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SOME ASPECTS OF THE BIOLOGY OF *PILAIRA SPECIES*

Thesis presented for an M.Sc. degree of the  
University of Durham

by

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June 1972



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## ABSTRACT

This thesis describes investigations into *Pilaira anomala*, *P. caucasica* and *P. moreaui*, also a brief review of previous work on these coprophilous mucoraceous fungi.

The isolation of *Pilaira* from dung is described, together with measurements of the diagnostic characteristics of a number of isolates. *P. anomala* was found to produce a vegetative 'growth ring' when the mycelium was briefly exposed to light. This phenomenon was associated with a marked temporary reduction in vegetative growth. *P. caucasica* and *P. moreaui* did not show any such conspicuous responses.

The carotene pigments present in *P. anomala* have been analysed in detail and related to analyses in other members of the Mucorales.

The morphology and location on the mycelium of the primordia of the sporangiophores has been studied. Sporangiophores were initiated by all three species in darkness, but a requirement for light by *P. anomala* for the development of the sporangium was found. The external morphology of the Stages of sporangiophore development are described, together with a brief study of the development of the columella and spores within the sporangium in all three species. The durations of the Stages of development have also been measured. The pattern of growth of the developing and mature sporangiophores has been studied.

The twisting of the mature sporangiophore during growth was measured in *P. caucasica* and demonstrated in the other two species. The production of 'stolons' - abnormal sporangiophores, has been described. A detailed analysis of the positive phototropism of the young and mature Stages of sporangiophore development has been made, including the reversal of tropism under liquid paraffin and a close examination of the location of the bending zone in all three species.

Finally, indication has been made of topics suitable for further investigation.

## INTRODUCTION

*Pilaira* (  $\pi\lambda\omicron\varsigma$  - a hat;  $\alpha\lambda\psi$  - I raise) was so named (Van Tiegham, 1875) because of the dramatic elongation of its sporangiophore in the final stage of its development. Although it was this physiological feature that led to its generic name, most studies on the genus have been confined to taxonomic and descriptive aspects. By contrast, *Pilobolus* which prior to 1875 included the genus *Pilaira*, had long been the object of intense physiological interest, notably due to its spectacular sporangium discharge, a feature lacking in *Pilaira*.

Extensive studies of the biology of some mucoraceous fungi have been made and in particular the genetics, growth, tropisms and other aspects of the physiology of *Phycomyces* have been closely analysed by many workers. There has been comparatively little attention to other genera, except for *Pilobolus*.

*Pilaira* has hitherto not found favour as an experimental organism. A series of investigations of the available species of this genus form the body of this thesis. Earlier work on these particular topics is dealt with at the beginning of each appropriate section.

As an introduction to the general biology of *Pilaira*, a review is presented here of some aspects of the physiology of *Pilaira* not further examined in this thesis.

In the few investigations made on *Pilaira*, before use was made of synthetic media, cultures were grown on dungs, agar media containing dungs or decoctions of dungs. Anderson (1933) used a decoction of rabbit dung in water to prepare an agar medium for the culture of *Pilaira anomala*.

The earliest culture of *Pilaira* on a semi-synthetic medium was by Schopfer (1935). He investigated the nutritional requirements of a number of thiamine-requiring fungi, including *Pilaira anomala*.



Schopfer (1935) showed that in both agar and liquid culture, *Pilaira anomala* grew feebly, giving a yield too small to be weighed and producing no aerial mycelium, on glucose-asparagine-thiamine medium (80 µg thiamine/100 ml). It grew well, giving 144 mg dry weight per 100 ml medium on glucose-asparagine-wheat-germ medium, producing aerial mycelium.

Schopfer in a further study (1938), using a defined medium, found that *P. anomala* grew almost equally well when supplied with 8 µg per 100 ml of the pyrimidine moiety (2-methyl-4-amino-5-aminomethyl-pyrimidine) of thiamine, as when given 8 µg per 100 ml of thiamine or a mixture of thiazole (4-methyl-5-(β-hydroxyethyl-thiazole) and pyrimidine (4 µg /100 ml of each). However, it would not grow on thiazole (8 µg /100 ml) alone, or without thiamine. In all cases the dry weights were lower (85 mg / 100 ml with thiamine or pyrimidine) than when wheat germ (Schopfer, 1935) supplied the needed growth factor (144 mg / 100 ml) and he described growth on synthetic media as 'feeble'. Only when wheat germ was supplied was aerial mycelium produced. Schopfer (1938) also investigated *Pilaira moreauxi* and showed that it differed from *P. anomala* in that it grew particularly poorly when supplied with 8 µg per 100 ml of the pyrimidine moiety alone and grew much better with 8 µg per 100 ml of thiamine (giving 153 mg / 100 ml medium), than with a mixture of 4 µg per 100 ml each of pyrimidine and thiazole. However, *P. moreauxi* was similar to *P. anomala* in that it grew feebly on 8 µg per 100 ml of thiazole alone, or without thiamine.

Leonian and Lilly (1938) also studied the nutrition of thiamine-requiring fungi, including *Pilaira moreauxi*. This exhibited no growth on a synthetic medium without thiamine, but grew on a semi-synthetic medium containing yeast extract or a synthetic medium containing amino acids and thiamine (5 µg / 100 ml). They investigated the effect on growth of different sources of nitrogen combined with thiamine.

Both urea and cyanide gave good growth. *P.moreaui* utilized glycine; dl-alanine; dl-iso-leucine; but none of the other amino acids tested were so well utilized. Leonian and Lilly also made use of *Pilaira moreaui* to assay thiamine in their experiments. They also found that pyrimidine and thiazole together stimulated the growth of *P.moreaui* in a 'nutrient' medium (i.e. synthetic medium and amino acids) but that there was no growth on thiazole alone and only 'poor to fair growth' on pyrimidine alone in the same medium. This suggested a restricted synthesis of thiazole by this organism, which was apparently unable to synthesize pyrimidine.

In a further investigation of *Pilaira anomala* as a pyrimidine-requiring fungus, Schopfer and Blumer (1940) showed also that pyrimidine (2  $\mu\text{g}$  /100 ml or 40  $\mu\text{g}$  /100 ml) only partially replaced thiamine (1.6  $\mu\text{g}$ /100 ml) and suggested that this confirmed that there might be restricted synthesis of thiazole (Schopfer, 1938). The yields were 60 mg per 100 ml of medium from both 2 and 40  $\mu\text{g}$  per 100 ml pyrimidine, but 104 mg per 100 ml from thiamine.

Fletcher (1970 a) showed that with *Pilaira anomala* much higher yields were produced on a medium of pH 7.3-7.5 than that found on the pH 5.0 of Schopfer (1938). The most favourable media contained casein hydrolysate and yeast extract. Whilst the former could be partially replaced by a mixture of eight amino acids and the latter by thiamine, the maximum utilization of glucose was only obtained on the undefined media. It would seem that thiamine is probably the only vitamin required, but the ideal medium for *P. anomala* has yet to be defined. Fletcher (1970 a) also showed that this organism had no requirement for a porphyrin.

## MATERIALS &amp; METHODS - GENERAL

'Special Techniques' are described at the head of each appropriate Experimental Section, including the use of media and other materials not described here.

*Media*

The two most commonly used agar media were: Sabouraud Maltose Agar (S.M.A.) and the same medium at one fifth strength (S.M.A./5). S.M.A. contained Maltose (Koch-Light) (40); Mycological Peptone 'Oxoid' (10) and No. 3 Agar 'Oxoid' (12), all g/l. It is evident that sufficient vitamin B<sub>1</sub> and mineral salts were provided by the above impure components. 15 ml of agar medium were used in petri dishes and 7.5 ml were used in tubes for slopes. The pH was not adjusted in either medium.

*Containers*

Sterile polystyrene petri dishes (Sterilin Ltd., Richmond, Surrey) (90 mm) were used for plates and 'Sterilin' sterile polystyrene 'Universal' bottles for slopes. Molten sterile agar was poured into dishes or bottles under aseptic conditions.

*Sterilisation*

Agar media were sterilised in glass test tubes, capped with aluminium caps, at 1-bar gauge pressure ( $103.4 \text{ kN/m}^2$ ) for 15 min.

*Inoculum*

Plugs of mycelium on agar were cut from the margins of young colonies on S.M.A. or S.M.A./5 by means of a flamed cork borer of approximately 3 mm diameter.

*Incubation*

For cultures in complete darkness, incubation was carried out in a dark room in an 'Electroheliol' incubator (model no. 28311) fitted with a contact thermometer, which gave a precise control over temperature. Except where stated, cultures were incubated at 25° C.

Illuminated cultures were maintained in a heated light-proof cabinet fitted with an 'Atlas' miniature (540 mm) 13 w fluorescent tube. The cabinet was maintained at approximately 25° C and fluctuations in temperature were monitored on an automatic recording thermometer (Cambridge Instrument Co,) which established that temperature variations rarely exceeded  $\pm 1^{\circ}$ . The light intensity within the cabinet ranged from between 50 and 150 lx, according to position.

In order to produce artificial light regimes (e.g. 6 h darkness and 18 h light every 24 h) the illuminated incubator was fitted with a Sangamo Weston 24 h dial synchronous timeswitch.

#### *Photomicrography*

All photographs shown in the figures of over x10 magnification were made with a Gillet and Sibert 'Photoconference' microscope which had a quartz iodine light source and was fitted with a voltage regulator and light meter. The instrument allowed a wide range of low and high power magnifications to be used. With x5 and x10 eyepieces bright field objectives of x3; x10; x40 and x90 (oil) could be used. With the same eyepieces, phase contrast objectives of x10; x40 and x90 (oil) could be used. In most cases the light source was covered with a dark green filter when black and white film was used. The maximum light intensity available was 750 lx.

#### *Macrophotography*

Photographs of the whole mycelium on plates shown in the figures up to x10 magnification were made with a Miranda 'Sensorex' camera fitted with a Pentax 'Macro Takumar' 50 mm f. 4 lens, which allowed photographs as large as natural size (1:1) on 35 mm negatives.

## EXPERIMENTAL SECTION I: TAXONOMY

Introduction

*Pilaira* was separated from *Pilobolus* by Van Tiegham (1875). It was described thus 'black sporangium with highly cutinized walls separated by columella from an evenly cylindrical sporangiophore without septum at base; absence of sub-sporangial swelling; mature sporangiophore collapses and there is no projection of spores'. Van Tiegham (1875) described two species: *P.anomala* and *P.nigrescens* (Table 1). Grove (1884) described *P.dimidiata* from canine dung. Morini (1904) described *P.saccardiana*, notable for having a branched sporangiophore; Ling-Young (1930) described *P.moreaui*, distinguished then by its large spores. Fletcher (1970 b) pointed out that the sporangial walls of *P.moreaui* were brown, thin and transparent and that the spores were very easily dispersed in water, i.e. the mucilage which invested them was much less viscous than that of *P.anomala*. Milko (1970) described *P.caucasica*, a species similar to *P.moreaui*, but with smaller spores. He also confirmed Fletcher's observations of the sporangial characteristics of *P.moreaui*.

Grove (1934) reported that *P.anomala* had been found on the 'dungs' of sheep, goat, hare, rabbit, goose, pig, donkey and horse. *P.moreaui* had been found on horse and rabbit dungs (Ling-Young, 1930) and *P.caucasica* on field-mouse dung (Milko, 1970).

TABLE 1

ORIGINAL DATA FOR SPORE & SPORANGIAL DIMENSIONS OF *PIAIRA* SPP.

Data	<i>P. anomala</i>	<i>P. caucasica</i>	<i>P. dimidiata</i>	<i>P. moreaui</i>	<i>P. nigrescens</i>	<i>P. saccardiana</i>
Spore Dimensions ( $\mu$ m )	8-12x 6-7	10-14x 6-8	12-14x 5-6	10-20x 5-12	5-6x 5-6	7-10x 7-10
Sporangial Diameter ( $\mu$ m )	120-250	100-250	100	300	-	90-130
Maximum Height of Sporangiophore (mm)	90-120	15-25	0.5-1.0	15-25	15-25	13-20
Authority	Van Tieghem (1875)	Milko (1970)	Grove (1884)	Ling-Young (1930)	Van Tieghem (1875)	Morini (1904)

## Taxonomic Methods

### *Isolation*

Dung pellets of herbivorous mammals and one bird (grouse) were collected in the field with sterile forceps (wrapped in the laboratory) and transferred to sterile 1 oz 'Universal' screw capped bottles. In the laboratory, the dungs were transferred to the following apparatus: a glass petri dish 87 mm in diameter, lined with a 90 mm filter paper disc and containing an inverted glass crystallising dish (80 mm diameter and 40 mm deep). (The apparatus had previously been sterilised in a hot air oven at 150° for at least one hour). The crystallising dish was lifted under aseptic conditions and the fresh dung was placed on the centre of the filter paper disc. The paper was moistened with 5 ml of sterile tap water and the dung was covered with the crystallising dish and the apparatus placed in a window. After 2-3 days, wispy grey aerial sporangiophores of *Pilaira* were frequently produced which elongated and deposited their sporangia inside the crystallising dish. These aerial structures including spores were transferred to the surface of sterile cow dung agar in petri dishes. These were incubated at 25° C for three days. Colonies were produced and 'plugs' were cut from the margins with a flamed 3 mm cork borer and used for inoculation on to agar slopes for stock cultures. The isolates regularly maintained (Table 2) are those which grew easily and were free from contaminants.

### *Maintenance Media*

Stock cultures were maintained in three different ways. Two replicates were cultured on slopes of agar medium giving vigorous growth. The most frequently used media were: 'Lab.Lemco'; 'Nutrient'; 'Sabouraud Dextrose' and 'Sabouraud Maltose' (all 'Oxoid'). Less vigorous growth was obtained on 'Potato Dextrose' ('Oxoid') and 2% malt extract ('Boot's').

A third culture was made on a 'butt' of corn meal agar 'Oxoid' (10 ml in a 1 oz 'Universal' bottle, unsloped). One of the two slopes was covered with sterile liquid paraffin, (Herb. I.M.I. Handbook, 1960) and stored at room temperature. The second slope was stored in a refrigerator at about 5°C together with the corn meal 'butt'. The corn meal 'butt' cultures were kept for three months before subculturing, but the slopes were subcultured at monthly intervals.

*Pilaira* was particularly easy to maintain provided that contamination by bacteria, other fungi and 'white mites' (Smith, 1960) was avoided.

The dung decoction agar as used by Page (1952) for the culture of *Pilobolus*, consisted of 200 g of air-dried cow dung, boiled in one litre of water, filtered and made up to volume; 0.5 g  $\text{KH}_2\text{PO}_4$  and 0.6 g  $\text{K}_2\text{HPO}_4$  were added and the final solution solidified with 12 g of 'Oxoid' No. 3 agar.

As an alternative to agar media, cylinders of approximately 20 mm diameter by 50 mm long were cut with a cork borer from large carrots or potatoes. Each cylinder was split longitudinally into two wedges and one was placed, wide end downwards into a 19 mm test tube. This was covered with an aluminium cap and autoclaved at 1-bar gauge pressure for 15 min. The wedge gave a large surface area for mycelial growth, similar to an agar slope.

#### *Measurement of spore and sporangiophore size*

The dimensions of spores were measured by taking loops of spores from S.M.A. slope cultures, and suspending them in lactophenol (Smith, 1960) on a slide under a coverslip. The linear dimensions of about 10 spores from each isolate were measured with an eyepiece micrometer under x400 magnification. The eyepiece micrometer was calibrated by means of a stage micrometer. Lactophenol is known to produce a minimum change in shape of fungal cells (Smith, 1960).

The diameter of sporangia was determined from measurements made from enlarged prints of photomicrographs of mature sporangiophores of each species.

The heights of Stage IIIb sporangiophores were determined similarly, but from prints of lower magnification.

#### Isolations and Measurements

Isolates of *P. anomala* were obtained from natural sources: dungs of rabbit, deer and horse; and from culture collections: Commonwealth Mycological Institute (C.M.I., all C.M.I. cultures are prefixed 'I.M.I.') and Centraalbureau voor Schimmelcultures (C.B.S.).

I was unable to find other species of *Pilaira* on the above natural sources nor did other 'dungs' of cow, sheep and grouse yield *Pilaira* species, although they were collected from habitats where *P. anomala* was found. Isolates of *P. moreaui* and *P. caucasica* were obtained from C.B.S.

Measurements pertaining to all the isolates maintained in culture are shown in Table 2. These agree in general with Table 1.

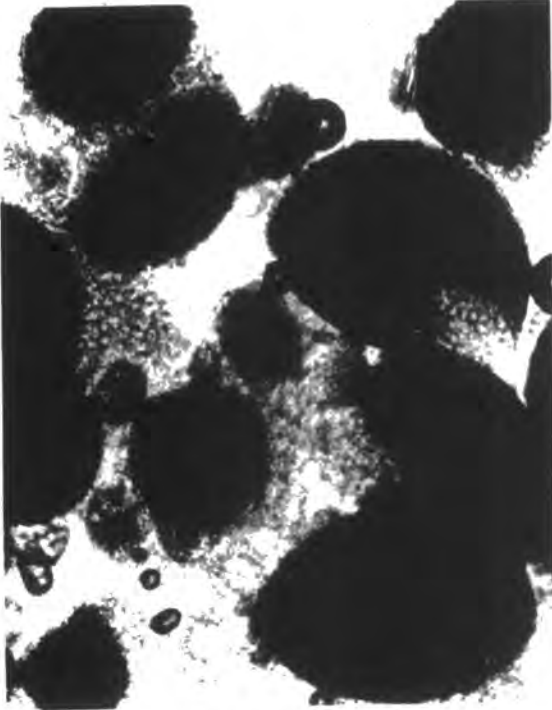
The three isolates studied in detail were: *P. anomala* I.M.I. 109387 from deer dung, the isolate of *P. caucasica* C.B.S. 523-68 and the isolate C.B.S. 101-26 of *P. moreaui*.

The *P. caucasica* and *P. moreaui* isolates here studied are the type cultures.

The differences between the sporangia and spores of these three species are shown in Fig. 1 (a-c).

FIG. 1

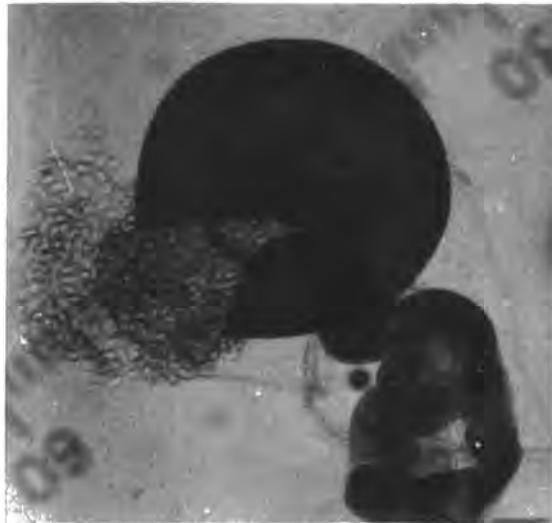
CHARACTERISTICS OF SPORANGIA AND SPORES OF *Pilaira* species.



*P. anomala* thick, black sporangial wall; spores held in viscous mucilage



*P. caucasica* thin, brown sporangial wall; spores easily dispersed



*P. moreaui* thin, brown sporangial wall; spores easily dispersed

TABLE 2  
DATA MEASURED FROM ISOLATES OF *PILAIHA* MAINTAINED IN CULTURE ON SABOURAUD MALTOSE AGAR (µm)

Species, Source & Culture Collection No.	Spore dimensions (means of 10 spores)		Spore diameter		Height of Stage IIb		s.e.m.			
	mean length	s.e.m.	mean width	s.e.m.	mean diameter	s.e.m.	n	n		
<i>Pilairia anomala</i>										
H.J.F. isolates deposited at C.M.I. & C.B.S.										
109387 (C.M.I.) (396.71 A:C.B.S.)	10.0	0	5.0	0.4	172	6.5	20	727	43	5
109386 (C.M.I.) (396.71 C:C.B.S.)	10.0	0	6.0	0.1	-	-	-	-	-	-
109388 (C.M.I.)	9.0	0.1	6.0	0.1	194	6.5	10	-	-	-
Other isolates from C.M.I.										
43,023	9.0	0.1	7.0	0.1	-	-	-	-	-	-
105,546	11.0	1.0	7.0	1.0	-	-	-	-	-	-
101,020 (a)	9.0	0.1	6.0	0.5	172	5.7	5	-	-	-
Isolates from C.B.S.										
'H.29'	7.5	0.2	5.9	0.2	-	-	-	-	-	-
131-23	8.1	0.4	6.2	0.3	-	-	-	-	-	-
424-70	9.6	0.4	7.1	0.3	162	4.8	10	-	-	-
695-60	8.8	1.1	5.6	0.3	-	-	-	-	-	-
<i>P. caucasica</i>										
523-68 (C.B.S.)	11.2	1.0	7.9	2.8	138	7.0	4	625	25	4
<i>P. moreaui</i>										
101-26 (C.B.S.) (109389 C.M.I.)	16.0	1.0	7.0	0.4	152	2.2	5	704	50	4
'H.30'	15.6	0.5	6.9	0.3	-	-	-	-	-	-

(n = number of specimens measured)

## EXPERIMENTAL SECTION II : THE EFFECT OF LIGHT ON VEGETATIVE GROWTH

Introduction

*Pilaira anomala* exhibited a light-induced growth ring; a rarely reported phenomenon in fungi (Fletcher, 1970 c). Moreover, the rate of growth was lowered by exposure to light on certain media. The highest rates of radial expansion of colonies in darkness ( $1.3 \text{ mm h}^{-1}$ ) were obtained on a coloured medium (Sabouraud Maltose Agar) described by Fletcher (1971). This favourable medium was used extensively in the studies, both at full strength and diluted.

Special Techniques

A plate of each of the three species of *Pilaira* was prepared using S.M.A./5, a colourless medium. Each plate was inoculated at two sites with 3 mm 'plugs' of mycelium on agar, cut from the margins of young colonies. The isolate of *P. anomala* used was C.B.S. 695-60, a different one from that used by Fletcher (1970 c). The inverted plates were incubated in darkness for 19 h at  $25^{\circ}\text{C}$ . Two diameters of each colony at right angles were then measured under red photographic safelight. One plate of each species was exposed to light from a miniature fluorescent tube filtered through 1%  $\text{CuSO}_4$  giving 150 lx at the plate, for 15 min. (This was a much lower intensity, for a shorter time, than the exposure used by Fletcher, 1970 c). Following this treatment, the plates were returned to darkness. Measurements were made at one hour from the commencement of the light period; at intervals over the next few hours, and twice on the following day. There were thus four measurements of colony diameter obtained at each time interval from each plate.

A reciprocal treatment was made on one plate of each species maintained in continuous light at 150 lx for 19 h, then transferred to darkness for 15 min and returned to light.

Control cultures of each species were maintained: one in continuous light at 150 lx and the other in continuous darkness (except for brief exposures to red photographic safelight for measurements of colony diameters). Measurements in all four treatments were made at the same time intervals.

### Experimental Observations

Table 3 gives the measurements of colony diameter (two from each colony) together with their means, obtained from the culture plate of each species under the four different treatments, at the time intervals shown. The overall growth rates are also shown. These means of colony diameters plotted against time are shown in Fig. 2.

It can be seen that there was a marked decrease in the rate of growth as a result of exposure to 15 min light in *P.anomala*. No pronounced decrease occurred in the other two species. The decrease in growth rate in *P.anomala* to a third of its previous rate, for a period of 5 h, was followed by a recovery to the original growth rate.

These changes in growth rate were associated with the formation of a marked growth ring in *P.anomala*. A feeble growth ring was present in *P.moreau* but a ring was scarcely visible in *P.caucasica*. (Fig. 3). The morphologically distinct ring occurred at a position on the colony corresponding to the colony margin at the time when growth was severely reduced, following exposure to light.

In the reciprocal treatment, a slightly increased growth rate occurred following transfer from light to darkness in *P.anomala*. This was followed by a return to the original growth rate. These changes in rate were also associated with the production of a feeble growth ring. In *P.caucasica* and *P.moreau* no significant change in growth rate was observed. Growth rings were not formed in *P.caucasica* or *P.moreau* as a result of this treatment.

