	7.1	7.3	7.6		0	0	0	
		5	7.6	6.8	7.1		0	0	0	
		6	7.1	6.0	6.6		0	0	0	
		7	7.6	5.1	6.6		0	0	0	
		8	7.1	6.6	5.1		0	0	0	
		9	7.3	5.1	6.8		0	0	0	
		10	5.7	7.1	5.1		0	0	0	
	4	0	13.7	12.0			1	2		
		1	11.1	13.4			1	2		
		2	12.3	11.9			2	2		
		3	13.7	11.1			1	1		
		4	12.0	12.3			2	1		
		5	12.3	12.3			1	1		
		6	13.7	11.1			1	0		
		7	12.3	11.1			0	0		
		8	11.1	12.0			0	1		
		9	11.9	12.0			0	0		
		10	11.1	11.1			0	0		

II.3 continued

5	0	22.9	21.4		
	1	22.6	23.8		
	2	22.1	22.6		
	3	20.4	22.6		
	4	23.7	20.9		
	5	22.1	22.1		
	6	23.8	20.8		
	7	22.1	20.8		
	8	20.8	20.4		
	9	11.9	12.0	0	1
	10	11.1	11.1	1	2
6	0	35.7	33.2	12	9
	1	34.2	35.8	10	11
	2	32.3	34.8	7	9
	3	23.9	31.6	13	6
	4	33.1	31.3	9	9
	5	34.7	29.1	7	10
	6	31.3	33.1	5	12
	7	31.3	33.1	8	9
	8	33.1	31.5	11	6
	9	30.7	31.7	5	7
	10	31.4	31.2	4	11

II.3 continued

7	0	26.0	27.4	27	32
	1	26.4	25.5	25	33
	2	24.6	27.4	26	17
	3	27.4	24.6	32	27
	4	26.4	24.6	27	26
	5	25.5	26.4	34	26
	6	26.0	25.5	13	24
	7	21.4	25.6	9	21
	8	26.6	26.6	25	13
	9	24.6	27.4	28	13
	10	25.5	26.4	8	27