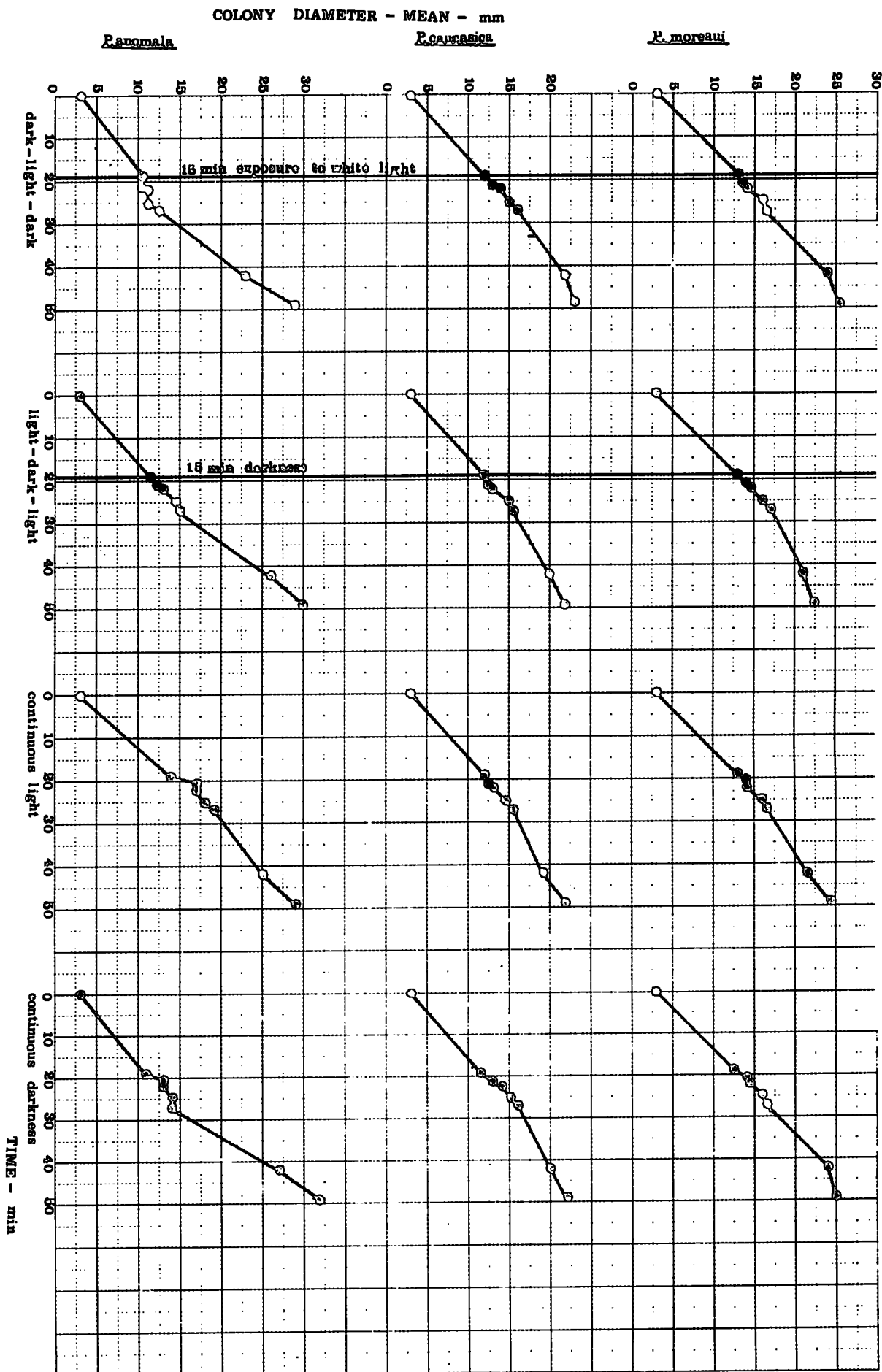


FIG 2 VEGETATIVE GROWTH OF *Ptilaria* species UNDER DIFFERENT LIGHT AND DARK CONDITIONS

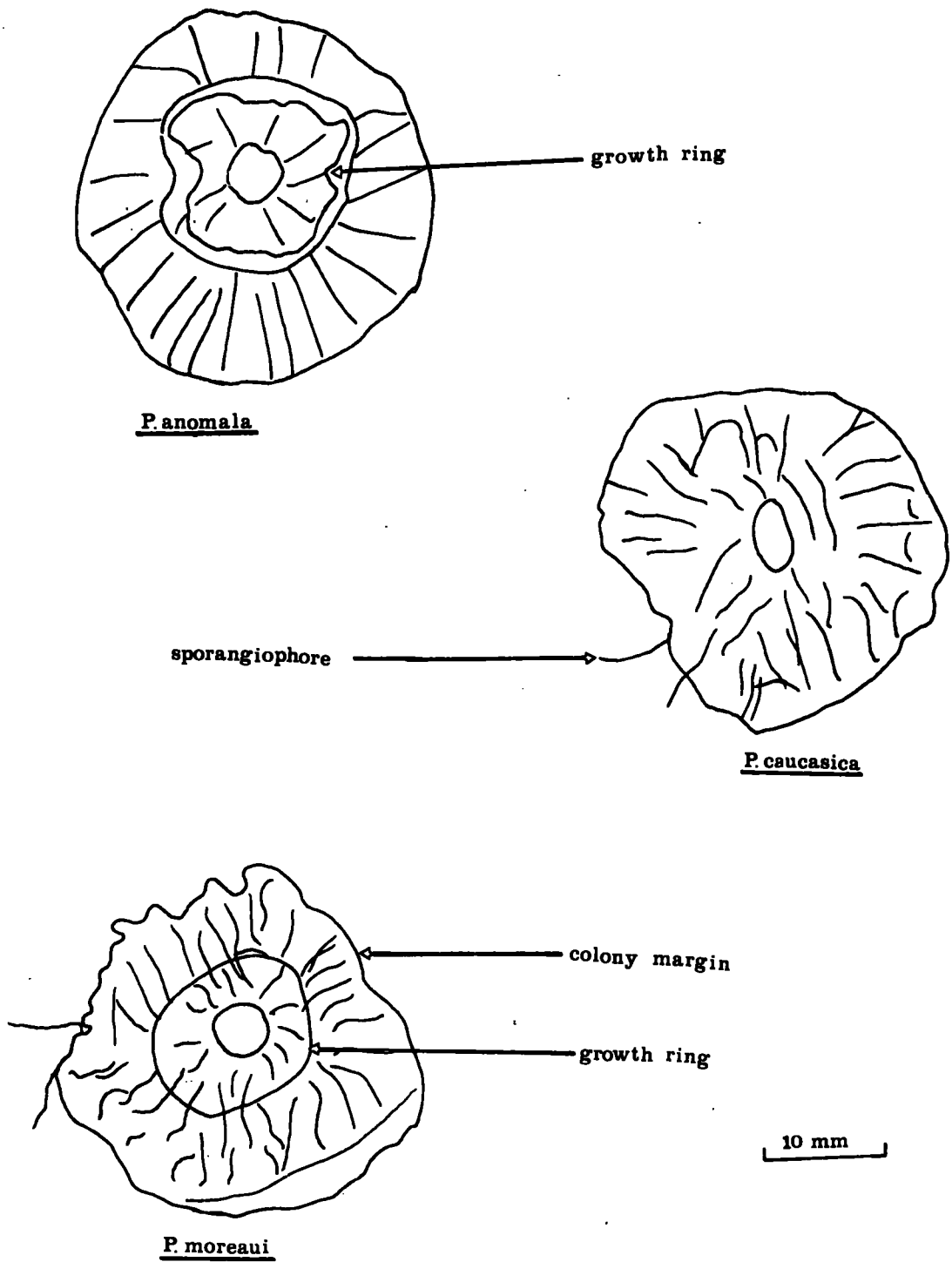


FIG. 3

PRODUCTION OF 'GROWTH RINGS' BY *Pilaira* species AFTER DARK/LIGHT/DARK TREATMENT.  
 (tracings from macrophotographs of colonies after 49 h incubation)

In the light and dark controls, growth in *P. caucasica* and *P. moreaui* was reasonably steady and approximately equal in rate. However in *P. anomala* growth was not uniform during the period of close observation (19-27 h) in both continuous light and continuous darkness. Where a trend was discernable, as in *P. anomala* : Light/Dark/Light, Continuous Light and Continuous Darkness, it was a transient increase and then diminution of growth rate over the period 20-27 h after inoculation. These changes in rate were much less than those in *P. anomala* resulting from the transfer from darkness to light and back, and moreover, growth rings were not observed in control cultures.

These results show that the production of a growth ring in *P. anomala* can be related to a decrease in the growth rate due to exposure to light. The appearance of less conspicuous growth rings in the other two species can be explained by the smaller changes in growth rate which occurred there.

#### Discussion

*P. anomala* differed from the other two species in that it showed marked changes in its vegetative growth rate when it was exposed to 15 min light at 150 lx, and a distinct growth ring was formed in the colony. These results are in accord with results previously described by Fletcher (1970 c). Less distinct rings with no evident change in growth rate were produced by the same treatment in the other two species and by the reciprocal transfers from light to dark and back to light.

The production of a growth ring is possibly related to a change in the branching pattern (Fletcher, 1970 c) and Trinci (1970).

The previously mentioned slight transient increase in the growth rate of many of the colonies over the period 20-27 h after the initiation of the experiment is puzzling, but very extensive investigation, with a better measuring system would be required to determine whether the phenomenon was reproducible and whether any circadian variation in growth rate was occurring following the exposure to light at the inoculation stage.

The only conclusion that can be drawn with confidence, is that in *P. anomala* a 15 min exposure to light in otherwise continuous darkness, brought about a reduction in growth rate, so that during the 5 h following exposure, colony expansion was at a rate of the order of a third of the rate that was maintained both before and over the period 8 to 30 h after the light treatment.

TABLE 3

MEASUREMENTS OF COLONY DIAMETER, AND MEANS, AT TIME INTERVALS SHOWN, TOGETHER WITH RATES OF GROWTH, UNDER DIFFERENT LIGHT AND DARK CONDITIONS FOR THE THREE SPECIES OF *PILAIHA*.

TREATMENT: DARK/LIGHT/DARK

<i>P. anomala</i>				<i>P. caucasica</i>				<i>P. moreaui</i>			
Time	mean	Time	mean	Time	mean	Time	mean	Time	mean		
h		h		h		h		h			
0	3	3	3	0	3	3	3	0	3	3	3
19	10	11	10	11	10.5	19	12.5	12.5	13	11	12
21	10	11	10	11	10.5	21	13	13	14	11.5	13
22	10.5	11.5	10.5	11.5	11.0	22	14	14	13.5	15	14
25	10.5	12	10.5	12	11.0	25	15	15	15.5	16.5	13.5
27	12	13	12	13	12.5	27	17	17	16	14	16
42	22	23.5	23	24	23	42	21	21	24	21	22
49	30	27.5	30	27.5	29	49	21	22	25	26.5	23.5
Overall rate of growth: 0.53 mm h <sup>-1</sup>				Overall rate of growth: 0.43 mm h <sup>-1</sup>				Overall rate of growth: 0.46 mm h <sup>-1</sup>			

THE HORIZONTAL LINE REPRESENTS THE TIME OF ADMINISTRATION OF THE 15 MIN EXPOSURE TO WHITE LIGHT.



TABLE 3 (cont.)

TREATMENT: CONTINUOUS LIGHT

<i>P. anomala</i>				<i>P. caucasica</i>				<i>P. moreawi</i>			
Time h		mean	Time h		mean	Time h		mean	Time h		mean
0	3	3	0	3	3	0	3	3	0	3	3
19	15.5	12.5	19	13	11	14	10	12	19	13	13.5
21	18	15	21	14	11	15	11	13	21	14	14.5
22	18.5	16	22	14.5	11.5	15.5	12.5	13.5	22	14	14
25	18.5	17	25	15.5	13	16.5	13	14.5	25	15.5	16
27	20	17	27	16.5	14	18	14	15.5	27	16	17
42	23	26.5	42	20	20	21	15	19	42	22	22.5
49	33	27	49	20	21.5	25	20.5	22	49	25	23
Overall rate of growth: $0.53 \text{ mm h}^{-1}$			Overall rate of growth: $0.38 \text{ mm h}^{-1}$			Overall rate of growth: $0.44 \text{ mm h}^{-1}$					



## EXPERIMENTAL SECTION III : ANALYSIS OF PIGMENTS

Introduction

A feature of the vegetative mycelium, sporangiophores and spores of *Pilaira* species is the marked production of yellow and orange pigments (Grove, 1934; Buller, 1934; Schopfer, 1938). This pigmentation was particularly marked in illuminated cultures on favourable media.

In these studies these general observations have been confirmed with a number of isolates of *P.anomala* and in the type cultures of *P.moreaui* and *P.caucasica*. The most detailed study has been made on the isolate I.M.I. 109387 of *P.anomala* and these results are presented here.

Special Techniques

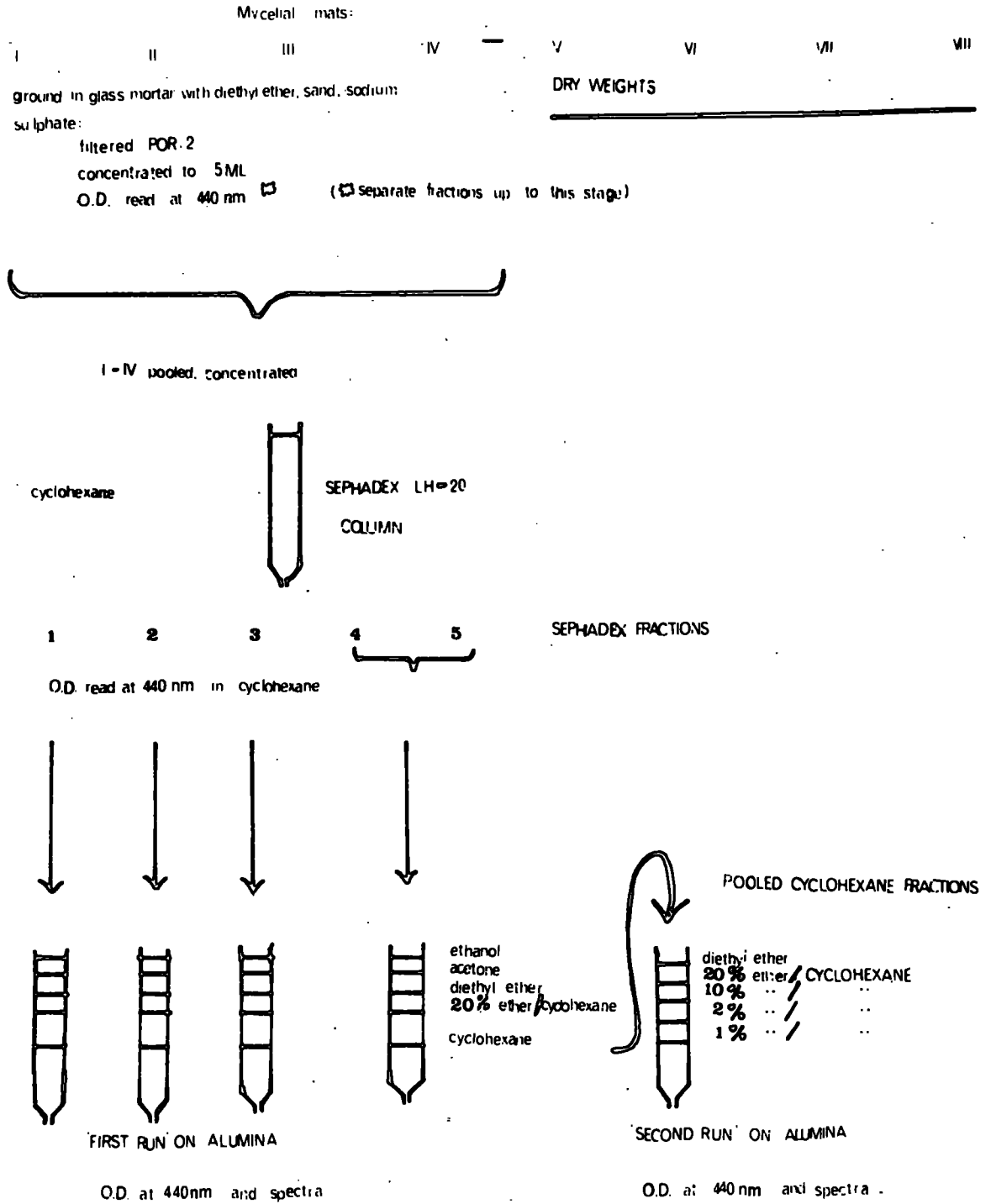
To obtain maximum pigmentation, cultures were grown on media containing glucose (10); salts:  $\text{KH}_2\text{PO}_4$  (1.0);  $\text{K}_2\text{HPO}_4$  (1.0);  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.5);  $\text{NaCl}$  (0.1);  $\text{CaCl}_2$  (0.1); (all g/l) and micronutrients: B (0.01); Cu (0.1); Fe (0.2); Mn (0.02) and Zn (2.0); (all mg/l), also casein hydrolysate 'Difco Bacto Casamino Acids' (5.0) and yeast extract 'Difco Bacto' (0.5), (both g/l).

50 ml of sterile medium were used in 1 litre Roux bottles capped with aluminium foil and inoculated with 3 plugs from S.M.A. These were laid flat in an illuminated cabinet (750 lx) at 25°C and incubated for 10 days.

The main stages of the extraction are summarised in the flow diagram (Fig. 4). The mycelial mats from a set of flasks were individually collected on to discs of Whatman No. 541 filter paper in a 50 mm Büchner funnel. The mats, which then appeared highly pigmented, were transferred to a glass mortar and an equal volume of acid-washed sand (May & Baker) was added.

FIG. 4

FLOW DIAGRAM OF CAROTENOID EXTRACTION AND ANALYSIS.



The mycelium was ground to a paste with 5 ml of diethyl ether. A volume of anhydrous sodium sulphate equal to that of the mycelial paste was incorporated into the mass and the whole mixed until homogeneous. This was scraped into a sintered glass Büchner funnel, porosity-2, attached to a filter flask. The mycelium-sand-sulphate-ether mixture was spread over the surface of the funnel and gentle suction applied. Extraction of the pigments from the mass continued with successive 5 ml volumes of diethyl ether until the eluate was colourless. This required from 25 ml to 50 ml of solvent. The final volume of each extract was recorded.

Samples were taken from these extracts and their optical density determined in the Hilger 'Uvispeck' H.700 at 440nm; after reference to a calibration curve a measurement of 'total carotenoid' in the extract was calculated. This was done for each extract in the set. All the extracts were pooled and concentrated by reducing the volume, under slight vacuum in an atmosphere of nitrogen, to less than 1 ml of diethyl ether extract. This concentrate was used as the starting material on a Sephadex LH-20 (Pharmacia G.B. Ltd) column.

A Pyrex column, 250 mm x 8 mm internal diameter was two thirds filled with a slurry of LH-20 in cyclohexane. As the last drop of cyclohexane drained into the column, the concentrated solution of the pigment in diethyl ether was applied, followed by elution of the column with cyclohexane. Collection of 2 ml fractions commenced with the loading of the starting material and continued until at least two fractions after the pigment band had apparently been eluted. Usually 10 fractions were collected. The first two fractions were colourless, the next five appeared to contain the pigment and the last three were colourless. All five colourless fractions were discarded. The optical density of each fraction was read at 440nm in a 5 mm cell. The optical density of the fractions showed that nearly all the pigment was in fractions 3 to 6. The rate of flow was 20 ml in 24 min.

Five 2 ml fractions were usually obtained from the Sephadex treatment. The first three were used separately and the fourth and fifth pooled before application to alumina columns. A Pyrex column: 250 mm x 8 mm internal diameter was half-filled with alumina (May & Baker 'Aluminium Oxide for Chromatography') and tamped down.

The whole of the 2 ml from the Sephadex extraction was run into the column and eluted with the following solvents of increasing polarity. In each case, the previous solvent was allowed to drain into the column before the subsequent one was run in. The solvents in order of application were: cyclohexane; 20% diethyl ether in cyclohexane; diethyl ether; acetone and absolute ethanol.

The eluate produced by each of the five solvents from the four alumina columns was pooled and its optical density at 440 nm was determined. As the starting volume was large and concentrated, an incomplete separation occurred on the first run.

The cyclohexane fraction was large and concentrated and its volume was reduced to less than 0.5 ml under slight vacuum in an atmosphere of nitrogen before being re-chromatographed on a second alumina column. Here it was eluted with increasing concentrations of diethyl ether in cyclohexane: 1.0 ; 2.0 ; 10.0 ; and 20.0% and finally pure diethyl ether. In each case a specific band was eluted before changing to the next concentration. The absorption spectra of the separate bands eluted from the two alumina columns were determined on a Unicam S.P. 800 automatic recording spectrophotometer. With those of very low concentration, the spectra were 'boosted' by means of a Beckman scale expansion recorder.

The bands containing  $\beta$ -carotene were identified by chromatography on silica gel (SG 81) and aluminium hydroxide (AH 81) loaded papers (Whatman 'Chromedia') of 50 x 300 mm. The spots were dried at the origin with a jet of nitrogen.

Crystalline  $\beta$ -carotene (Roche Products Ltd) was used as a standard.

Two separate solvent systems were used with the ascending technique:

light petroleum (40/60) alone, and benzene: light petroleum (40/60) in the ratio 1:4. Separation was complete in 30 min.

#### Preliminary Procedure

Preliminary extractions showed that all the pigment could be extracted by fat solvents and that none were water soluble. Grinding with diethyl ether was found to be the most effective method and this was used for analysis of the pigments present in *P. anomala*.

A diethyl ether extract was dried over sodium sulphate, concentrated, made up to a known volume and its optical density read at 440 nm. The standard Saponification (Davies, 1965) to remove triglycerides which may interfere with subsequent chromatography was not used because it was found that much of the pigment was destroyed. As an alternative, filtration through Sephadex LH-20 was tried. This is a gel filtration medium developed for the separation of molecules of comparatively low molecular weight in organic solvents.

In preliminary trials it was necessary to establish that the LH-20 fractions were free from contaminating lipids and that the molecular species being separated were solely carotenoids. Infra-red spectra were therefore determined (Freeman, Lindgren, Ng and Nichols, 1957).

Fractions from the Sephadex (2 ml) were evaporated to dryness and redissolved in a minimum volume of carbon disulphide. They were transferred to a 0.1 mm rock salt cell and the spectra were read across the range: 2.5 to 15.0  $\mu\text{m}$ . The spectra obtained were compared with various pure lipids markers run separately:  $\beta$ -carotene; oleic acid; stearic acid and cholesterol, all in carbon disulphide. The solvent was also run separately. By comparing the spectra of the pure lipids, together with that of the solvent and that of the fractions from the Sephadex column, it was shown that the Sephadex fractions were free from fatty acids and cholesterol.

The spectra of the coloured LH-20 fractions agreed with that of  $\beta$ -carotene. This would seem to confirm the expected separation of lipid molecules on this gel on the basis of their molecular weights:  $\beta$ -carotene (537); oleic acid (282); stearic acid (284) and cholesterol (387).

#### Analytical Results

*Pilaira anomala* cultures grown, harvested and extracted under the conditions described above and summarised in Fig. 4 were subjected to qualitative and quantitative analysis of their carotenoid pigments.

Table 4 presents the data relating to these harvested cultures, the diethyl ether extracts and estimate of 'total carotenoid contents'.

The diethyl ether extracts were pooled and the concentrated solution applied to a Sephadex LH-20 column and eluted with cyclohexane. Table 5 shows the data relating to this separation.

TABLE 4

## INITIAL EXTRACTION OF CAROTENOIDS FROM HARVESTED CULTURES

Culture	Volume of diethyl ether (ml)	O.D. at 440 nm	$\mu\text{g/ml}$	Total $\mu\text{g}$ of carotenoid	mg dry wt. of mycelium
I	50	0.19	0.8	40	
II	26	0.34	1.4	36	
III	35	0.28	1.1	39	
IV	30	0.47	1.9	57	
V					110
VI					108
VII					89
Total				172	307
mean yield				(43)	(102)
per culture					

Therefore the carotenoid content of the mycelium ( $\mu\text{g}$  pigment/mg dry weight) is 0.42  $\mu\text{g}/\text{mg}$  (43/102)

TABLE 5

## SEPARATION ON SEPHADEX LH-20

Successive 2 ml coloured fractions eluted with cyclohexane

Fraction	O.D. at 440 nm (5 mm cell)
1	0.81
2	2.59
3	2.55
4	0.74
5	0.10

An accurate estimation of carotenoid content was not possible from these results since the optical density of fractions 2 and 3 was too high to be read from the calibration curve. Each 2 ml Sephadex LH-20 fraction was further separated into bands of individual carotenoid on an alumina column. The bands were eluted with solvents of increasing polarity. Fractions 4 and 5 from Sephadex LH-20 were pooled. Data pertaining to the alumina columns are shown in Table 6.

TABLE 6

## FIRST SEPARATION OF ALUMINA

Solvent	Fractions from Sephadex LH-20 (Table 5)				O.D. at 440nm of pool of fractions from each solvent. 10 mm cell
	1	2	3	4/5	
	Volumes (ml)				
Cyclohexane	2.35	2.5	4.8	1.25	2.48
20% diethyl ether in cyclohexane	3.4	3.9	3.3	1.4	0.257
Diethyl ether	3.3	5.0	4.8	1.4	0.194
Acetone	3.2	2.5	3.8	1.5	0.145
Ethanol	-	-	5.5	-	0.058

Table 7 provides additional data pertaining to Table 6

TABLE 7

## QUANTITIES OF CAROTENOIDS FROM FIRST ALUMINA SEPARATION

Solvent	O.D. at 440 nm	Total vol. (ml)	µg/ml	Total carotenoid µg
Cyclohexane	2.48	10.9	10.3	112*
20% diethyl ether in cyclohexane	0.257	12.0	1.3	14.4
Diethyl ether	0.194	14.5	0.9	13.2
Acetone	0.145	11.0	0.6	6.6
Ethanol	0.058	5.5	0.2	1.1

\* Approximate estimate

Table 8 shows the absorption maxima for each fraction from the first separation on alumina, determined with a Unicam S.P. 800 automatic recording spectrophotometer.

TABLE 8

## ABSORPTION MAXIMA OF FRACTIONS IN TABLE 7

Solvent	nm		
Cyclohexane	420	452	480
20% diethyl ether in cyclohexane	430	460	490
Diethyl ether	-	445	470
Acetone	430	450	480
Ethanol	390	430	460

The 9.0 ml of the cyclohexane fraction were concentrated at reduced pressure under nitrogen and re-chromatographed on alumina. The elution data and absorption maxima are shown in Table 9.

TABLE 9

## SECOND SEPARATION ON ALUMINA

Solvent (% diethyl ether in cyclohexane)	Volume ml	O.D. at 440 nm 5 mm cell	µg/ml	Total carotenoids µg	Absorption maxima
1.0	1.7	0.038	0.2	0.68	undetectable
2.0	8.8	1.26	6.1	107.2	420 455 480
10.0	2.7	0.091	0.4	1.35	undetectable
20.0	2.1	0.285	2.3	9.68	430 460 490
100.0	1.9	0.177	1.5	5.70	- 465 -

A combined summary of the carotenoid fractions with similar absorption maxima, obtained from both alumina separations, is given in Table 10. This shows that 159.9 µg of carotene have been recovered from the original 172 µg of extracted pigment (Table 4). This represents a recovery of 93%.

TABLE 10

## SUMMARY OF FRACTIONS

Absorption maxima	Total $\mu\text{g}$	% in whole extract	Tentative identification of pigment
- 445 470	13.2	8.25	$\alpha$ - carotene
420 452 480	113.8	71.0	$\beta$ - carotene
430 460 490	25.18	16.19	$\gamma$ - carotene
(400) (420) 465	5.70	3.6	neurosporene
undetectable	2.03	1.27	-
	159.91	100.31	

Since all the initial extract remained in the epiphase when a cyclohexane solution was shaken with 90% aqueous methanol, it seems likely that the pigments are all carotene and that xanthophylls are absent.

The identification of  $\beta$ -carotene was confirmed by chromatography on loaded papers in at least two solvent systems with an authentic sample of crystalline  $\beta$ -carotene. The absorption maxima also corresponded with that of the crystalline  $\beta$ -carotene in at least two solvents.

The tentative identification of the remaining pigments (Table 10) was based on the tables of Davies (1965) showing the order of elution from alumina by solvents of increasing polarity and the absorption maxima in the appropriate solvent. Quantitative estimations of individual pigments were made from the calibration graph for  $\beta$ -carotene.

## DISCUSSION

The observation of an overall yellow appearance in the mycelium was not made until Schopfer (1938) grew *Pilaira* spp. in liquid culture. It may have been that the use of dark coloured materials such as dung, by earlier workers, had masked any yellow pigmentation in their cultures. It therefore required the separation of the fungus from the medium by harvesting, for the pigment to be observed. This suggestion is supported by the fact that the yellow pigment was more conspicuous in compact dry mycelium than when the mycelium was suspended in a liquid medium.

In the analyses of pigments described in this thesis, whole mycelial mats grown in the light were used. The harvested pigmented mycelium consisted of two components: vegetative and asexual. The pigments tend to be located in older vegetative hyphae, in Stage III sporangiophores and spores. The description in Grove (1934) that the sporangiophore was 'yellow at first', must refer to Stage III, where, having reached maximum diameter, the sporangium becomes brightly yellow before the upper portion blackens and cutinises. No attempt was made to suppress sporulation or to separate vegetative from asexual structures at harvest.

Previous workers with *Pilaira* had not attempted to analyse the yellow pigment. Schopfer (1938) described it as a 'carotene' and presumably based this identification merely on its solubility in fat solvents and its colour. The pigments of *Pilaira* have been described in greater detail in this thesis.

In the detailed analysis of the carotenoids of *Pilaira*, there is a parallel with other fungi: *Blakeslea*; *Phycomyces* and *Syzygites*. Unfortunately there is no detailed analysis of *Pilobolus*. Bünning (1937) referred to the pigment of *Pilobolus* as 'carotene'. A comparison between the carotenoid composition of *Pilaira* and other fungi is shown in Table 11.

Table 11 shows the close affinity between the pigment composition of *Pilaira* and that of *Syzygites*, although they are members of different families. It is quite clear that *Pilaira* does not resemble *Phycomyces* in its pigment composition. The almost exclusive production of  $\beta$ -carotene by *Phycomyces* makes it quite different. *Pilaira* differs markedly from *Blakeslea* in that the latter produces lycopene. *Blakeslea* also differs from the other organisms in a much lower concentration of  $\beta$ -carotene.

TABLE 11

CAROTENOID COMPOSITION OF SOME FUNGI

OF THE ORDER OF MUCORALES

Organism	<i>PILAIRA</i> <sup>a</sup>	<i>SYZYGITES</i> <sup>b</sup>	<i>PHYCOMYCES</i> <sup>c</sup>	<i>BLAKESLEA</i> <sup>d</sup>
Family	Pilobolaceae	Mucoraceae	Mucoraceae	Choaneophoraceae
Total carotenoids ( $\mu\text{g/g}$ )	420	600	>3,000	2,189
% of total carotenoids				
$\beta$ - carotene	71	71	95	53
$\alpha$ - carotene	8	0	2	0
$\gamma$ - carotene	16	24	1	27
lycopene	0	5	1	14
neurosporene	4	0	0	trace
unidentified	1	-	-	-

a, Fletcher, 1969a; b, Wenger and Lilly, 1966; c, Goodwin, 1952 and Lilly et al. 1957 ; d, Thomas and Goodwin, 1967 and Sutter and Rafelson, 1968.

### Introduction

In the related *Pilobolus*, the precursor of the sporangiophore is the trophocyst, a distinct 'cell' cut off from the vegetative hyphae, from which the sporangiophore itself arises. In *Pilaira* and *Phycomyces*, no trophocyst occurs, the sporangiophore is merely an aerial branch of a vegetative hypha and moreover is in coenocytic continuity with the vegetative hyphae.

### Special Techniques

Photomicrographs were made of suitable areas of mycelium bearing primordia, examined directly with phase contrast under a X 10 objective or through a cover glass under a X 40 objective. Observations and measurements were made from enlarged prints.

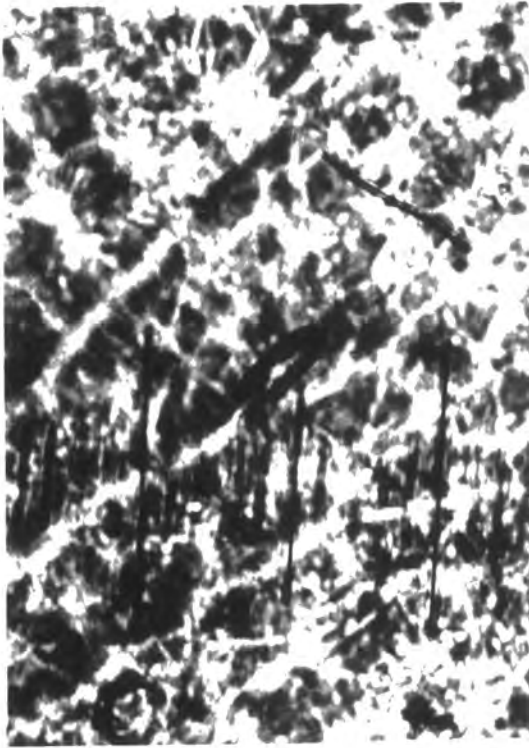
### Observations and Measurements

Primordial sporangiophores of *P. anomala* were found to resemble the hyphae from which they arose, in that their diameters were similar (Table 12). The primordia arose from main radial hyphae and not from the ultimate terminal branches (Fig. 5). The primordia differ from substrate hyphae physiologically in that they show distinctive properties such as weak negative geotropism and marked positive phototropism (Fig. 6).

The whole sporangiophore at this stage is like an enormous hyphal tip, in that it is vacuolated, except at the apex where there is continuous protoplasm (Fig. 7). The size difference between sporangiophores and the aerial vegetative hyphae can be seen in Fig. 8, where the base of a sporangiophore is in the same focal plane as the hyphal tips that have emerged from the medium. This is shown in Table 12.

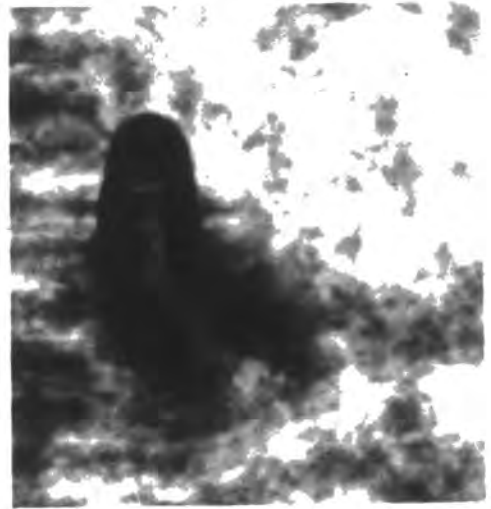
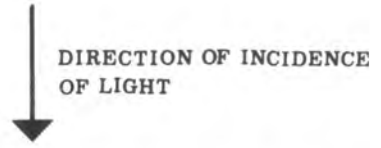
There do not appear to be any major differences between the sporangiophore primordia of the three species.

SPORANGIAL PRIMORDIA OF *Pilaira anomala*



X200

FIG 5 Primordium and radial hypha

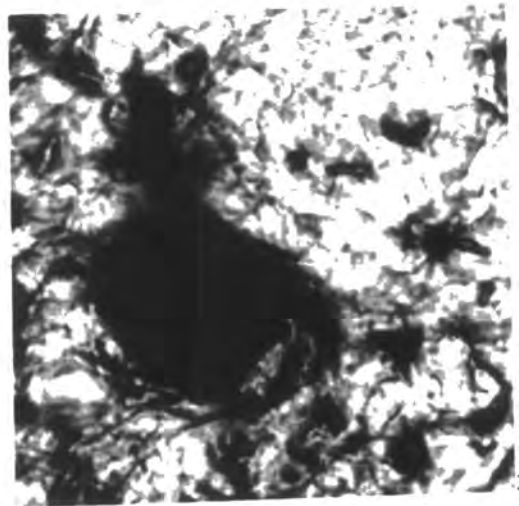


X 500

FIG 6 Primordium tip



FIG 7 Older primordium tip showing continuous protoplasm and vacuole X 500



X 500

FIG 8 Primordium base and aerial hyphae

TABLE 12

Dimensions of newly emerged sporangiophores of *P. anomala* and the hyphae from which they have arisen, and neighbouring terminal hyphae (Fig.8).

μm

Primordium length				Primordium diameter				Hyphal diameter				Aerial vegetative hyphae diameter			
70	80	92	70	25	25	30	30	30	40	32	30	3	3	3	3

### Discussion

It would seem from the observations later in this Section (IV B) that sporangiophores are not produced until a period of maturation has elapsed in the vegetative hyphae. This maturation period may be required for the sporangiophores to produce protoplasm with physiological properties quite distinct from that of vegetative hyphae, as regards tropic and other phenomena. This is all the more remarkable because the sporangiophore is a coenocyte in open continuity with the same vegetative hypha from which it arose and yet the sporangiophore has developed its distinctive properties.

## EXPERIMENTAL SECTION IV B:

## THE LOCATION OF SPORANGIOPHORE INITIALS ON THE MYCELIUM

Introduction

The transition from vegetative to asexual development in *Pilobolus* is a major step in morphogenesis. The formation of trophocysts in that genus involved the flow of protoplasm towards the site of trophocyst development (Page, 1956). Sporangiphore initiation in *Pilaira* does not involve such conspicuous morphological changes, but the origin of sporangiphores still marks a new phase of development.

Special Techniques

Enlarged prints (as used for other sporangiphore investigations) were studied. Measurements were made, with a mm rule, of the distances of the sporangiphore initials from the margin, and these measurements converted to the actual distances, from the magnification factor of the prints.

Measurements

Table 13 shows the location of the initial Stage I sporangiphores in relation to the margin of the colony on two different media (for *P. anomala*) and in three different light regimes for all three species.

It can be seen from Table 13 that on both media and under all light conditions, the sporangiphores of *P. anomala* are formed much further from the margin than those of the other two species. This partly resulted from the colonies of *P. anomala* being uniformly circular, while those of the other two were indented. This indentation seems to be related to their poorer growth on the S.M.A./5 medium, as compared with growth of *P. anomala*. As vegetative growth precedes sporangiphore development, the faster the vegetative growth, the wider the sporangiphore-free margin, provided that a constant maturation time elapses before sporangiphore initiation. This is also borne out by the wider sterile margin on S.M.A. over that on S.M.A./5 in *P. anomala*.

The light regimes do not appear to have any great effect on the width of the sterile zone and this would be related to the absence of any difference in vegetative growth under the three regimes.

### Discussion

It would seem that a sufficient period of vegetative maturation is required before sporangiophores can arise from mycelium. As pointed out earlier in this Section, sporangiophores are formed from main hyphae of a certain diameter. These are only formed beyond a minimum distance from the purely vegetative margin, which consists of small branching hyphae in direct contact with the substrate.

TABLE 13

SPECIES & MEDIUM	LOCATION OF INITIAL STAGE I SPORANGIOPHORES (WIDTH OF STERILE ZONE) (mm, from nearest vegetative margin)				
	<u>Light Regimes</u>				
	CONTINUOUS LIGHT (means in brackets)	CONTINUOUS DARK	DAYLIGHT		
<i>P. aroma</i> ta	S.M.A.	6/6/6/6/7.3 8/7.3/8/8	6.9/6.3/8.1/6.9/7.5 7.5/7.5/8.8/7.5/6.3	5.3/4.7/6.7/6/4.7 5.3/6/6/6/5.3	(5.6)
	S.M.A./5 Colony A	3.5/3/2.5/3/2 3.5/4/2/2/1/4.5	3/2/1/2/2.5/3/3.5/3.5 3.5/3.5	4/3/4/2.5/2.5/6.5 4/4.5/4/4.5	(4.0)
Colony B		4/4/3.3/2.7/3.3/3.3 3.3/2.7/3.3/2.7/4/1.3	-	-	(3.5)
	<i>P. caucasi</i> ca				
S.M.A./5		1/1/0.8/1.5/1 1/1/1/1/1	0.5/0.5/1/0.5/0.5 1.0/0.5/0.5/0.5/0.5	0.8/0.8/0.3/0.1/0.5/0.8	(0.6)
	<i>P. moreau</i> i				
S.M.A./5 Colony A		0.8/1/1/0.8/0.8 1/0.5/1/0.3/0.5/1	1/1/1/1/0.3/0.3 0.5/0.5/0.5/0.5	0.5/0.5/0.5/1/0.5 0.5/0.5	(0.6)
	Colony B	2/1/1/0.5/1/1/1	1/0.5/0.5/0.5/0.5 0.5/0.5/1/0.5/0.5	-	(0.6)
Colony B					
		2/1/1/0.5/1/1/1	-	0.8/0.5/0.5/0.5/0.8 0.1/0.5/0.5/0.5/0.5	(0.5)

EXPERIMENTAL SECTION IV C:  
FACTORS CONTROLLING THE INITIATION & MATURATION OF  
SPORANGIOPHORES

Introduction

The effect of nutrition and the requirement for light in the initiation and maturation of sporangiophores of *Pilobolus kleinii* was described by Page (1952). The light requirements of this genus were reviewed by Carlile (1965). The factors determining sporangiophore production in *Phycomyces* were discussed by Bergman et al. (1969). A brief description of sporangiophore development in *Pilaira* was given by Fletcher (1969 b).

Experimental techniques

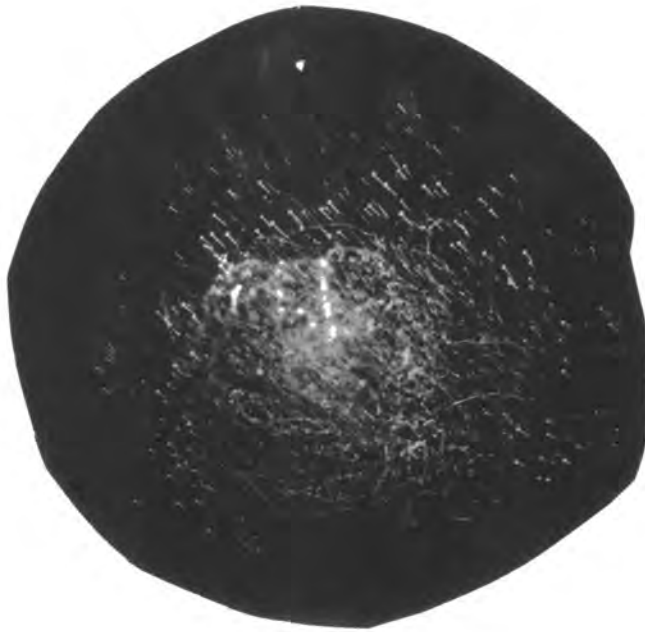
Cultures of the three species of *Pilaira* were grown in darkness for two days on S.M.A./5 in petri dishes. These were used for providing the inoculum: 3 mm plugs cut with a sterile cork borer from the margins. Plates were inoculated with one plug into the centre of the agar medium, which was either S.M.A./5 or S.M.A. The plates were placed either in continuous light at 150 lx, continuous darkness or exposed to the natural alternation of daylight and night (Aberdeen, May - approximately 7 h darkness; 17 h light), all light regimes were at approximately 25°C.

The plates were examined and measured after 48 h and in some cases macrophotographs were made of colonies bearing sporangiophores. From enlarged (X 2) prints of the photographs, counts were made of the number of the different Stages of sporangiophore present at the time of observation, in the margin and submargin of the colony, in advance of the region of extensive elongation of the sporangiophore (Fig. 9). In other cases, estimates were made by observations with a dissecting binocular microscope, of the numbers of sporangiophore at each Stage.

FIG 9

COLONY OF *P. anomala* GROWN ON SMA IN NATURAL DAYLIGHT

(showing production of sporangiophores at various stages, in margin and submargin)



x 2

For reasons set out fully in Section V A of this thesis, the following Stages of sporangiophore development have been recognised:

- I conical tipped sporangiophore up to Stage when elongation ceases temporarily,
- IIa papilla at tip, less than diameter of sporangiophore,
- IIb sporangial primordium swelling, equal to sporangiophore diameter at first and then exceeding it,
- IIIa sporangium formed, spores develop within it and columella appears, sporangium reaches maximum diameter, yellow,
- IIIb sporangium at maximum diameter, upper wall cutinised and blackening,
- IV sporangiophore recommences elongation.

Previous work in this field is referred to in Section V A.

Using photographic records, Stages I and IIIb may be readily identified. It therefore proved expedient to combine Stages IIa and IIb with IIIa, since these three Stages appeared light in the print and were not readily distinguishable from each other.

#### Experimental Observations

The counts of the numbers of sporangiophore of each of the three species at each Stage are presented in Table 14. It can be seen from this Table that in all three species on S.M.A./5, light is required for the maturation of the sporangiophores beyond the initial Stage I. Stage I sporangiophores, however, are initiated in darkness on both media, and under both light regimes in all three species. On S.M.A., both *P. caucasica* and *P. moreaui* produced sporangia in darkness, while in *P. anomala*, no Stages beyond Stage I were produced on this medium in the absence of light. Mature sporangia were produced in all three species in continuous light and daylight on both media. There were however, differences in the pattern of the numbers of the Stages of sporangiophore development of the three species, under the two 'Light Regimes' (Table 14).

TABLE 14

Counts of 50 marginal and submarginal sporangiophores of three species at each Stage on S.M.A. and S.M.A./5, after 48 h exposure to the illumination regime shown, at 25°C.

REGIME, SPECIES & MEDIUM		SPORANGIOPHORE STAGES			
		I	IIa/b & IIIa	IIIb	IV
<u>Continuous Light</u>					
<i>P. anomala</i>	S.M.A.	21	29	0	0
	S.M.A./5	6	30	11	3
<i>P. caucasica</i>	S.M.A.	(SUCCESSION OF STAGES PRESENT)			
	S.M.A./5	23	19	0	8
<i>P. moreaui</i>	S.M.A.	(SUCCESSION OF STAGES PRESENT)			
	S.M.A./5	32	0	0	18
<u>Continuous Darkness</u>					
<i>P. anomala</i>	S.M.A.	50	0	0	0
	S.M.A./5	50	0	0	0
<i>P. caucasica</i>	S.M.A.	(EQUAL NUMBERS OF STAGES I AND IV)			
	S.M.A./5	50	0	0	0
<i>P. moreaui</i>	S.M.A.	(MOSTLY STAGE I, A FEW STAGES IIIb & IV)			
	S.M.A./5	50	0	0	0
<u>Daylight</u>					
<i>P. anomala</i>	S.M.A.	13	31	4	2
	S.M.A./5	34	12	3	1
<i>P. caucasica</i>	S.M.A.	(MANY STAGE IV, FEW STAGE I, SOME OTHER STAGES)			
	S.M.A./5	11	15	14	10
<i>P. moreaui</i>	S.M.A.	(MOSTLY STAGE III, A FEW STAGE I & STAGE II)			
	S.M.A./5	15	7	8	20

## Discussion

In the production of sporangiophores, *Pilaira* species show some similarities to species of *Pilobolus*. In *Pilobolus kleinii*, sporangia were produced only under conditions of alternating light and darkness (8 h light: 16 h darkness) on media containing thiamine or the thiazole moiety (Page, 1952). Also in *Pilobolus*, different responses were given by different species: in *P. kleinii* light was required for trophocyst initiation, in *P. crystallinus* trophocysts were produced in darkness together with elongated Stage I sporangiophores, but no further maturation occurred, while in *P. sphaerosporus* complete sporangiophore development was carried out in darkness (Carlile, 1965).

In *Pilaira* species sporangiophores are initiated without light, on dilute and concentrated media. In *P. anomala* further development does not occur in the absence of light, but the other two species can produce mature sporangia in darkness on a suitable medium. It could be possible that the effect of light is equivalent to an adequate supply of some nutritional factor, although this is not the case in *Pilobolus kleinii* where light and thiamine operate on different stages of development (Page, 1952).

## EXPERIMENTAL SECTION V A:

## EXTERNAL MORPHOLOGY OF STAGES OF SPORANGIOPHORE DEVELOPMENT

Introduction

Fletcher (1969 b) described the Stages of development of *P. anomala*. These now require redefinition in the light of further studies presented here.

Special Techniques

Techniques similar to those in Section V C were used.

Observations

The new terms proposed are represented in Fig. 10. Stage II has been subdivided into IIa and IIb, to separate the transient primordial papilla (IIa) which precedes the more obvious swelling of the very young sporangium (IIb). Stage III has been subdivided so that the former III can be redesignated IIIa and the former IVa redesignated IIIb. The terms IVa and IVb (Castle, 1942) are unacceptable here in the absence of anticlockwise rotation of the sporangiophore - a feature of the IVa of *Phycomyces*. The failure to detect anticlockwise rotation is described in Section VI. As the rotation in the three species of *Pilaira* has been observed to be clockwise, the rapidly elongating phase (described in detail in Section VIII) is designated Stage IV. This Stage is terminated when dehiscence and detachment of the sporangium takes place and Stage V is attained.

These Stages have been described for *P. anomala* (Fig. 10) and are the same for *P. caucasica* (Fig. 11) and *P. moreaui* (Fig. 12). There is no difference between the three species in the external appearance of the development from Stages I to IIIb. There is also no difference in the next Stage of development, in which the sporangium borne aloft by the rapidly elongating sporangiophore dehisces from the columella (Stage IV) the whole still held together by mucilage (Fig. 13).

FIG 10

KEY TO TERMINOLOGY USED IN DESCRIPTION OF STAGES OF SPORANGIOPHORE DEVELOPMENT

(based on *P. anomala*)



STAGE I



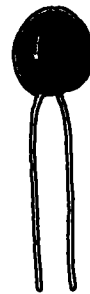
STAGE IIa



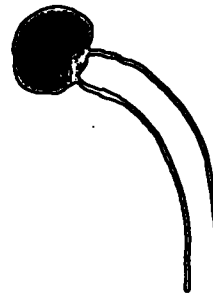
STAGE IIb



STAGE IIIa



STAGE IIIb



STAGE IV



STAGE V

200  $\mu$ m

FIG 11

STAGES OF SPORANGIOPHORE DEVELOPMENT OF *P. caucasica*

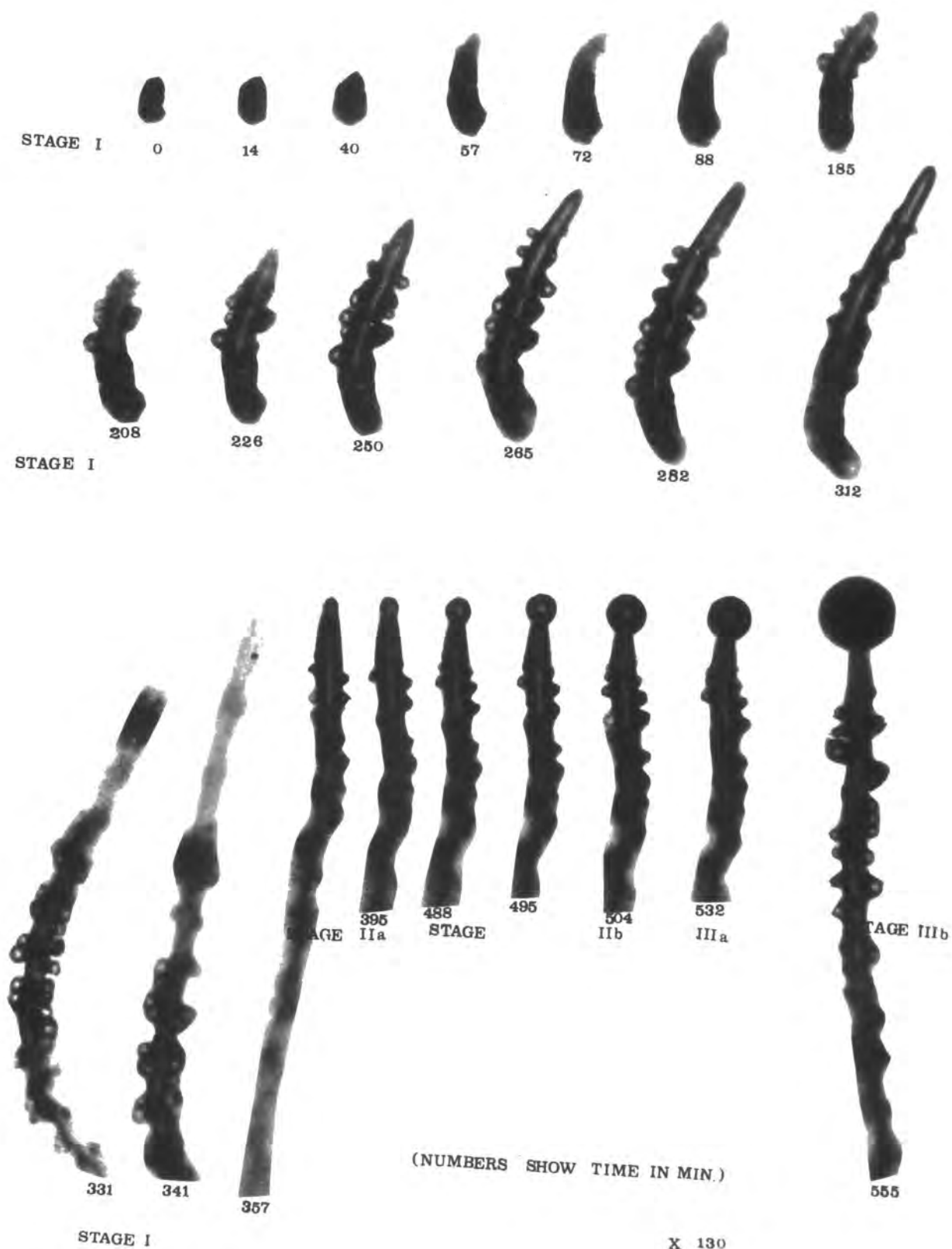


FIG 12

STAGES OF SPORANGIOPHORE DEVELOPMENT OF *P. moreaui*

(NUMBERS SHOW TIME IN MIN.)

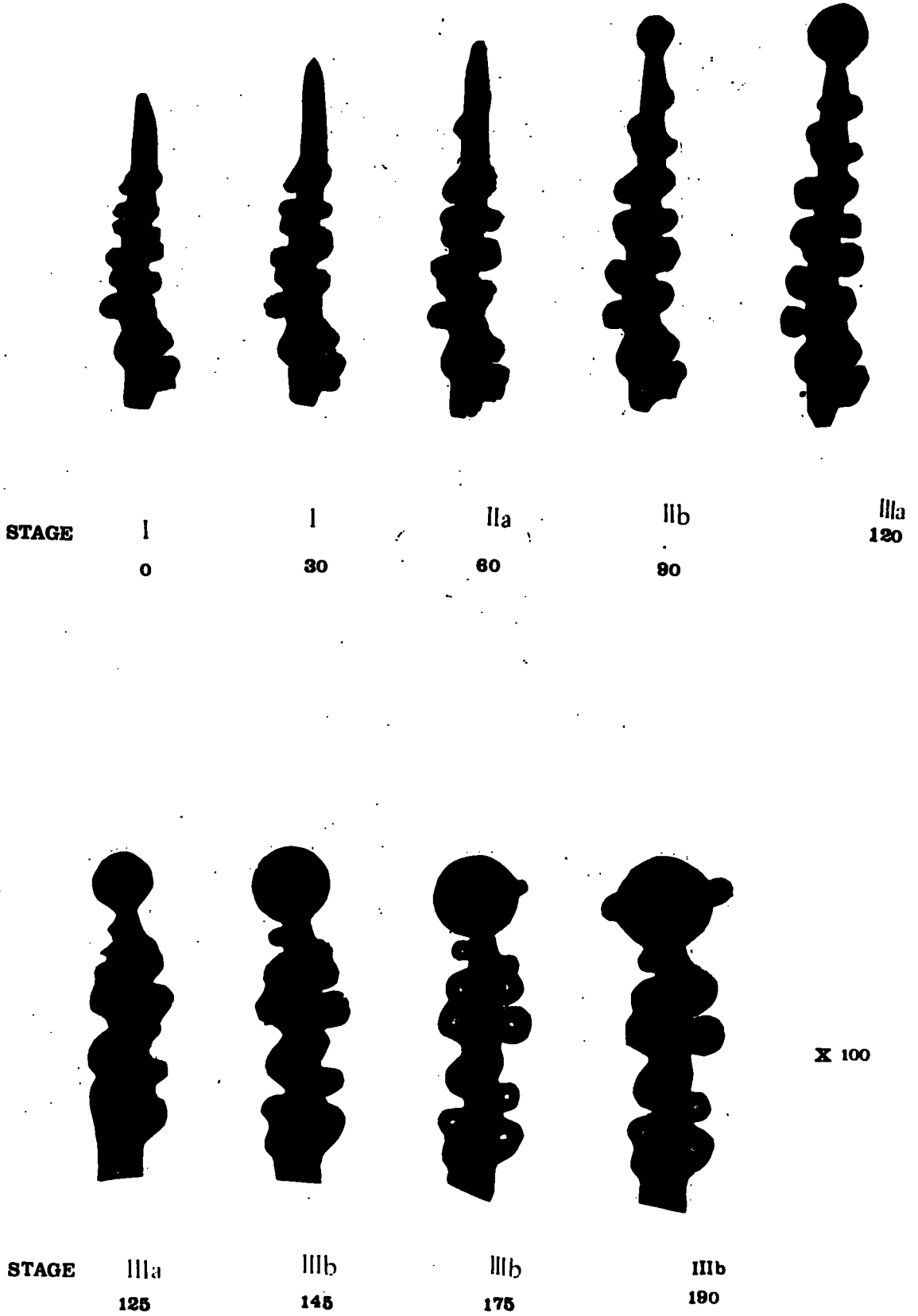


FIG 13

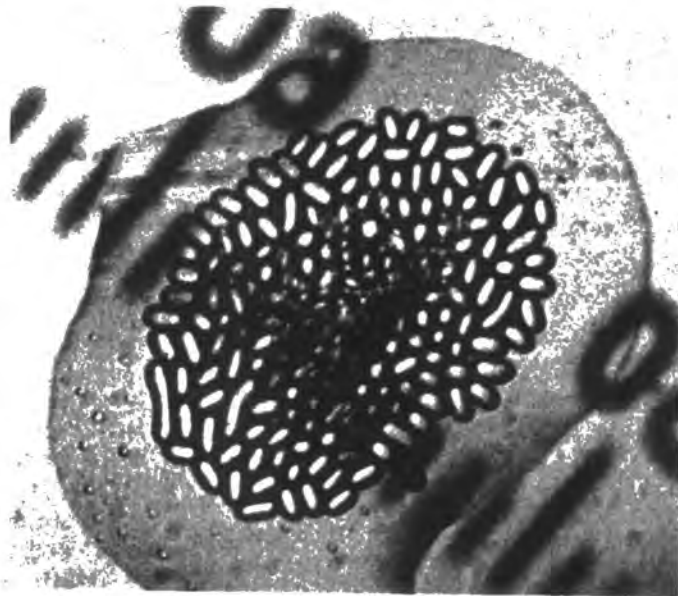
STAGE IV SPORANGIOPHORE OF *P.moreaui* showing dehiscence of sporangium and ring of mucilage investing spores.



X 250

FIG 14

DISPERSED SPORES OF *P.moreaui*, free from sporangial wall.



X 500

The marked ring of extruded mucilage is particularly well shown.

The final phase of sporangiophore development occurs when the sporangium separates from the columella; this Stage V is equivalent to the forcible discharge in *Pilobolus*. This detachment in *Pilaira* occurs when the sporangiophore has elongated so as to bring the sporangium into contact with a hard surface (Ingold, 1971). There is a minor difference between *P.anomala* and the other two species in that in *P.anomala* the sporangium and spores remain together and the sporangial wall 'roofs over' the spores when it becomes attached to a surface. In the other two species, the mucilage appears to be less viscous (although apparently no less efficient in holding the sporangium to the columella during dehiscence) and the spores are easily dispersed from the sporangium. This results in a droplet of spores being separated from the main mass at the time of impact. Fig. 14 shows this phenomenon for *P.moreaui* and a similar effect occurs in *P.caucasica*.

#### Discussion

The developmental Stages of *Pilaira* sporangiophores resemble those of *Phycomyces*, in the transition from I to IV. The absence of an anticlockwise Stage IVa is a notable feature of *Pilaira*. The papilla (Stage IIa) formed in the course of sporangial swelling is also seen in *Phycomyces*. *Pilaira* more closely resembles *Pilobolus* in the later phases of its development (IV and V). The blackened upper portion of the sporangium, dehiscence of the sporangium, and the mucilage surrounding the spores are similar in both genera, but the subsporangial bulb is absent in *Pilaira*.

The morphology of dispersal in *Pilaira* is unique, although an example of the ubiquitous mechanism, the 'stalked spore drop' (Ingold and Zoberi, 1963). It is very different from the forcible discharge mechanism of *Pilobolus*, but an interesting intermediate form between the two genera has been discovered in *Utharomyces* (Boedijn, 1958).

In this organism, a subsporangial vesicle like *Pilobolus* is formed, but when mature, the sporangium is merely set free by disintegration of the subsporangial vesicle.

It would seem that the lack of a firm mucilagenous attachment of the spores to the sporangium in *P.moreaui* and *P.caucasica* might mean that these two species are not so well adapted to the coprophilous habit as is *P.anomala*. This might account for the fact that they appear to be less common and widely distributed than the ubiquitous *P.anomala*. The isolation of *P.moreaui* from nature has only been recorded twice (Ling Young, 1930 and Hadlok, 1972, personal communication) and *P.caucasica* only once (Milko, 1970).

## EXPERIMENTAL SECTION V B :

## FORMATION OF COLUMELLA AND SPORES

Introduction

Mature sporangia (Stage IV) consist of a partially-cutinised wall (depending on species) surrounding a mass of aplanospores, separated from the vacuole of the elongated portion of the sporangiophore by a convex wall, the columella. Detailed light microscope studies have been made on the sporangial structure in other members of the Mucorales, by, for example, Swingle (1903) and Cutter (1942 a & b) and with the electron microscope by Bracker (1966).

A columella is by definition: 'a sterile central axis, within a mature fruit body' (Ainsworth, 1963). It is delimited by a boundary, consisting of both wall and plasmalemma, from the sporogenous zone of the sporangiophore. The lower portion of a sporangium, devoid of spores, but including the columella, is sometimes termed the apophysis. This is by definition: 'a swelling or swollen filament e.g. at the end of a sporangiophore below the sporangium in Mucorales' (Ainsworth, 1963).

The limited purpose of this study was to locate the internal structures of columella and spores in the sporangium in situ, without sectioning or staining, in order to relate these structures to the Stages of development already described.

Special Techniques

The technique finally adopted for the observation of the sporangiophores was the removal of a piece of the agar about 10 x 10 mm bearing mycelium and sporangiophores. It was then placed in a plastic petri dish and flooded with liquid paraffin, so that the sporangiophores were totally immersed. They could then be viewed by transmitted light and their internal structures seen through the transparent wall. If the high power objective was used, a coverslip was floated on to the agar. Inertness of the liquid paraffin was shown by the fact that the guttation of the sporangiophores continued for some hours.

## Observations

It was found that at Stage IIb there was no wall apparent within the sporangium, but the columella, as formed in Stage IIIa, appears to occupy the greater part of the volume that the sporangium filled at Stage IIb. It seems that the columella was differentiated in the protoplasm of the sporangium with a volume that must have occupied a substantial part of the sporangium at that stage - between Stages IIa and IIb. A feature of all three species was the distinct columella 'plasm' and the rough edge to its inner boundary. The increase in volume of the sporangium appears to be in the region between the columella wall and the outer wall of the sporangium (Table 15).

Connections between the new columella and the old walls are visible at Stage IIIa. Before the end of Stage IIIa, the sporangium is almost fully expanded and the different zones are fixed for the mature sporangiophore (IIIb and IV). From IIIa onwards, the columella becomes the distinctive 'hollow sphere' within the sporangium, so that the sporogenous zone is the upper peripheral portion. The lower portion (an apophysis) appears in the sporangium, presumably by the retraction of the spore mass within the sporangium causing the upper contents to move slightly away from the columella in IIIb or early in Stage IV, Fig. 16 (*P. caucasica*).

Spores are apparently differentiated by Stage IIIb to IV, but there is some evidence that a period of dormancy intervenes before germination can take place (Fletcher, 1971). No differences in the occurrence of the columella and sporulation have been observed in the three species (Fig. 16).

FIG 15

KEY TO TABLE 15 AND DETAILS OF SPORANGIUM AND COLIMELLA.

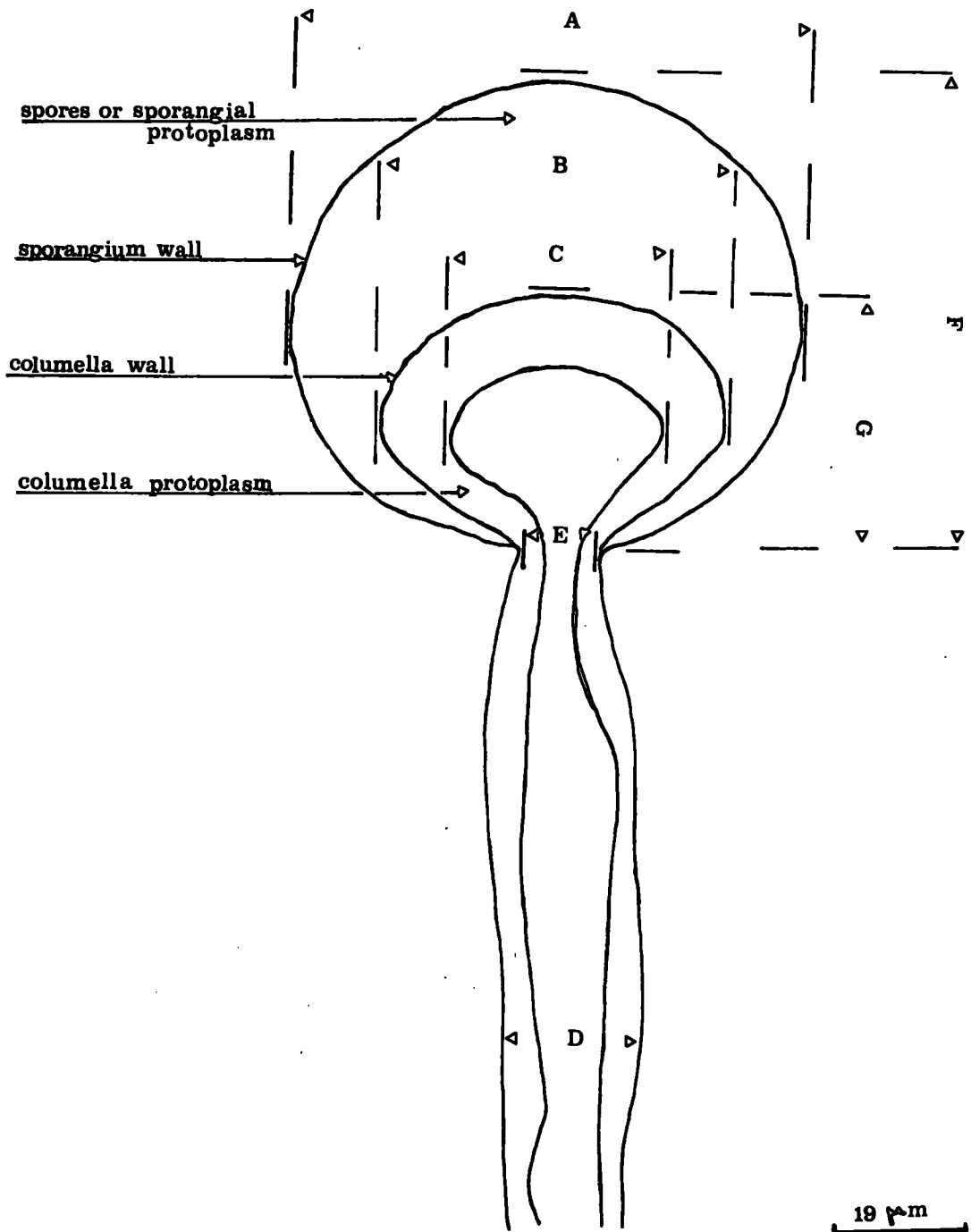


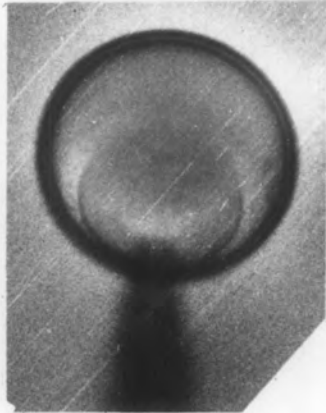
FIG 16

PHOTOMICROGRAPHS OF SPORANGIOPHORES OF *Pilaira species*,  
showing development of sporangium and columella.

P. anomala



IIb



IIIa



IIIb

P. caucasica



IIb

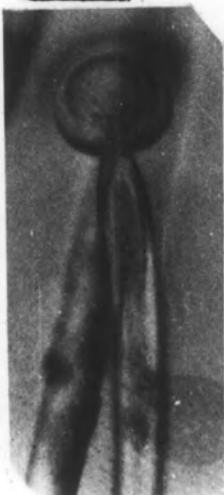


IIIa



IV

P. moreaui



IIb



IIIa



IIIb

X 250

## Discussion

Further clarification of the details of sporangium and columella development requires the use of thin sections and preferably electron microscopy.

It is evident that substantial transfer of material must take place to provide for the pronounced increase in volume of the sporangium between Stage IIa and IIIb.

TABLE 15

DIMENSIONS: A to G (Fig. 15) of sporangiophores from actual measurements from prints of known magnification to nearest 5  $\mu\text{m}$ .

(  $\mu\text{m}$  )

*P. anomala*

Stage IIb	A	B	C	D	E	F	G
<b>Sporangiophore</b>							
1	80	50	40	40	25	70	60
2	70	40	40	50	25	70	50
3	60	25	25	30	20	60	40
<b>Stage IIIa</b>							
<b>Sporangiophore</b>							
4	210	130	50	50	20	200	120
5	105	60	20	25	20	100	50
6	120	60	20	25	20	120	90
7	100	60	50	20	20	90	60
8	160	60	30	30	20	140	80

TABLE 15 (cont.)(  $\mu\text{m}$  )*P. caucasica*

Stage IIb	A	B	C	D	E	F	G
<b>Sporangiophore</b>							
1	60	-	50	20	10	60	-
2	61	50	30	30	15	60	40
3	100	-	60	30	20	100	-
4	60	50	40	30	20	60	50
5	70	60	35	25	15	65	60
<b>Stage IIIa</b>							
<b>Sporangiophore</b>							
6	160	100	80	40	20	140	120
7	80	60	40	20	10	80	40
8	80	60	40	25	10	80	40
<b>Stage IIIb</b>							
<b>Sporangiophore</b>							
9	125	75	50	30	15	110	60

TABLE 15 (cont.)

(  $\mu\text{m}$  )*P. moreauxi*

Stage IIb	A	B	C	D	E	F	G
<b>Sporangiophore</b>							
1	60	50	50	30	15	60	45
2	80	-	50	70	40	80	50
3	80	70	50	50	30	90	80
4	70	50	25	20	10	70	45
5	120	80	30	35	20	100	85
6	90	75	60	40	20	100	80
7	100	90	-	40	30	90	80
 <b>Stage IIIa</b>							
<b>Sporangiophore</b>							
8	95	60	40	-	15	90	60
9	130	70	40	30	20	120	100
10	150	120	50	50	25	140	80
11	100	70	40	30	20	100	60

## EXPERIMENTAL SECTION V C:

## THE DURATION OF THE STAGES OF SPORANGIOPHORE DEVELOPMENT

Introduction

The successive Stages of development of the sporangiophores of *Phycomyces* have been fully described by Castle (1942) based on the studies of Errera (1884). The stages of development of *Pilobolus* were described by McVickār (1942). A combination of the terminology used by both Castle (1942) and McVickar (1942) was used in the description and classification of the Stages of *Pilaira* laid out in Section IV C.

Special Techniques

The majority of these studies were made on cultures grown on S.M.A./5 inoculated with one 3 mm plug. They were inverted and exposed to alternating light and darkness (18/6 h) at 25°C and 150 lx in an illuminated incubator. They were then transferred to the stage of the photomicroscope, lid upwards. The lid was wiped free from condensation with tissue. Selected Stages were observed over prolonged time periods and photomicrographs taken, usually at approximately 20 minute intervals. At the same time other sporangiophores appeared on the negatives and their development could be followed on the enlarged prints. This particularly applied to the initiation of new Stage I sporangiophores. In this way the durations of up to four replicates of each Stage of the three species were obtained.

Observations

The full primary data, consisting of the times over which each particular sporangiophore was observed, for the three species at each Stage of development, are given in Table 16. The values for the estimated durations and their means, for the successive Stages in the maturation of individual sporangiophores, are given in Table 17. The total time taken for the sporangiophores of each species to reach maturity is also given.

These times are subject to the inaccuracy of the method of observation, which was based on photomicrographs taken at intervals, so that in the transient Stages an error of the order of 10-15 min is probable.

From Table 17 it can be seen that Stage I is the longest of the developing Stages. It varies in duration between the three species. It is terminated by a rapid change, when elongation ceases and a papilla (IIa) appears at the previously conical tip. This is a transient Stage in all three species, the papilla continuing to swell until it is larger than the hyphal diameter and is now IIb. This is also a transient Stage, while the sporangium enlarges (IIIa). Here the columella and spores are formed, described in Section V B. Finally the upper part of the wall cutinises, the sporangium reaches maximum diameter and enters the resting phase (IIIb). This Stage is the second longest in duration and again varies with species. The whole development process is longest in *P. caucasica*.

### Discussion

There is a marked specific difference in the duration of sporangiophore development in the three species, caused by considerably longer Stage I and Stage IIIb durations in *P. caucasica* and longer Stage I in *P. moreaui* than those in *P. anomala*. Although the development of the sporangiophores is very rapid between Stages I and IIIa, presumably a period of maturation is required (IIIb) before the spores are fully developed.

It has been observed (Fletcher, 1969 b) that a diurnal rhythm is shown by *P. anomala* with respect to sporangiophore development under natural conditions of alternating day and night. Further work is needed to determine what modification in time course occurs when the cycle of development described here for sporangiophores under continuous illumination is modified to a natural diurnal cycle.

TABLE 16

## DURATION OF STAGES OF SPORANGIOPHORE DEVELOPMENT

Where the first observation available was the time at which a Stage was present, that time was accepted as the beginning of the Stage. When a time at which a Stage had not been attained was known and it had been attained at the next observation, then a time mid-way between these two observations was taken as the commencement of the Stage in question. Similarly, when a Stage was known still to be present at one observation and to have been passed at the next observation, a time mid-way between these was taken as the end of the Stage in question.

Data relating to actual observations of sporangiophores attimes shown.

*P. anomala*

Sporangiophore Stage	Time of observation				Estimated Duration (minutes)	Observation Intervals (minutes)
	Previous Stage	Observations of Particular Stage		Next Stage		
		INITIAL	FINAL			
Stage I	-	16.10	20.55	-	> 285	} 0/25/50/210/240/ 260/285
	-	16.10	19.40	-	> 210	
	-	14.30	17.40	18.05	> 203	} 0/15/35/60/80/105/ 125/145/170/190/ 215/230
	14.45	15.05	18.20	-	> 205	
Stage IIa	17.40	18.05	18.05	18.20	20	} 0-230, as above " "
	14.30	14.45	14.45	15.05	17	
	14.30	14.45	14.45	15.05	17	
	15.50	16.15	16.15	16.35	22	
Stage IIb	14.45	15.05	15.05	15.30	23	"
	14.45	15.05	15.05	15.30	23	"
	16.15	16.35	16.35	16.55	20	"
	17.40	18.05	18.05	18.20	20	"
Stage IIIa	15.05	15.30	15.50	16.15	45	"
	15.05	15.30	15.50	16.15	45	"
	15.05	15.30	15.50	16.15	45	"
Stage IIIb	15.50	16.15	18.05	18.20	130	"
	15.50	16.15	18.05	18.20	130	"
	16.55	17.20	18.20	-	> 72	"
	15.50	16.15	18.20	-	> 137	"

EXHIBIT 17  
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TABLE 16 (cont.)

*P. caucasica*

Sporangiophore Stage	Previous Stage	Time of observation		Next Stage	Estimated Duration (minutes)	Observation Intervals (minutes)
		Observations of Particular Stage INITIAL	FINAL			
Stage I	-	10.30	18.45	18.54	>500	0/14/40/57/72/ 88/185/208/226/ 250/265/282/312/ 331/341/357/ 395/488 as above + 495/504/532
	10.44	11.10	16.25	17.05	348	
	11.58	13.35	18.54	19.22	381	
Stage IIa	18.45	18.54	19.25	19.30	38	0-532, as above
	13.42	14.01	14.01	14.23	19	0/1/2/22/44
	11.00	11.25	11.25	11.50	25	0/25/50/80
	10.05	10.40	10.40	11.10	30	0/40/75/105
Stage IIb	20.27	20.30	20.52	-	> 23	0/2/5/8/11/15/ 19/27
	11.25	11.50	11.50	12.20	27	8/13/38/68
	16.35	16.55	17.20	17.40	45	0/30/50/70/95/ 115/135/160/180
	15.30	15.50	15.50	16.15	23	
Stage IIIa	15.50	16.15	16.55	17.20	65	0/30/50/70/95/ 115/135/160/180
	-	14.40	15.10	15.30	> 40	
	11.50	12.20	12.20	13.50	60	0/25/50/80/170
	12.10	13.25	13.25	14.35	72	0/40/75/105/140/ 165/240/310/365
Stage IIIb	11.10	11.45	13.25	14.35	152	0/40/75/105/ 140/165/240
	10.40	11.10	13.25	14.35	155	
	14.01	14.23	16.03	16.19	120	0/1/20/42/74/88/ 105/130/136/142/ 158/167/178/184/186/ 191/196/206/210
	14.01	14.23	16.19	16.38	138	

TABLE 16 (cont.)

*P. moreaui*

## Time of Observation

Sporangiophore Stage	Previous Stage	Observations of Particular Stage		Next Stage	Estimated Duration (minutes)	Observation Intervals (minutes)
		INITIAL	FINAL			
<b>Stage I</b>						
	-	11.00	15.40	16.00	> 290	} 0/165/240/260/ 280/300
	-	11.00	15.20	15.40	> 270	
	-	12.00	16.00	16.25	> 253	} 0/105/180/205/ 240/265
	-	12.00	16.00	16.25	> 253	
<b>Stage IIa</b>						
	-	09.30	09.30	10.05	18	0/25
	-	09.30	09.30	10.05	18	0/25
	15.30	15.50	15.50	16.10	20	0/20/40
	15.30	15.50	15.50	16.10	20	0/20/40
<b>Stage IIb</b>						
	09.30	10.05	10.05	10.40	35	0/25/70
	09.30	10.05	10.05	10.40	35	0/25/70
	10.05	10.40	10.40	11.10	32	0/25/70
	14.40	15.30	15.30	15.50	35	0/50/70/90/125
<b>Stage IIIa</b>						
	10.05	10.40	10.40	11.10	32	} 0/25/70/100
	10.05	10.40	10.40	11.10	32	
	15.40	16.00	16.30	16.45	48	20/50/65/80
	16.30	17.00	17.00	17.30	30	0/30/60
<b>Stage IIIb</b>						
	10.40	11.10	12.10	13.25	113	} 0/30/65/90/165
	10.40	11.10	12.10	13.25	113	
	10.40	11.10	12.10	13.25	113	
	10.40	11.10	12.10	13.25	113	

TABLE 17

Estimated durations of intermediate Stages of sporangiophore development  
(minutes)

Stage	<i>P. anomala</i>					<i>P. caucasica</i>					<i>P. morreari</i>				
	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	
I	> 285	> 210	> 203	> 205	226	> 500	348	381	-	410	> 290	> 270	> 253	> 253	267
IIa	20	17	17	22	19	38	19	25	30	24	> 18	> 18	20	20	19
IIb	23	23	20	20	22	> 23	27	45	23	29	35	35	32	35	34
IIIa	45	45	45	-	45	65	40	60	72	59	32	32	48	30	35
IIIb	130	130	> 72	> 137	117	152	155	120	138	141	113	113	113	113	113
	Total: <u>429</u>					Total: <u>663</u>					Total: <u>468</u>				

## EXPERIMENTAL SECTION V D:

## GROWTH OF 'YOUNG' SPORANGIOPHORES

Introduction

The sporangiophores of *Phycomyces* (Bergman et al., 1969) and *Pilaira anomala* (Fletcher, 1969 b) elongate in two phases: firstly during Stage I, followed by a period of little or no elongation, and again later during Stage IV.

In both genera, the period of no elongation is associated with the development of the sporangium in Stage II-III, followed by a cessation in the expansion of the sporangium, when all growth ceases (Stage IIIb of *Pilaira*).

Special Techniques

The techniques employed were the same as those described in Section V C.

Growth Curves

Detailed studies of only *P.caucasica* and *P.moreaui* have been made here. *P.caucasica* shows the most abrupt cessation of growth in the four sporangiophores studied (Fig. 17 a-d). The rapid phase of growth of Stage I was terminated by the development of a sporangial primordium (Stage IIa). Further elongation was then temporarily arrested. The growth during part of Stage I of two sporangiophores of *P.moreaui* is shown in Fig. 18 (a and b); these measurements were taken during the development of a phototropic curvature of these sporangiophores. There was a slightly accelerating phase of growth during the observed period, even when the bending had reached a maximum.

In Fig. 19 which shows the whole growth period of a Stage I of *P.moreaui*, the cessation of elongation is more gradual than in *P.caucasica*, but by the end of the observed period, the sporangiophore has ceased elongation.

sporangiophore height above fixed marker ( $\mu\text{m}$ )



FIG 17  
GROWTH CURVES OF 'YOUNG' SPORANGIOPHORES OF *P. canaliculata*.

sporangiophore height above fixed marker ( $\mu\text{m}$ )

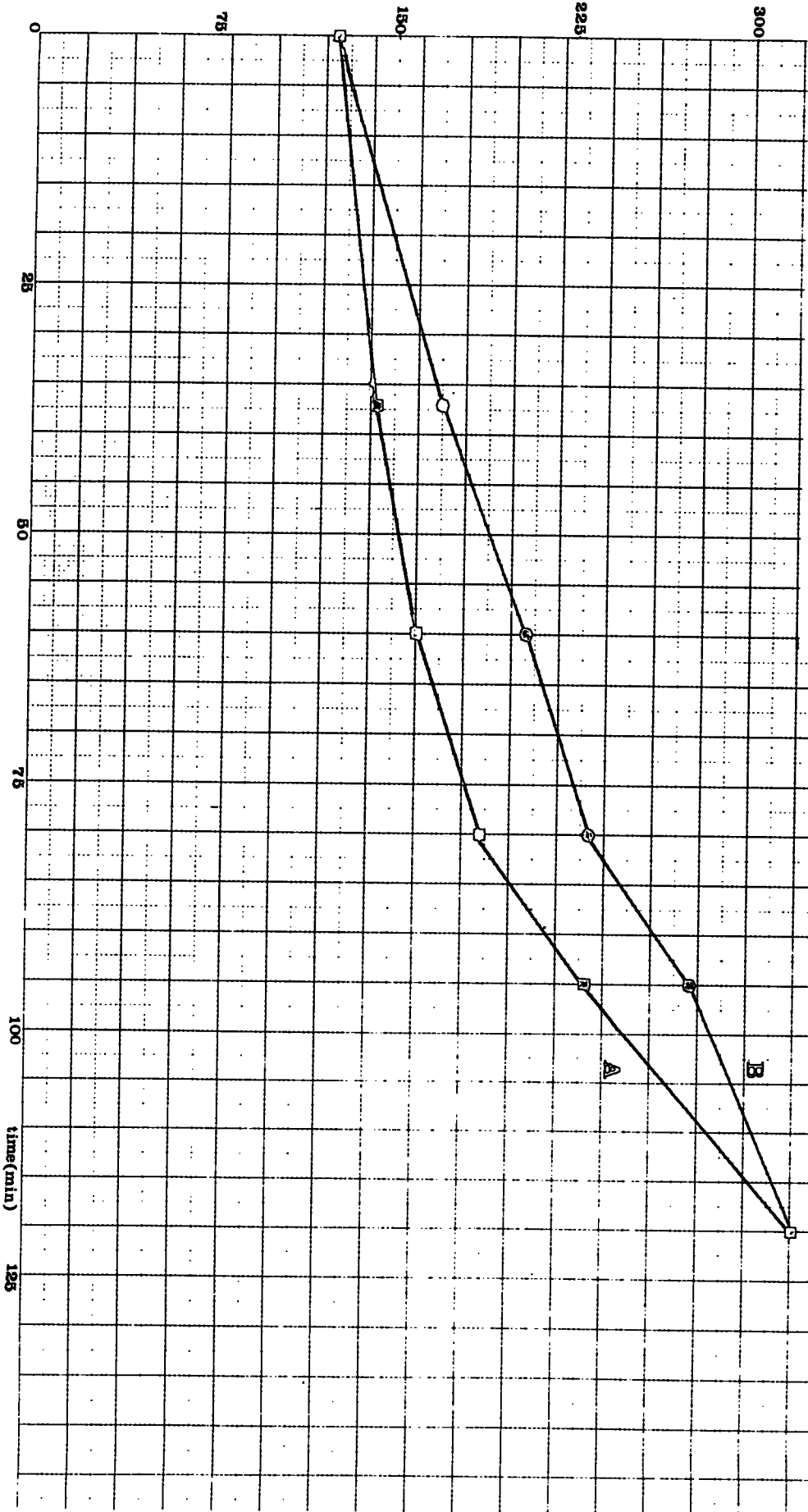


FIG 18  
GROWTH CURVES OF STAGE I SPORANGIOPHORES OF *P. MORREANA*.

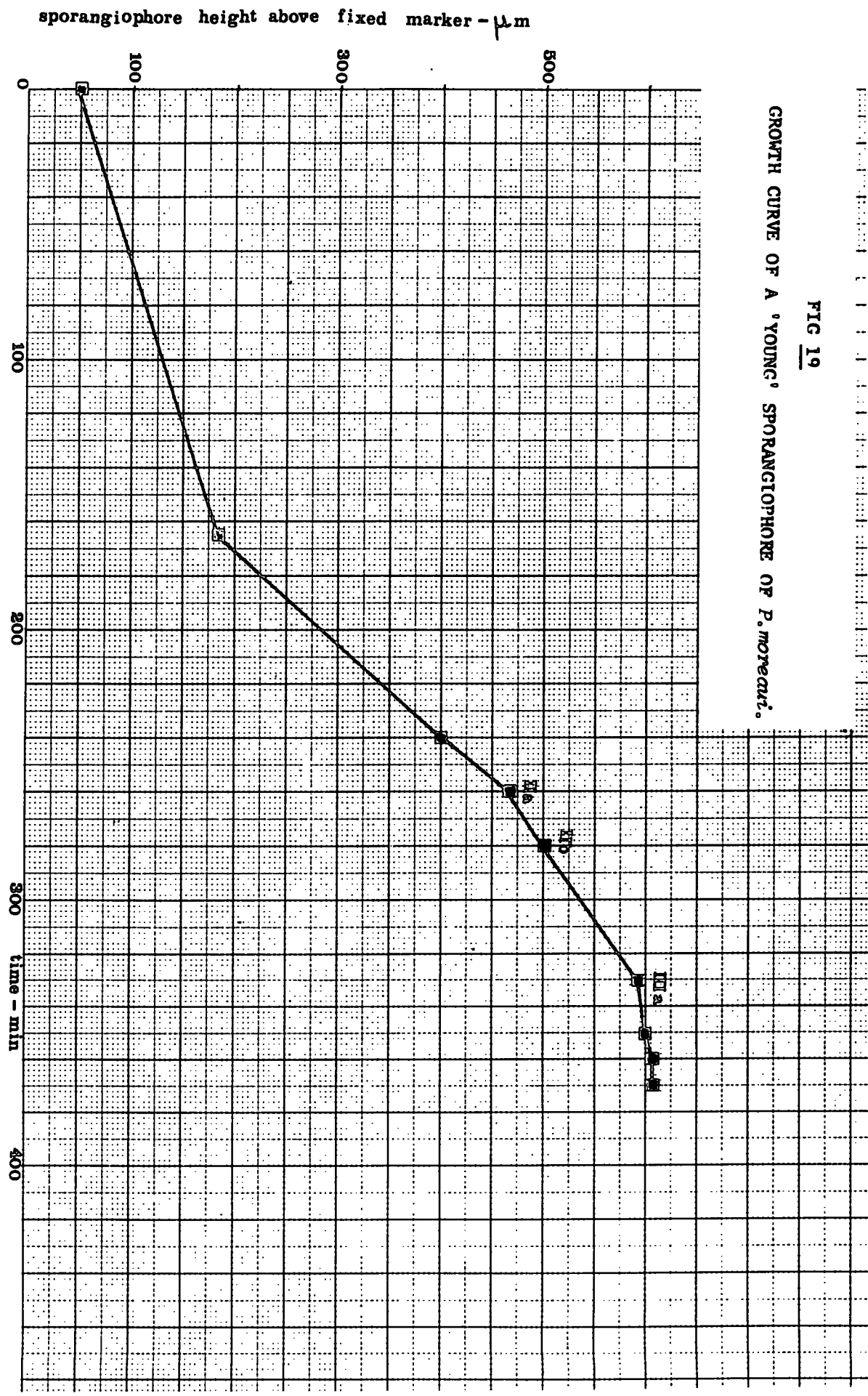


FIG 19  
 GROWTH CURVE OF A 'YOUNG' SPORANGIOPHORE OF *P. moreletii*.

In other sporangiophores, continuous slow elongation during Stages IIa-IIIa was observed under the conditions studied.

### Discussion

In most sporangiophores studied, extension growth ceased for a time at Stage IIIb. The continuing growth during Stages IIa-IIIa in *P.moreaui* is in contrast with the abrupt cessation at Stage IIa in *P.caucasica*. *P.caucasica* had a longer period of elongation in Stage I and this may be correlated with the prompt termination of growth when sporangium formation was initiated.

## EXPERIMENTAL SECTION VI:

## TWISTING OF THE STAGE IV SPORANGIOPHORE DURING GROWTH

Introduction

Twisting of Stage IVb sporangiophores of *Phycomyces* during elongation is a feature of their growth (Bergman et al, 1969). The phenomenon does not appear to have been reported for *Pilaira*, except for a very brief reference to its occurrence by Fletcher (1969 b).

Special Techniques

Similar procedures, with cultures on agar, were followed as for Phototropism (Section IX). Sporangiohores were illuminated by a beam incident on their apex in the direction of their long axis. Their behaviour was followed over periods from a half to over an hour, with photomicrographs taken at intervals.

Use was made of the presence of naturally occurring water droplets on the sporangium, as markers, and sporangiophores were selected on which this feature was present. The extent and nature of the rotation was followed from enlarged prints of up to X 100 magnification. In order to check the angles of rotation viewed horizontally, a model was made of the sporangium and droplets at the same magnification (Fig.20). The rates of growth were determined from the original prints of the sporangiophore.

Observations and Measurements

Only one sporangiophore from one species, *P. caucasica*, has been analysed in detail for twisting (Table 18). This sporangiophore was followed from Stage IIIb to Stage IV (Fig. 21). It can be seen that linear extension growth was hardly detectable, perhaps in part because it was growing obliquely upwards towards the camera. Although elongation was only just commencing, twisting was very marked.

FIG 20

MACROPHOTOGRAPHS OF MODEL OF *P. caucasica* SPORANGIOPHORE IN  
FIG 21, showing sporangium and marker droplet.

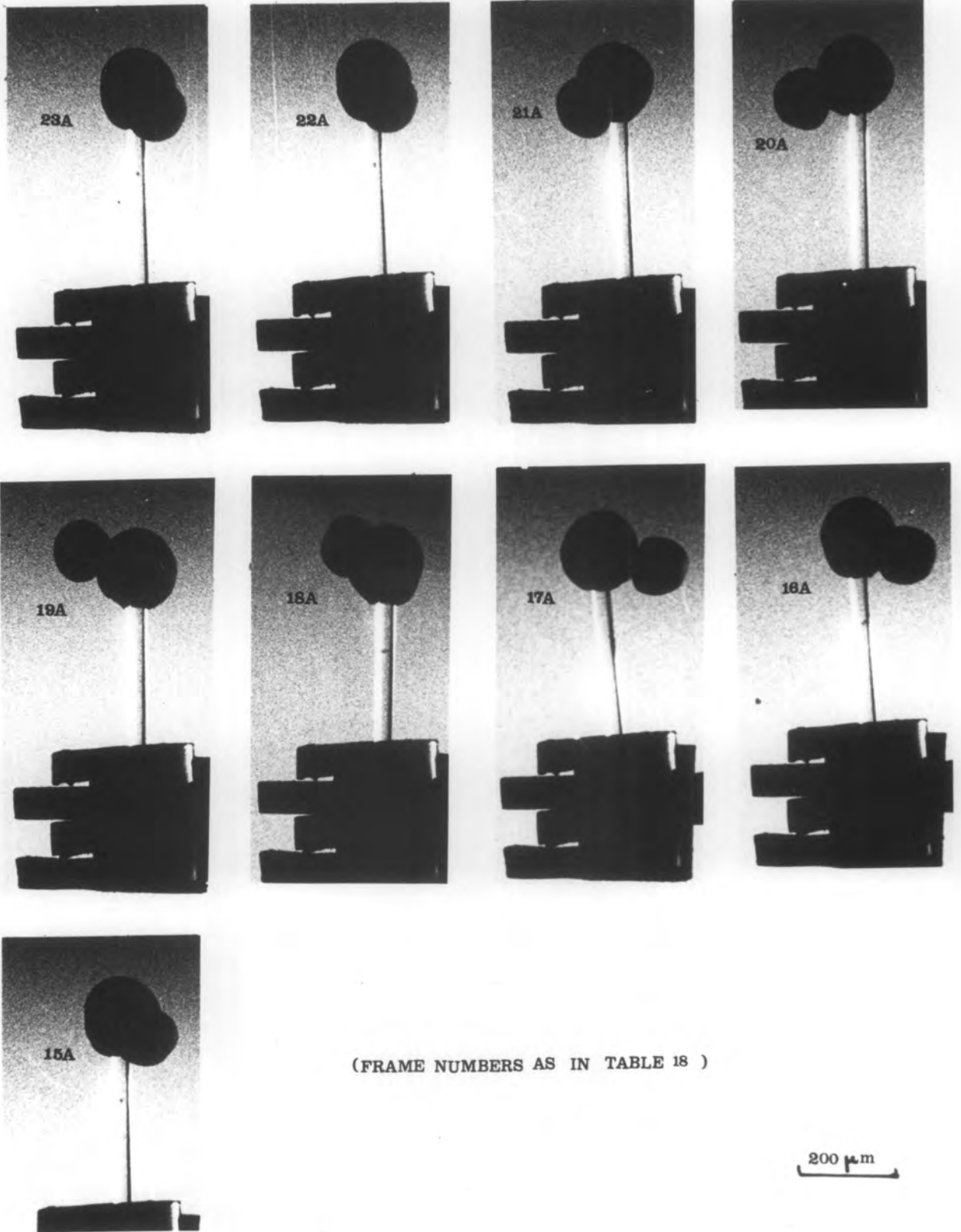
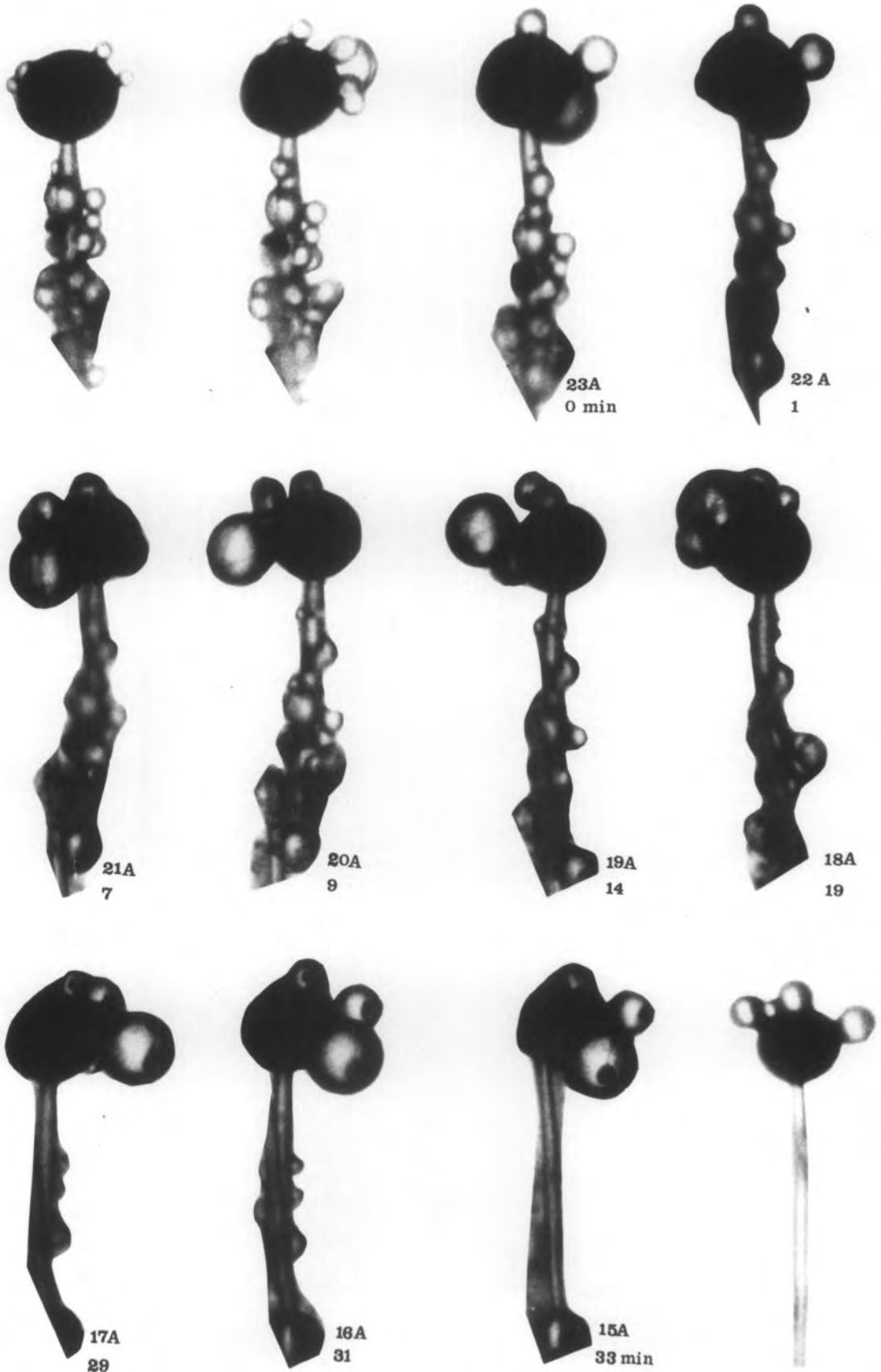


FIG 21

PHOTOMICROGRAPHS OF STAGE IV SPORANGIOPHORE OF *P. caucasica*,  
showing twisting of sporangium.



FRAME NUMBERS & TIMES (MIN) ARE SHOWN

X 100

Fig. 21 shows the sporangiophore of *P. caucasica* with its distinct water droplets, and their changes in position. Estimates of the rate of rotation were made from the other data presented in Table 18. If it is assumed that the water droplet was displaced between 14 and 19 min from the commencement of observation, and the apparent rate of rotation over that interval is ignored, then the rate of rotation shows a steady increase over the observed period. At the end of the observed period the rate of rotation of the marker corresponded with a complete revolution in about 17 minutes, and it was apparently still accelerating. Throughout these observations twisting occurred in a clockwise direction when viewed from above. This particular sporangiophore was observed from late Stage IIIb into Stage IV. This development is shown in Fig. 22. At no Stage was anticlockwise rotation observed.

Twisting in this way has also been observed in *P. anomala* (Fig. 23) and *P. moreaui* (Fig. 24). In the latter figure, there is the complication of a tropic component of growth.

FIG 22

PHOTOMICROGRAPHS SHOWING THE TRANSITION FROM STAGE IIIb to IV IN ANOTHER SPORANGIOPHORE OF *P. caucasica*.

(The elongation of Stage IV was associated entirely with clockwise twisting).



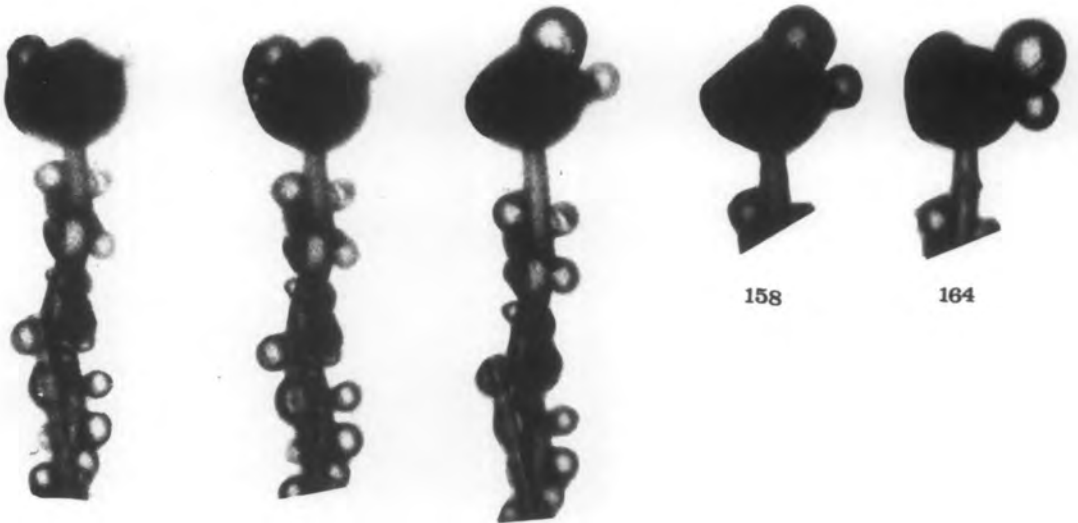
STAGE IIIb

68

95

110

116



STAGE IIIb

122

138

STAGE IV

157

clockwise  
twisting

158

164

X 100

(NUMBERS SHOW TIME IN MIN)

FIGS 23 & 24

PHOTOMICROGRAPHS OF SPORANGIOPHORES OF *P. anomala* AND  
*P. moreaui* TO SHOW TWISTING.

FIG 23 *P. anomala*

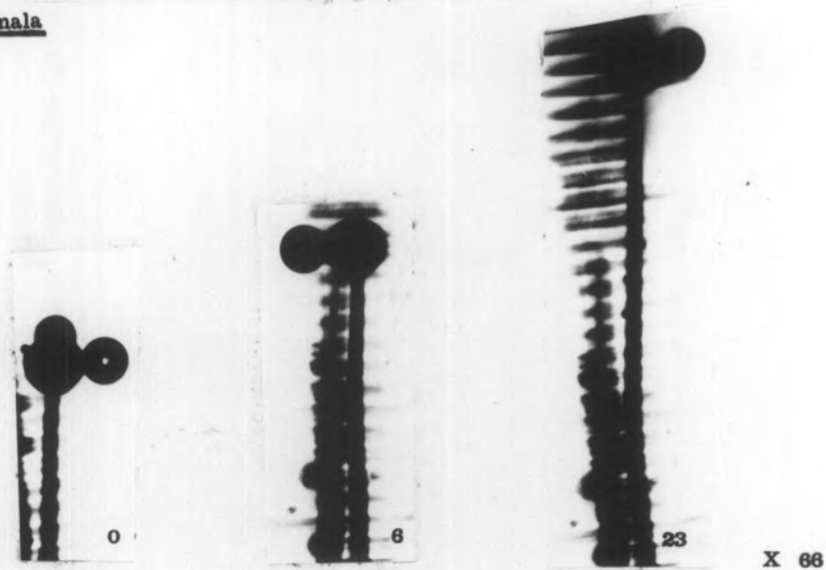


FIG 24 *P. moreaui*

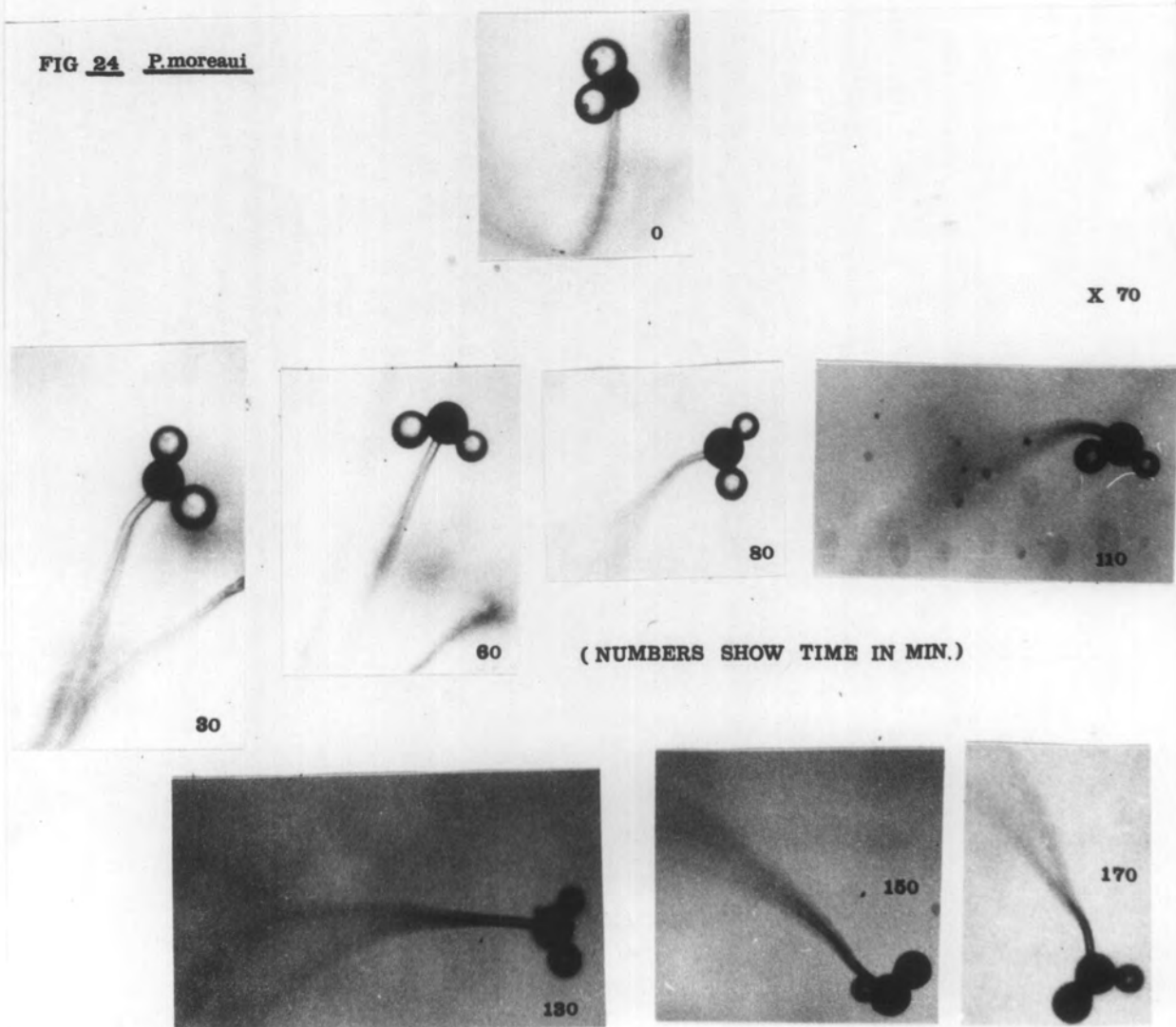


TABLE 18

## ROTATION OF THE SPORANGIUM OF A STAGE IV

SPORANGIOPHORE OF *P. caucasica*

Frame No.	Time (min)	Degrees of rotation from 0 min	Rate of rotation of marker during preceding <sub>1</sub> interval (degrees min <sup>-1</sup> )
23A	0	0	-
22A	1	5	5
21A	7	50	8
20A	9	70	10
19A	14	125	11
Suspected displacement of marker droplet during this interval			
18A	19	150	(5)
17A	29	285	13.5
16A	31	315	15
15A	33	360	22.5

( ) = Misleading Estimate

## Discussion

All three species show helical growth in Stage IV sporangiophores, made conspicuous by the rapid rotation of the sporangium itself. *Pilaira* appears to be similar to *Phycomyces* in this phenomenon, which is believed to be due to the intussusception of new wall materials at a constant angle. As in *Phycomyces*, this process has yet to be satisfactorily explained, but the phenomenon is probably related to the orientation of the chitin microfibrils of the primary wall. No information is yet available concerning the ultrastructure of *Pilaira* walls.

## EXPERIMENTAL SECTION VII :

## 'STOLON' PRODUCTION

Introduction

An anomalous feature of the asexual development of the three species of *Pilaira* is the formation of 'stolons' on some nutrient media. These appear to be arched sporangiophores which collapse on to the agar surface and may or may not produce abortive sporangia. Originally observed by chance, these structures can regularly be produced on deficient media.

Special Techniques

These structures have been observed both by photomicrography and by macrophotography.

Observations

In *P. anomala* 'stolons' were first observed on sub-optimal synthetic agar media (Fletcher, 1970 a) containing for example, glucose, salts and amino acids, but lacking natural sources of growth factors such as yeast extract or peptone. On such media, an early check in the radial expansion of the vegetative hyphae was usually observed, and if illuminated, mature sporangiophores were developed. Many of these were found to be abortive: they lacked spores and failed to exhibit negative geotropism or positive phototropism. These sporangiophores frequently collapsed beyond the vegetative margin of the colony and new vegetative growth took place from them (Fig. 25).

In some cases, the 'stolon' had a distinct aerial portion, in others it collapsed all along its length. The tip of the stolon was sometimes conical like a Stage I sporangiophore and terminated on the agar giving rise to simple vegetative hyphae. In other cases, the stolon terminated in a sporangium (Stage IV) which gave rise to complex branches on the agar. Fig. 26 shows an example of a sterile aerial 'stolon' terminating in simple vegetative hyphae, and Fig. 27 shows an abortive sporangium giving rise to dendroid branches which may have arisen from spores.

FIGS 25-27

'STOLON' PRODUCTION IN *P. anomala*.

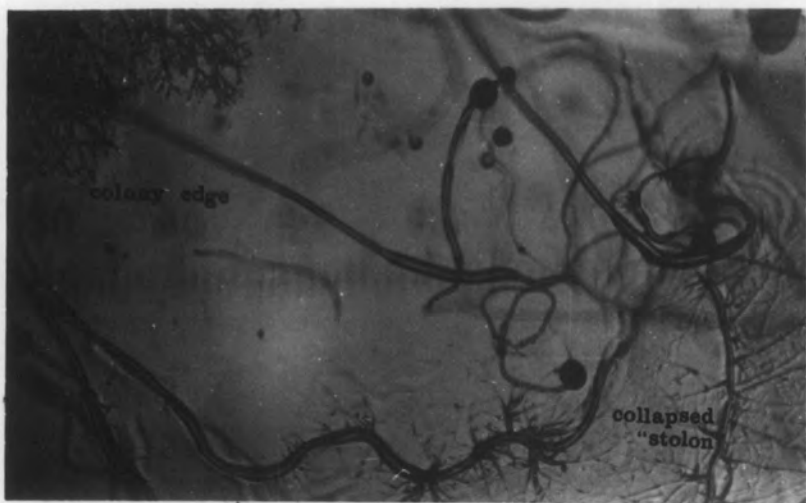
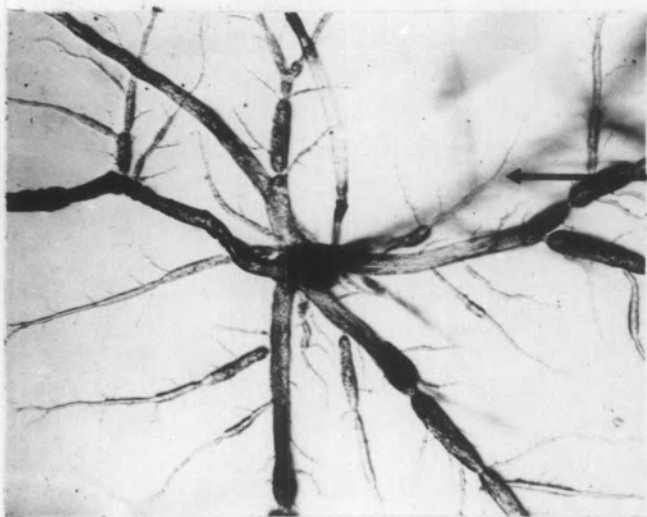


FIG 25

"STOLON" GIVING RISE TO VEGETATIVE LATERAL ON AGAR.

x 30



aerial  
"stolon"

FIG 26

"STOLON" TERMINATING AS VEGETATIVE HYPHAE.

x 120

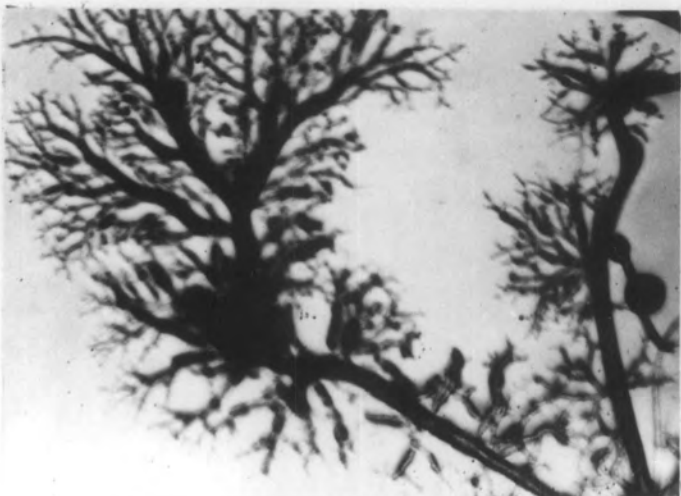


FIG 27

"STOLON" TERMINATING AS ABORTIVE SPORANGIA.

x 120

The proximal part of this 'stolon' is completely collapsed on the agar and is giving rise to lateral hyphae.

'Stolon' formation was induced in *P.moreaui* by growth on water agar and Fig. 28(a-d) shows various aspects of this:

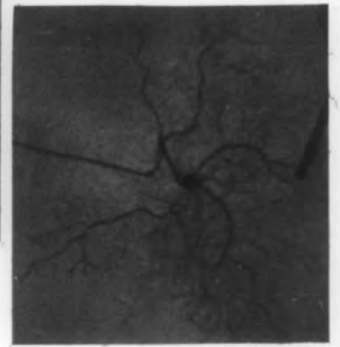
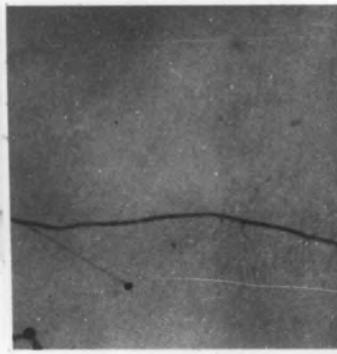
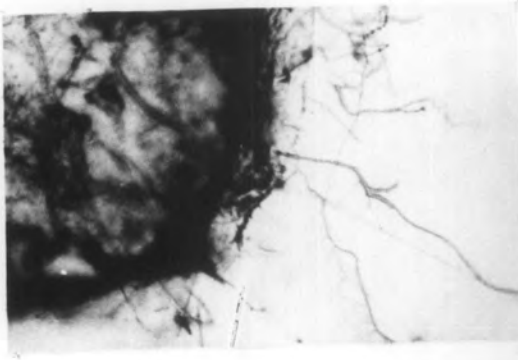
- (a) 'Stolon' emerging from plug.
- (b) 'Aerial' portion of 'stolon' with minor lateral branches on the agar.
- (c) Terminal portion of 'stolon' with abortive sporangium and terminal colony.
- (d) Spore formation has occurred in one sporangium.

It was found possible to obtain 'stolon' production in the three species by inoculation of water agar plates with plugs cut from healthy colonies on S.M.A./5. In one such experiment, a set of 2% malt agar plates were also inoculated for comparison. The latter medium was sub-optimal for *P.anomala* but the other two species grew better on it (Table 19). It was not however an ideal medium for any of the three species, since the margin was not evenly developed to form a smoothly circular colony, as on S.M.A. On 2% malt agar, 'fans' of hyphae tended to be developed, breaking up the margin in all three species. Sporangiphore development appeared normal on the 2% malt agar with the exception of a few 'stolons' formed near the margins of the colony, presumably a symptom of the break-up of the margin. However, the sporangiophores which gave rise to these 'stolons' were much more robust than those on water agar media (Fig.29).

On water agar, in *P.caucasica* and *P.moreaui*, normal vegetative mycelium was not produced. The 'colony' was made up entirely of secondary mycelium developed from arched 'stolons' derived from the inoculum plug. There were also some depauperate Stage IV sporangiophores. This extreme situation was not found in *P.anomala* (C.B.S. 695-60) on water agar, where a sparse but genuine primary mycelium was developed. It was however, almost sterile and the few sporangiophores that were formed did not exceed the vegetative margin of the colony and form secondary colonies.

FIG 28 (a-d)

'STOLON' FORMATION IN *P. moreaui* ON WATER AGAR

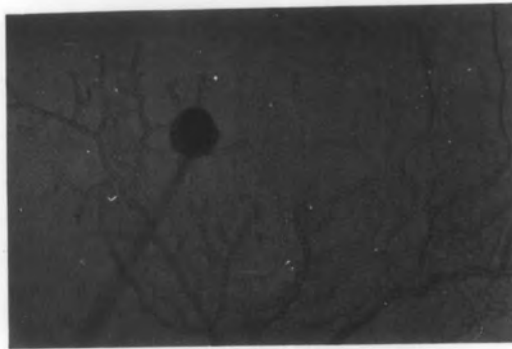


x 40

(a) 'stolon' emerging  
from plug

(b) 'aerial' portion  
with laterals

(c) terminal portion



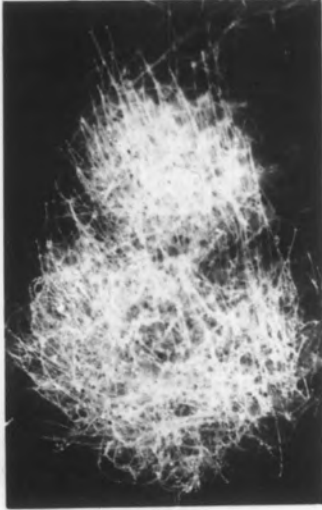
x 60

(d) terminal portion of another 'stolon'  
and one 'normal' sporangium

FIG 29

'STOLON' PRODUCTION BY *Pilaira species* ON 2% MALT AGAR  
AND WATER AGAR.

2% MALT AGAR



Panomala

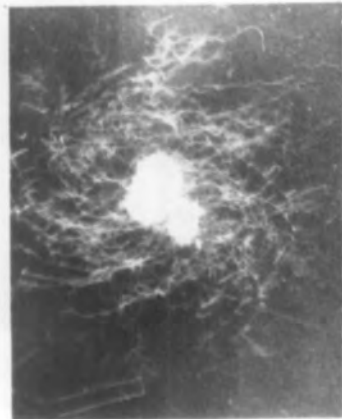
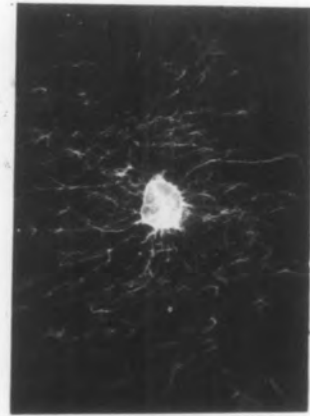
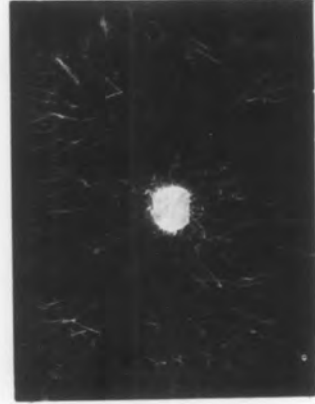


P. caucasica



P. moreaui

WATER AGAR



x 1.5

Thus the 'stolon' is the response to poor nutrition and shows regenerative properties of the aerial sporangiophores at various stages of their development.

TABLE 19

Rates of radial growth of colonies on 2% malt agar for *Pilaira* spp.  
(mm h<sup>-1</sup>)

Time after inoculation	<u><i>P. anomala</i></u>	<u><i>P. caucasica</i></u>	<u><i>P. moreaui</i></u>
(h)			
0-96	0.16	0.38	0.42
96-118	0.15	0.32	0.42

### Discussion

This phenomenon might have a function under natural conditions of allowing a poorly developed colony, on an exhausted fragment of substratum and unable to produce healthy sporangiophores, to give rise instead to 'stolons' which might vegetatively prolong the life of the colony.

## EXPERIMENTAL SECTION VIII :

## THE RATE OF GROWTH OF STAGE IV SPORANGIOPHORES

Introduction

Linear growth rates in *Phycomyces* have been extensively reported, but no quantitative study has been undertaken for the genus *Pilaira*.

Special Techniques

Measurements and observations have been made on enlarged prints of sporangiophores photographed for other studies, such as Sections V and IX.

Growth Curves

The growth of Stage IV sporangiophores commences in the same way as many similar structures, with an exponential phase giving rise to a steady linear phase of rapid elongation growth. It is uncertain whether dispersal of the sporangium occurs during this linear phase or during a subsequent decline in growth.

The growth of Stage IV commences when a Stage IIIb has completed its period of cessation of both elongation and sporangial expansion. Initially, elongation was very slow: a rate of less than  $1 \mu\text{m min}^{-1}$  was followed by a rapid increase in rate, to a steady maximum of about  $40 \mu\text{m min}^{-1}$  (Fig. 30). This seems the normal rate for *P.morecavi* and probably also for *P.caucasica*.

Fig. 31 shows the linear phase in *P.anomala* of about  $38 \mu\text{m min}^{-1}$ . This rate continues for many hours until the sporangiophore collapses.

The linear rates of growth for *P.anomala* are generally higher than for the other two species. Rates of over  $200 \mu\text{m min}^{-1}$  have been recorded, but approximately  $50 \mu\text{m min}^{-1}$  seems normal.

When sporangiophores are elongating rapidly they become attenuated, possibly thinner walled and are very liable to collapse.

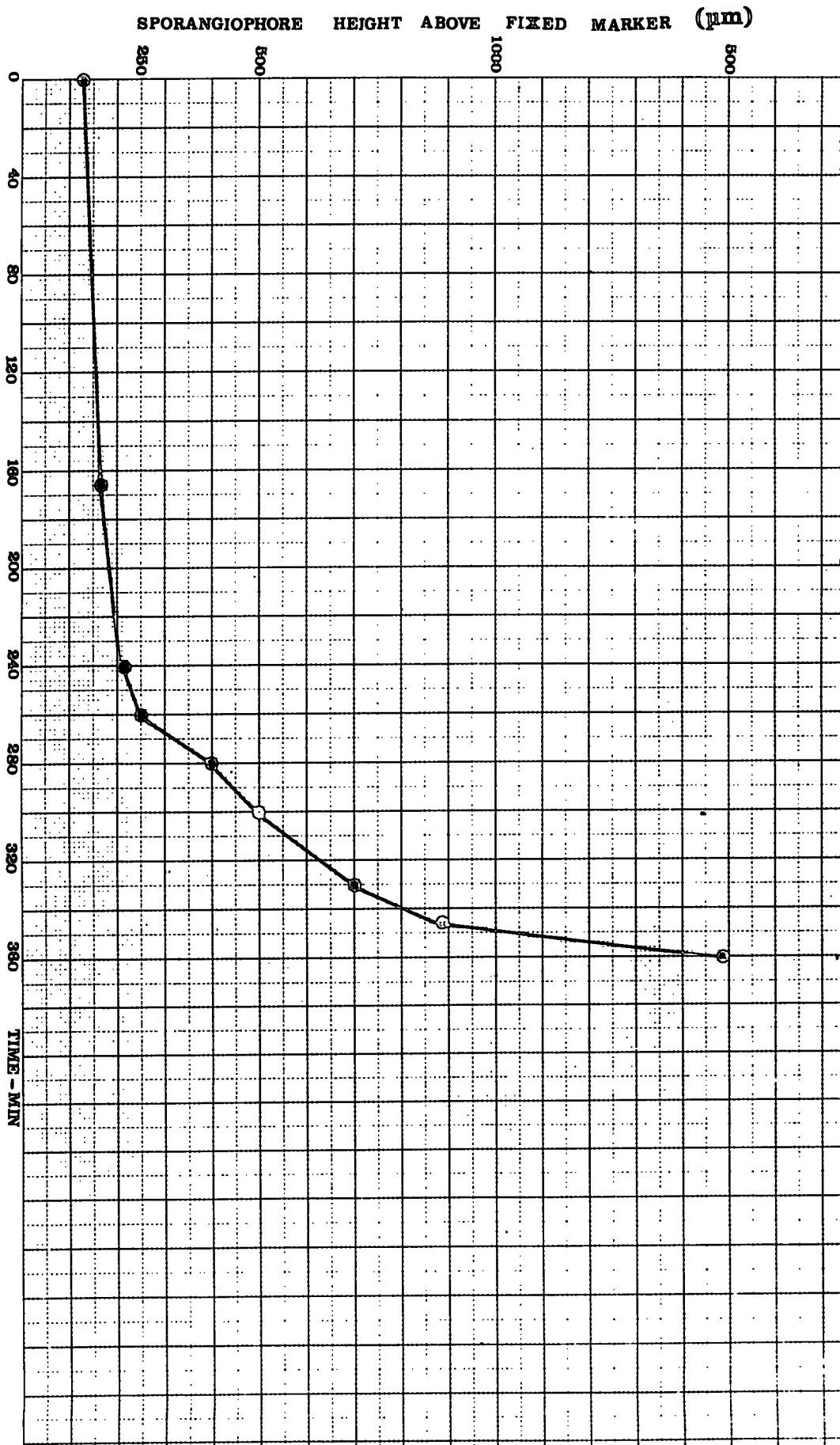
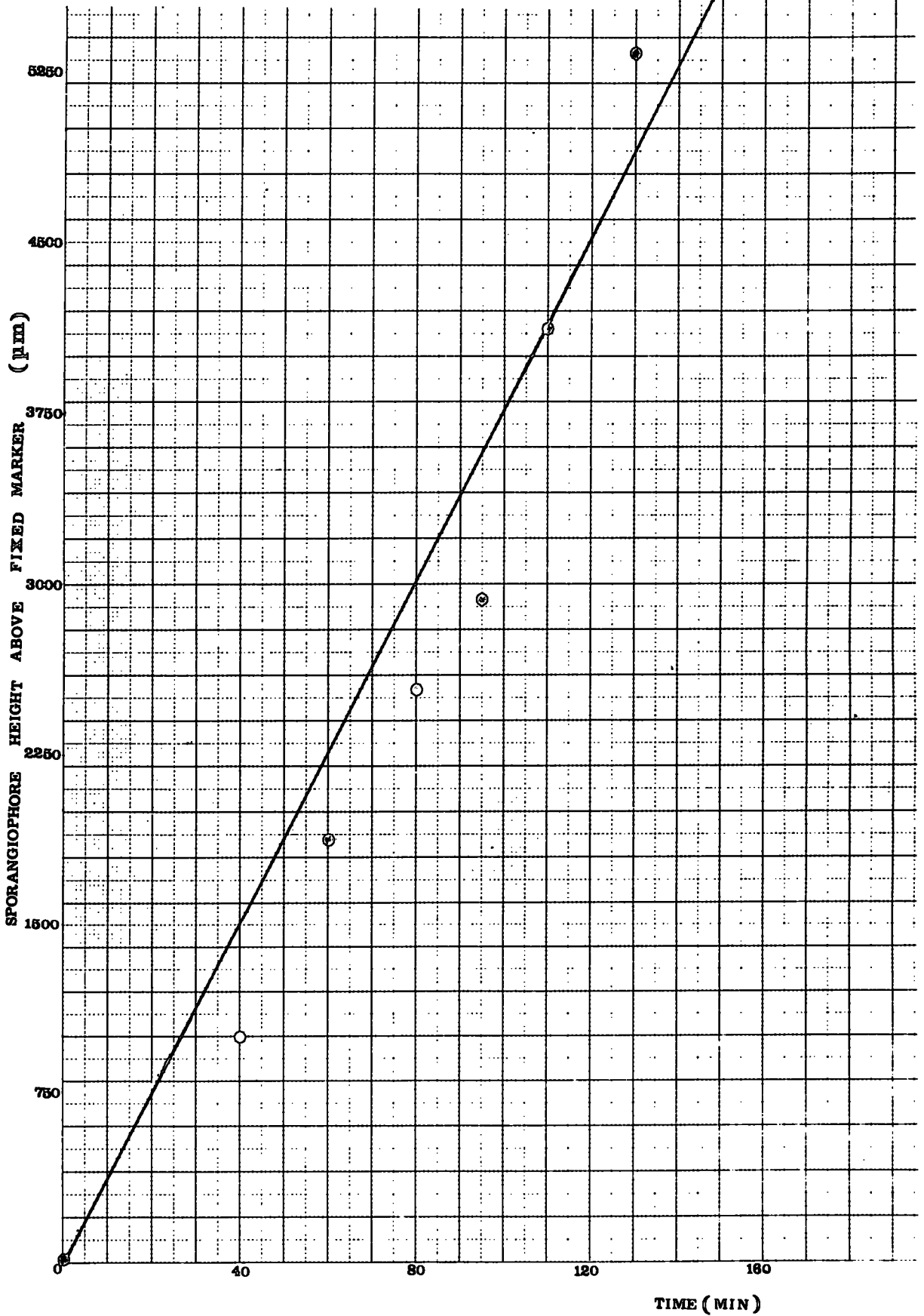


FIG 30  
GROWTH CURVE OF 'EARLY' STAGE IV SPORANGIOPHORE OF *P. notogauri*.

6000

FIG 31

GROWTH CURVE OF 'LATER' STAGE IV SPORANGIOPHORE OF *P. anomala*.



### Discussion

The sporangiophores of *Pilaira* species exhibit remarkably high rates of growth considering their small size. While not achieving the duration of extension growth of the sporangiophores of *Phycomyces*, their rapid elongation over a short period is quite outstanding. This is obviously of ecological advantage to the organism, since this elongation of the sporangiophores is the only probable means of dispersal of the spores from the mycelium developed on the dung surface.

## EXPERIMENTAL SECTION IX :

## PHOTOTROPISM

Introduction

There have been many accounts of the extensive investigations of phototropic behaviour in *Phycomyces* and to a much lesser extent in other fungi, including *Pilobolus* spp. These include Banbury (1959); Ingold (1962); Shropshire (1963); Carlile (1965); Page (1965); Castle (1966); Page (1968); Bergman et al. (1969); Webster (1970) and Carlile (1971). A detailed study of the positive phototropism of the sporangiophore of *Phycomyces blakesleeanus* has been made by Castle (1962). An important aspect of the phototropic behaviour, mentioned by most reviewers, is the light growth response, together with the study of the reversal of phototropism by immersion in liquid paraffin.

Experimental Techniques

For the study of phototropism, plates were examined through their lids in a horizontal position on the microscope stage. A suitable area of the mycelium was selected in which there were sporangiophores of either Stage I or Stage IV, as required.

A Watson 6v rheostat-controlled microscope lamp provided the unilateral light source at 150 mm from the plate. This was focussed on the sporangiophore being observed giving a flux of approximately 50 lx at the sporangiophore. The phototropic curvature was recorded by photomicrography during the whole or part of the response to unilateral light. An objective of X 3 and an eyepiece of X 10 were generally used, which gave an overall magnification of X 15 on the 35 x 24 mm negative, due to the optics of the camera attachment.

In some cases, enlarged prints of about X 100 - 200 of the actual sporangiophore were made and direct tracings obtained, or positive contact transparencies, made directly from the negative, were projected on to paper and then traced. The latter gave magnifications up to X 350.

For each particular experiment, the dish was orientated so that the selected sporangiophore was initially growing horizontally in a direction perpendicular to the unilateral beam of light. The subsequent positive or negative phototropic curvature of the sporangiophore occurred in an approximately horizontal plane and could be measured by observation through the microscope from above. Using Castle's terminology (1962), the bending of the sporangiophores was analysed by determining the slope angle ( $\Phi$ ) along the sporangiophore at distances ( $S_y$ ) upwards from a fixed point on the sporangiophore, below the growth zone. In some cases this fixed point was a guttation droplet that was present throughout the period of observation; in other cases, a well-defined morphological feature near the sporangiophore on the surface of the substrate served the same purpose.

The slope angle ( $\Phi$ ) was plotted against  $S_y$ , in order to locate the bending zone. Also the plots of sporangiophore length against time, and slope angle at the tip against time, were prepared. Up to four sporangiophores of Stage I and Stage IV of each species were analysed.

For the investigation of the reversal of the lens effect, plates were selected which bore suitable sporangiophores and the cultures were then covered with a layer of liquid paraffin. The subsequent development of a negative phototropic curvature could then be followed either by unilateral illumination in the incubator or by direct observations of a particular sporangiophore with the dish positioned on the stage of the microscope.

#### Experimental Observations

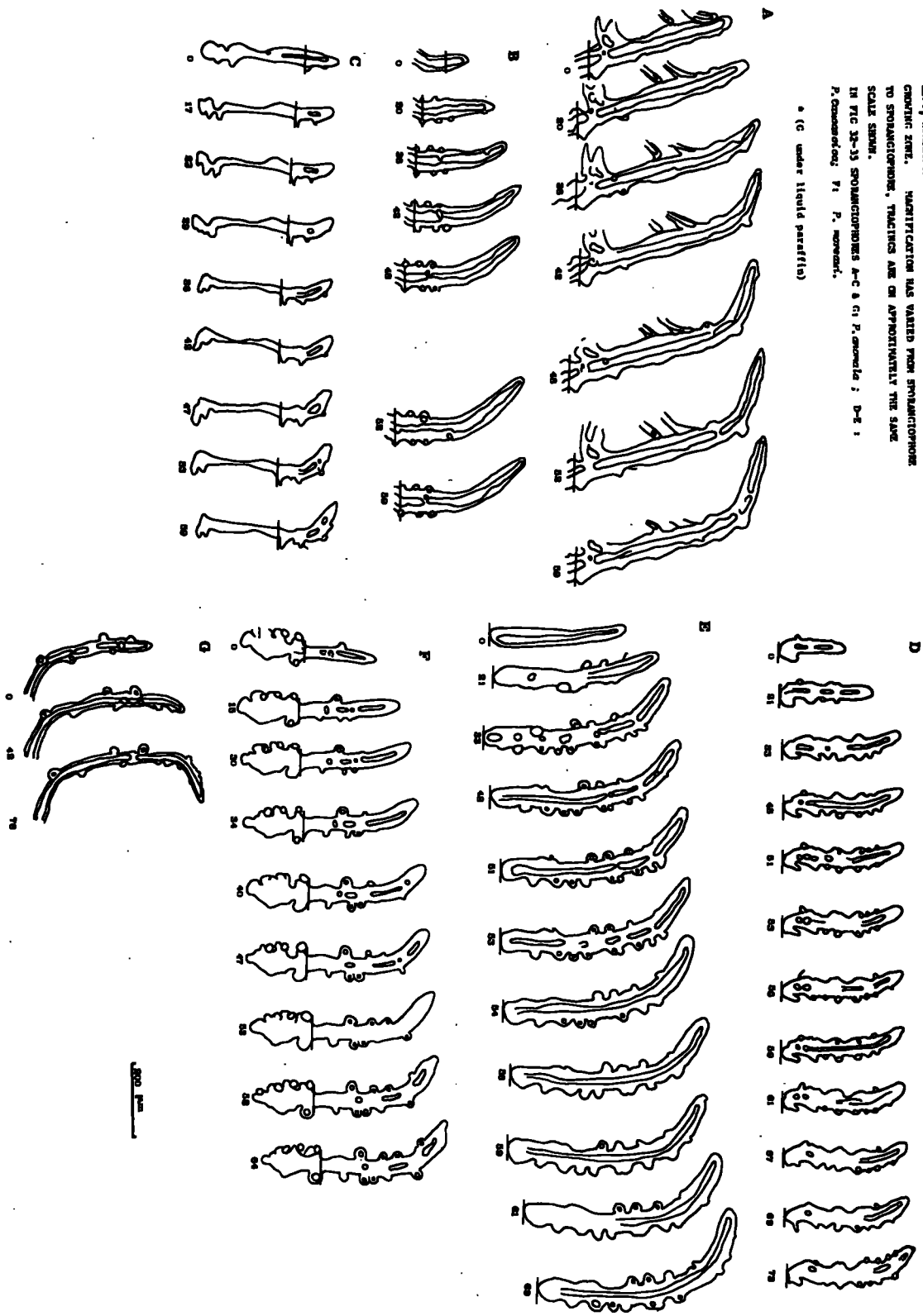
##### POSITIVE PHOTOTROPISM OF STAGE I SPORANGIOPHORES IN AIR: *P. anomala*

The data relating to location of bending in phototropic sporangiophores are given in Tables 20 and 21 (A-C). The tracings of the photographic record of the three bending sporangiophores are shown in Fig. 32 (A-C).

TRACTING FROM PHOTOGRAPHS OF STAGE 1 SPONGIOPHORES OF *Plectra* species UNDERGOING PHOTOTROPIC BENDING IN ALIVE AT THE INTERVALS SHOWN (MIN). INCIDENT LIGHT HORIZONTAL FROM LEFT; HORIZONTAL BAR SHOWS LOCATION OF FIXED MARKER BELOW GRAPHIC ZONE. VARIATION HAS VARIED FROM SPONGIOPHORE TO SPONGIOPHORE. TRACTING ARE ON APPROXIMATELY THE SAME SCALE SHOWN.

IN FIG 23-25 SPONGIOPHORES A-C & G1 *P. gonocela*; D-F: *P. gonocela*; G2 *P. mowbrayi*.

\* (C under ileoid paraffin)



The slope angle in relation to height above a fixed point, below the bending zone of each sporangiophore, at time intervals during the observation period, is shown in Fig. 33 (A-C). Table 22 (A-C) summarises information relating to phototropic bending from Tables 20 & 21.

The elongation of the three sporangiophores under observation, in relation to time during the bending period, is shown in Fig. 34 (A-C). There is a correlation between the length of the bending zone (Table 21) and the maximum and average rate of elongation, but no correlation between the length of the bending zone and the rate of bending (Tables 21 and 22). These measurements exhibit a wide range in the three sporangiophores.

It can be seen from Fig. 33 (A-C) and Table 21 that bending initially occurred just below the tip of the sporangiophore. As bending continued, the bend migrated down the sporangiophore, while the upper part appeared to cease bending, although prior to this there was some upward migration of the bending zone (Table 21). The sharpness of the migration as shown in Table 21 and Fig. 33 appeared to be abrupt, due to the measurements only being made at widely spaced intervals along the sporangiophore. This downward migration demonstrates in *Pilaira* the observation of Castle (1962) on Stage IV of *Phycomyces*. The bending zone lengthened as bending proceeded, usually reaching a maximum at maximum bending.

Fig. 35 (A-C) shows that the bending of the sporangiophore in relation to time exhibits an S-shaped curve. The rate in most cases rises sharply, reaches a maximum and declines during the period of observation, as the axis of the tip of the sporangiophore approaches the direction of the beam.

#### *P. caucasica*

The data relating to this species are shown in Tables 20-22 (D & E) and Figs. 32-35 (D & E). In this species, neither sporangiophore showed the downward migration of the bend.

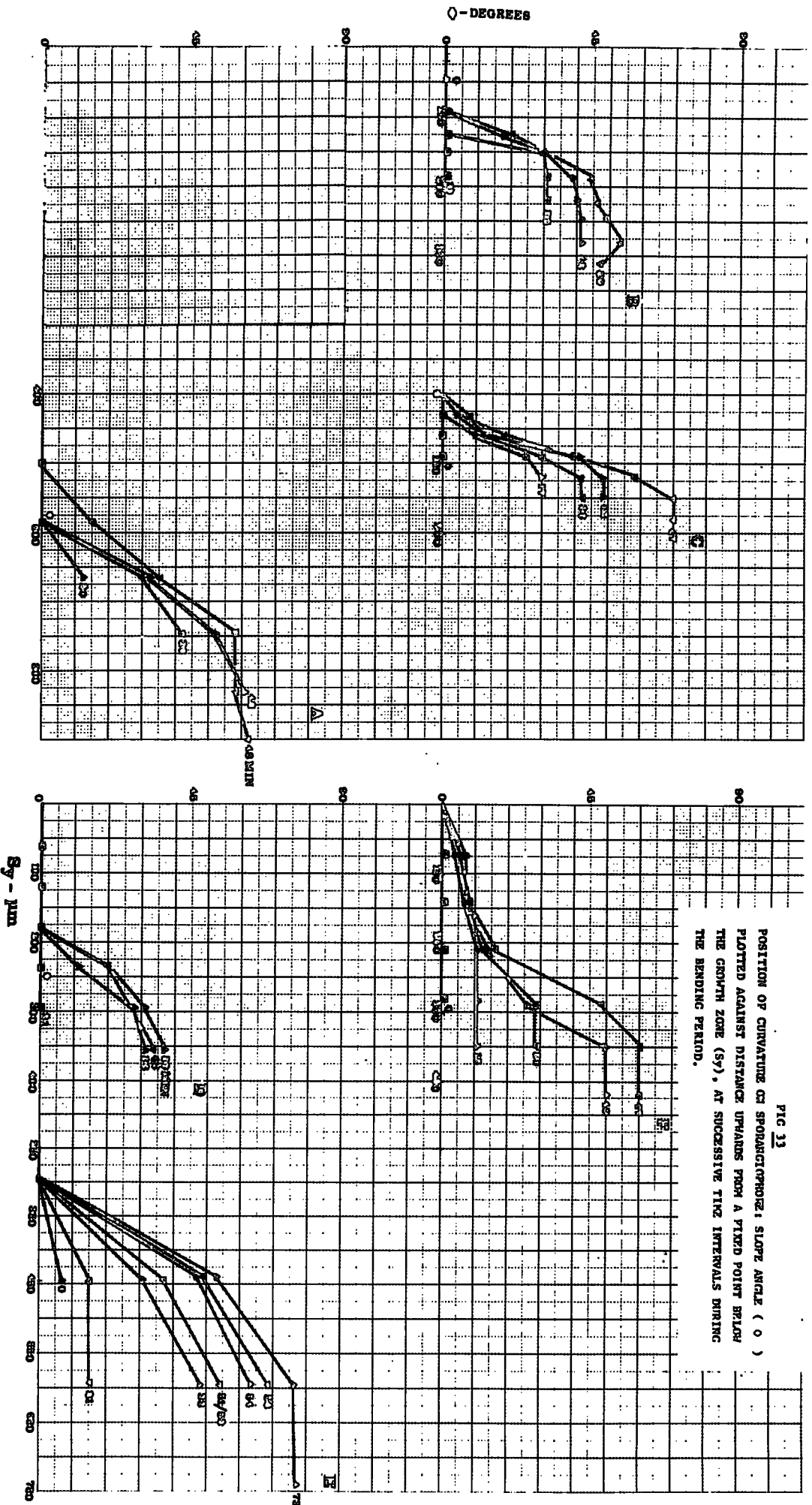


FIG 34  
GROWTH CURVES OF STAGE I SPORANGIOPHORES UNDERGOING PHOTOTROPISM.

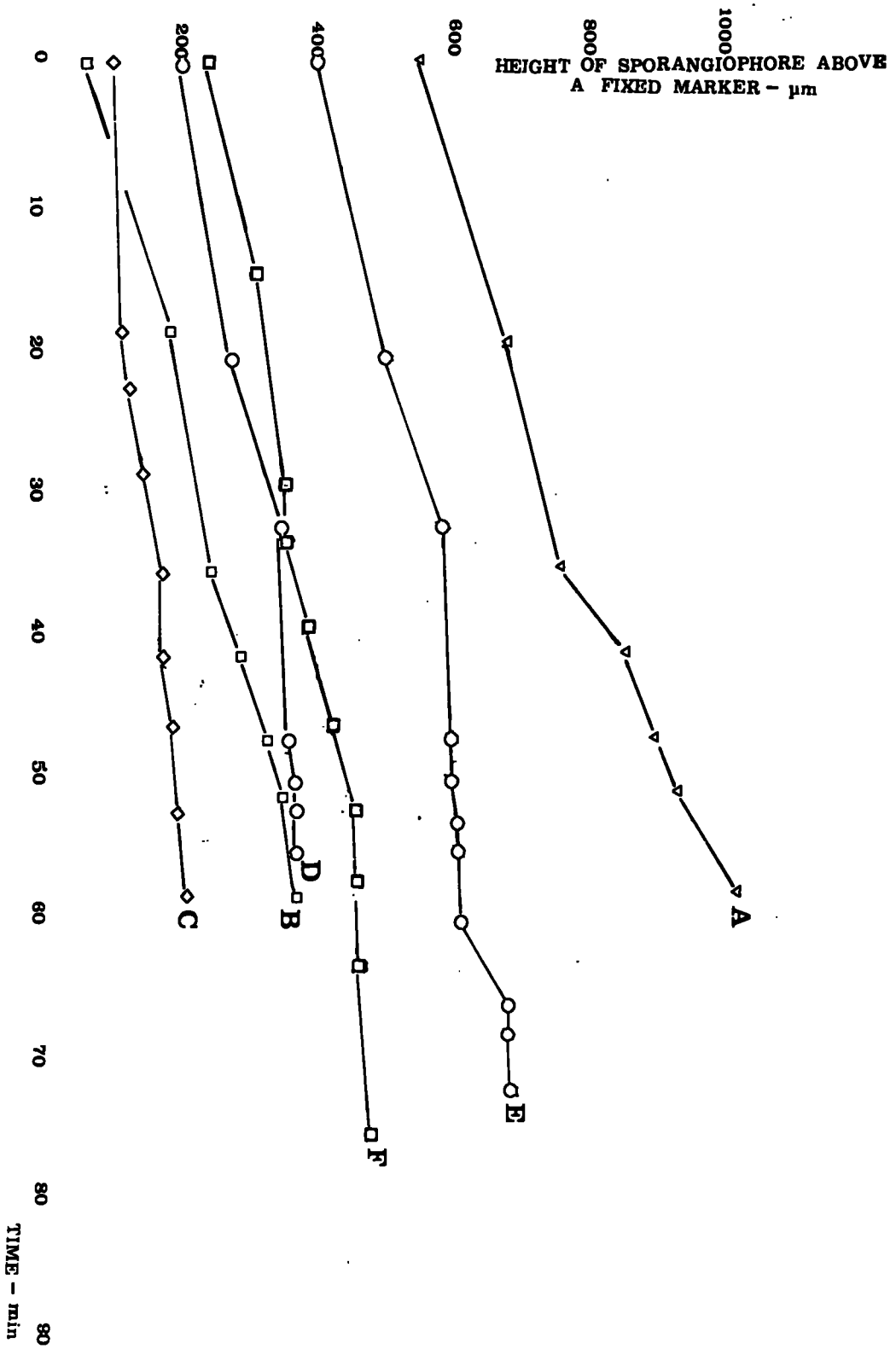
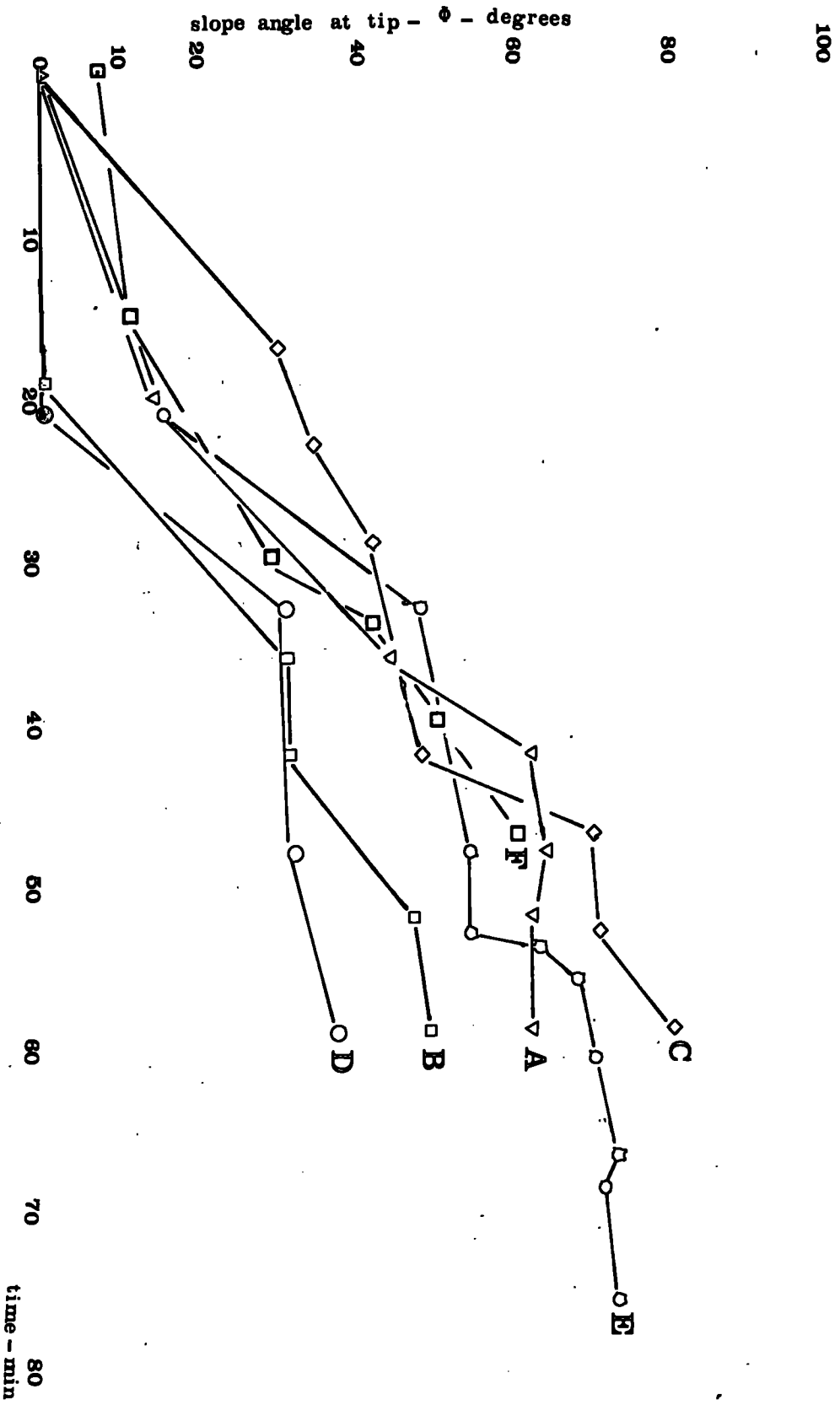


FIG 35  
BENDING OF STAGE I SPORANGIOPHORES IN RELATION TO TIME.



This can be seen in both Table 21 and Fig. 33. In the larger sporangiophore, the tightness of the bending is particularly marked, being confined to the same region throughout the observed period. In these sporangiophores there is also a correlation between the rate of bending and the length of the bending zone: the faster growing sporangiophore had a longer bending zone. The bending in relation to time also follows a similar pattern to that found in *P. anomala*.

*P. moreaui*

The data relating to the bending of the sporangiophore are given in Tables 20-22 (F) and Figs. 32-35 (F). The pattern of bending is more like *P. caucasica* than *P. anomala*, in that the bending zone shows no signs of downward migration and upward migration was confined to the first half of the observed period (Table 21 F). The other aspects of elongation and bending are similar to the other two species.

TABLE 20

LOCATION OF PHOTOTROPIC BENDING OF STAGE I SPORANGIOPHORES OF *P. anomala*  
 AT SUCCESSIVE TIME INTERVALS: SLOPE ANGLE ( $\phi$ ) ALONG SPORANGIOPHORE  
 AT DISTANCE UPWARDS FROM A FIXED MARKER (Sy).

## SPORANGIOPHORE A

Height above  
 fixed marker \*

( $\mu\text{m}$ )	500	582	664	746	828	910
Time (min)	Angle in degrees:					
0	0	0				
20	0	0	12			
36	0	0	30	42		
42	0	0	32	52	61	
48	0	15	35	58	58	62
52	0	20	34	58	61	61
59	0	20	31	45	56	61

## SPORANGIOPHORE B

Height above  
 fixed marker \*

( $\mu\text{m}$ )	93	124	155	186	217	248	279	310	341
Time (min)	Angle in degrees:								
0	0	0	0						
20	0	0	0	0					
36	0	0	30	30	37				
42	0	18	30	39	40	41	41		
48	0	16	23	28	38	42	42	42	
52	0	20	29	32	37	45	47	47	47
59	0	16	30	44	46	49	53	47	47

TABLE 20 (continued)

## SPORANGIOPHORE C

Height above  
fixed marker \*(  $\mu\text{m}$  )

30 60 90 120 150 180

Time  
(min)

Angle in degrees:

0	0	0	0			
17	0	10	25	30		
23	0	12	24	34		
29	0	11	24	42	42	
36	8	12	30	42	42	
42	4	12	41	48	48	
47	4	18	39	58	70	70
53	5	10	38	59	71	71
59	10	10	33	53	73	80

## SPORANGIOPHORE D

Height above  
fixed marker \*(  $\mu\text{m}$  )

148 296 444 592 740

Time  
(min)

Angle in degrees:

0	0	0	7		
21	0	0	15	15	
33	0	0	31	48	
48	0	0	31	54	
51	0	0	37	54	
53	0	0	37	54	
54	0	0	47	63	
56	0	0	48	68	
59	0	0	46	64	
61	0	0	50	70	
67	0	0	53	73	73
69	0	0	53	71	71
73	0	0	53	76	76

TABLE 20 (continued)

## SPORANGIOPHORE E

Height above  
fixed marker \*

( $\mu\text{m}$ )	59	118	177	236	295	354
Time (min)	Angle in degrees:					
0	0	0	0	0		
21	0	0	0	0	0	
33	0	0	0	11	28	31
48	0	0	0	20	28	32
59	0	0	0	20	31	37

## SPORANGIOPHORE F

Height above  
fixed marker \*

( $\mu\text{m}$ )	70	140	210	280	350	420
Time (min)	Angle in degrees:					
0	0	0	0	0		
15	3.5	7	11	11	11	
30	5	8	12	29	29	
34	8	8	12	28	42	
40	7	7	13	26	50	50
47	5	8	15	49	60	60
53	5	6	13	47	54	54

\* The points at which angles of deviation of sporangiophore from direction at the base were measured, represent points at regular fixed intervals above a fixed base point. The magnitude of the intervals is appropriate to the scale employed.

TABLE 21

## LOCATION AND LENGTH OF BENDING ZONE

Time (min)	* Lower Limit ( $\mu\text{m}$ )	o Upper Limit ( $\mu\text{m}$ )	Length of bending zone ( $\mu\text{m}$ )	Rate of growth of sporangiophore ( $\mu\text{m}\cdot\text{min}^{-1}$ )	†Rate of bending of sporangiophore (degrees $\text{min}^{-1}$ )
SPORANGIOPHORE A					
0	0	0	0	-	-
20	623	623	0	6.3	0.6
36	623	705	82	4.7	1.9
42	623	787	164	16.7	3.7
48	541	869	328	6.7	0
52	541	869	328	8.7	0
59	541	869	328	11.8	0
SPORANGIOPHORE B					
0	0	0	0	-	-
20	0	0	0	6.5	0
36	139	201	62	3.2	1.8
42	108	232	124	6.7	1.8
48	108	232	124	5.7	0.5
52	108	263	155	7.0	0.5
59	108	294	186	3.3	0.5
SPORANGIOPHORE C					
0	0	0	0	-	-
1.7	45	105	60	0.9	1.5
23	45	105	60	1.5	1.5
29	45	105	60	3.0	1.5
36	15	105	90	4.0	0
42	15	105	90	0	1.8
47	15	135	120	2.4	2.4
53	15	135	120	1.5	0
59	15	165	150	2.0	1.5
SPORANGIOPHORE D					
0	0	0	0	-	-
21	0	0	0	3.3	0
33	207	325	118	6.2	2.6
48	207	325	118	0.4	0.7
59	207	325	118	0	4.5

TABLE 21 (continued)

Time (min)	* Lower ( $\mu\text{m}$ )	o Upper ( $\mu\text{m}$ )	Length ( $\mu\text{m}$ )	Rate of growth ( $\mu\text{m min}^{-1}$ )	†Rate of Bending (degrees $\text{min}^{-1}$ )
SPORANGIOPHORE E					
0	370	370	0	-	-
21	370	518	148	4.5	0.4
33	370	518	148	6.8	2.7
48	370	518	148	0.5	0.3
51	370	518	148	2.7	0.3
53	370	518	148	0	0.3
54	370	518	148	7.0	4.7
56	370	518	148	0	4.7
59	370	518	148	0	0.5
61	370	518	148	0	0.5
67	370	518	148	11.0	0.5
73	370	518	148	0	0.5
SPORANGIOPHORE F					
0	0	0	0	-	-
15	35	175	140	4.7	1.0
30	35	245	210	2.3	1.0
34	35	315	280	0	3.5
40	35	315	280	5.6	3.5
47	35	315	280	5.0	3.5
53	35	315	280	5.8	0
58	35	315	280	0	0

\* Point midway between lowest point at which bending was apparent and point immediately below, at which bending was not apparent.

o Point midway between highest point at which bending was apparent and point immediately above, at which bending was not apparent.

† Increase in the angle between direction of axis at the tip and direction of axis at the base, in relation to unit time.

TABLE 22

DATA RELATING TO GROWTH AND BENDING OF STAGE I SPORANGIOPHORE IN AIR

Sporangiophore	Average rate of growth ( $\mu\text{m min}^{-1}$ )	Average rate of bending * (degrees $\text{min}^{-1}$ )	† Reaction time (min)	Total period of observation (min)
<i>Pilaira anomala</i>				
A	7.8	1.3	20	59
B	5.1	0.8	36	59
C	1.8	1.4	17	59
Mean	4.9	1.1	24	59
<i>P. caucasica</i>				
D	2.8	0.7	33	73
E	3.0	0.7	21	73
Mean	2.9	0.7	27	73
<i>P. moreuxi</i>				
F	2.9	1.3	15	76

\* over the whole period to attainment of maximum curvature

† to the first detectable curvature

## POSITIVE PHOTOTROPISM OF STAGE IV SPORANGIOPHORE IN AIR

The reaction time is shorter at this Stage in all three species. The rate of bending is often faster; this is consistent with a higher rate of elongation.

*P. anomala*

The data relating to these sporangiophores are shown in Tables 23-25 (A-D) and Figs. 36-39 (A-D), similar to these for Stage I sporangiophores.

Downward migration of the bending is marked in these sporangiophores, but the bending zone is further away from the base of the sporangium (equivalent to tip of the sporangium in Stage I). There is no correlation between the length of the bending zone and the rate of growth of the sporangiophore, nor the latter and the rate of bending. Sporangiophore B appears to show some recovery of its bending after 7 min, which is seen in the change in the distribution of the slope angle (Fig. 37) but the possibility exists that this sporangiophore had dropped towards the agar, twisting out of the plane of measurement and producing an apparent reduction in curvature (Table 23 B).

*P. caucasica*

The data relating to these sporangiophores are shown in Tables 23-25 (E-F) and Figs. 36-39 (E-F). Sporangiophore E is the upper part of a long, very rapidly growing cell. It shows the location of the origin of the bend, but was followed over too short a period of time to observe migration of the bend. This was shown more markedly in sporangiophore F, followed over a longer period. Although the rate of growth of E was nine times faster than that of F and its rate of bending is approximately five times greater than F, the sporangiophore E had a shorter bending zone than F. There is therefore no correlation between the parameters for growth and bending in these sporangiophores.

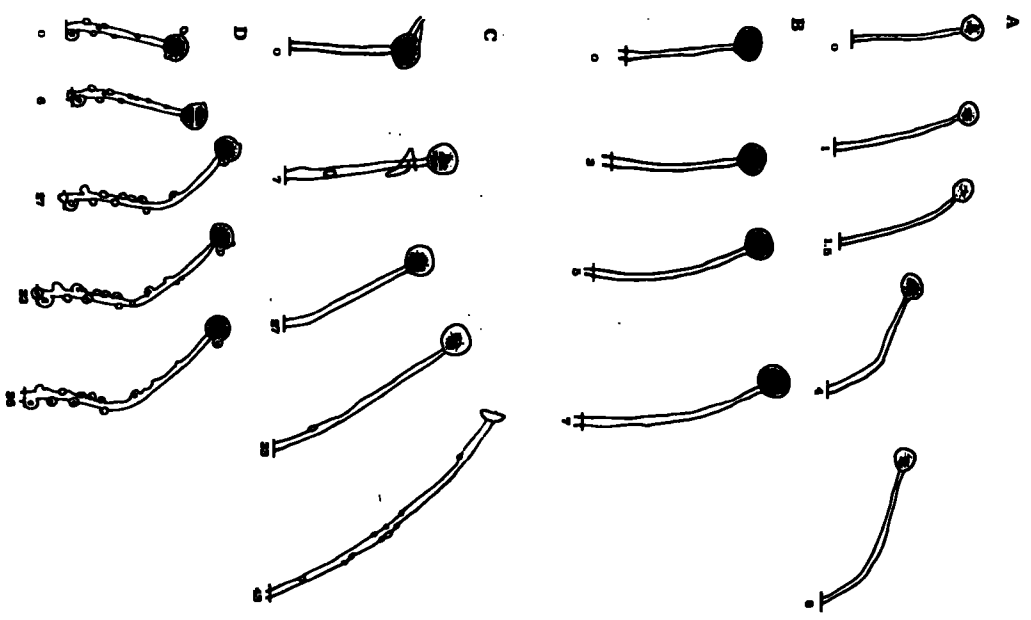
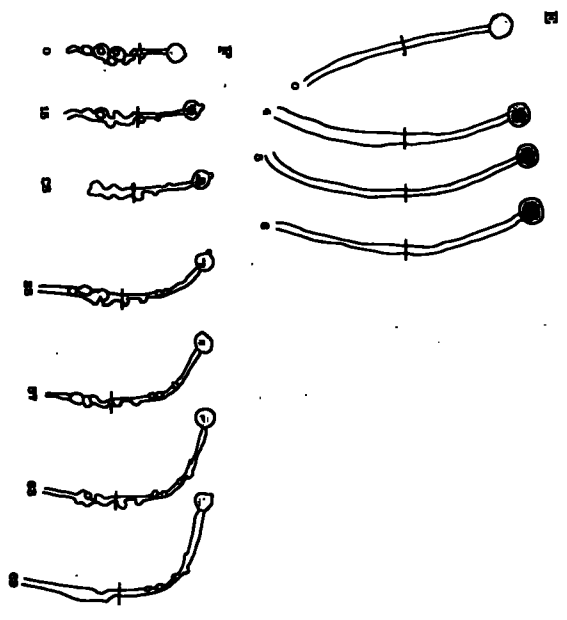
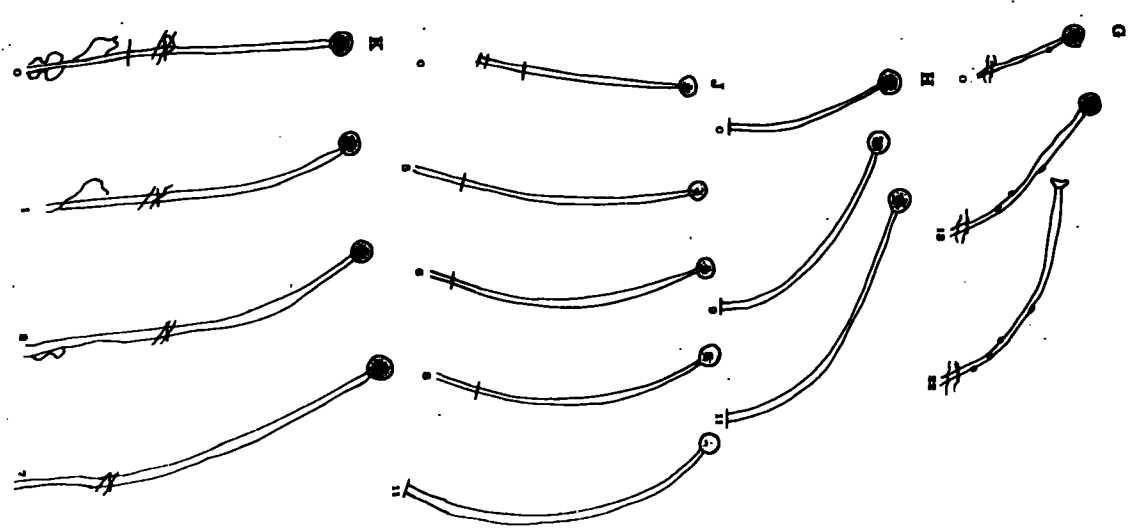


FIG. 26  
 TRACTINGS FROM PHOTOGRAPHIC STAGE 14 SPORANGIOPHORES OF *P. leucophaea* UNDER VARIOUS PROTOZOIC BEHAVIOR IN AIR AT THE INTERVALS SHOWN (HR). INCIDENT LIGHT HORIZONTAL FROM LEFT; HORIZONTAL BAR SHOWS LOCATION OF FIXED MARKER BELOW GRAPHIC LINE. MAGNIFICATION HAS VARIED FROM SPORANGIOPHORE TO SPORANGIOPHORE, TRACTINGS ARE ON APPROXIMATELY THE SAME SCALE SHOWN.  
 IN PICS 36-39 SPORANGIOPHORES A-D1 *P. grandis* 1 B-F1 *P. grandis* 2  
 G-K 1 *P. leucophaea*



400  $\mu$ m



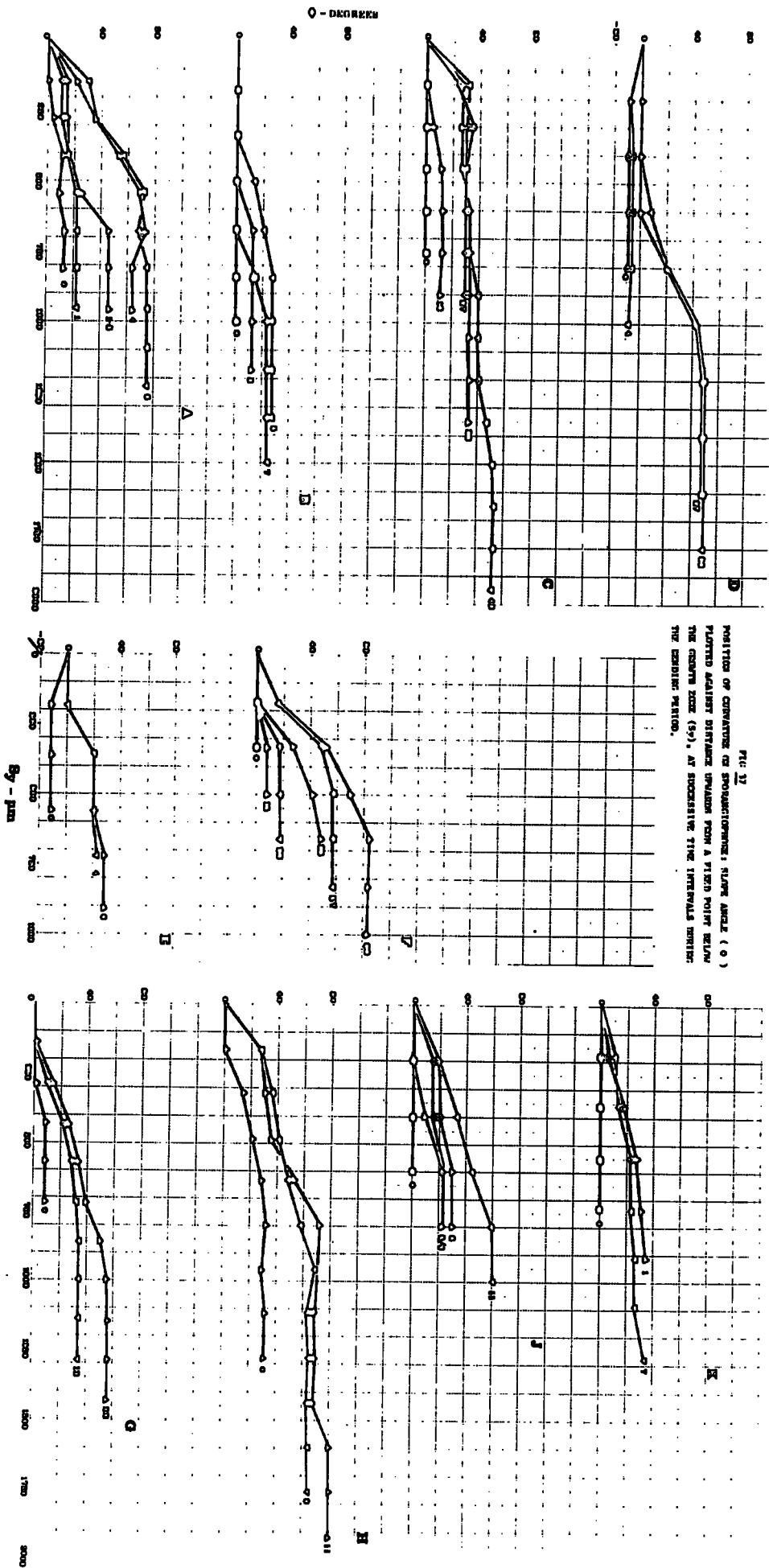


FIG. 12  
 POSITION OF CENTERS OF PROGLOTTINEMES, SLUGS AND ANGLES (0) PLOTTED AGAINST DISTANCE (S<sub>x</sub>) FROM A FIXED POINT BELOW THE CENTER AXIS (S<sub>y</sub>), AT SUCCESSIVE TIME INTERVALS DURING THE EXCYSTMENT PERIOD.

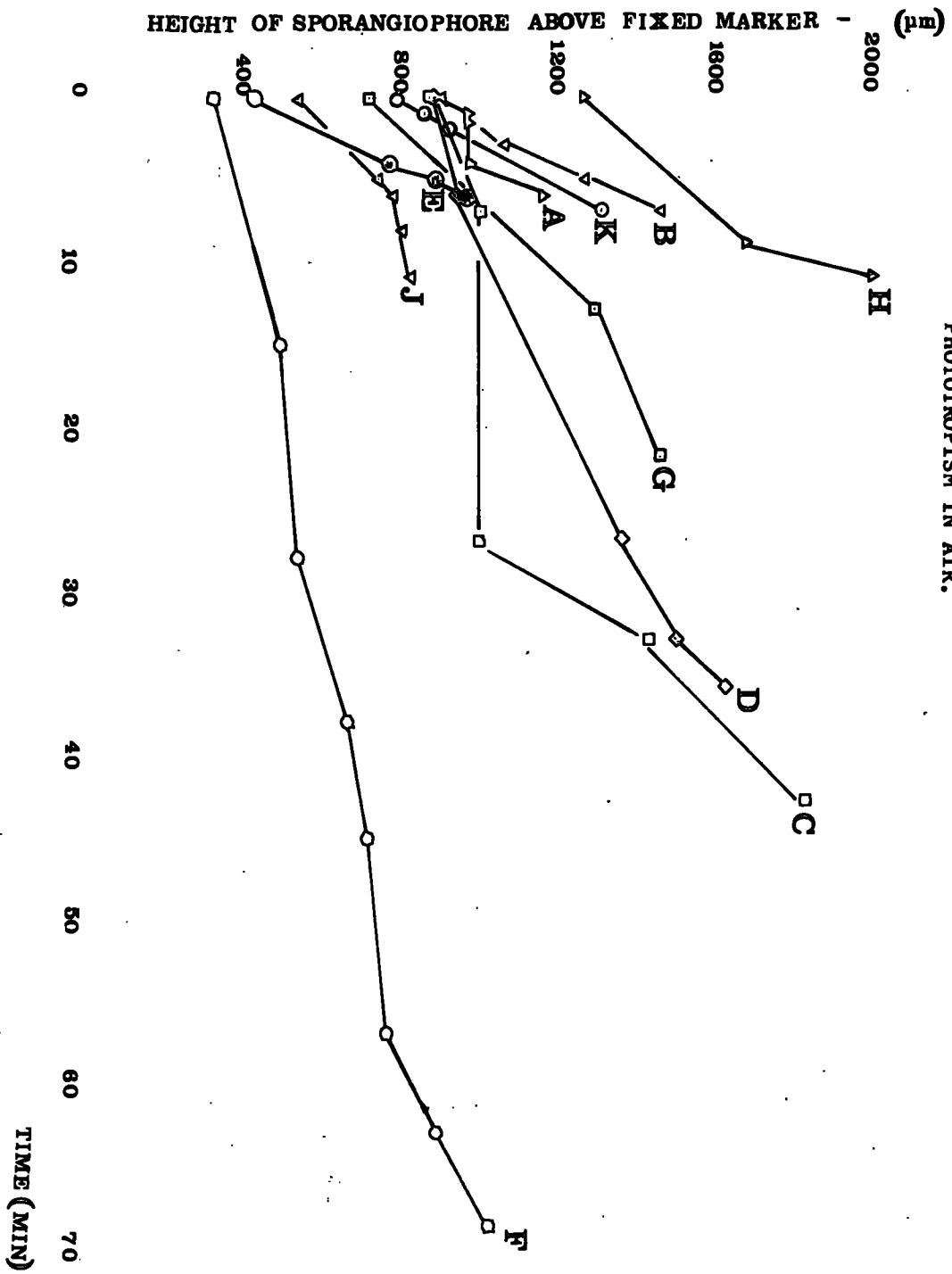
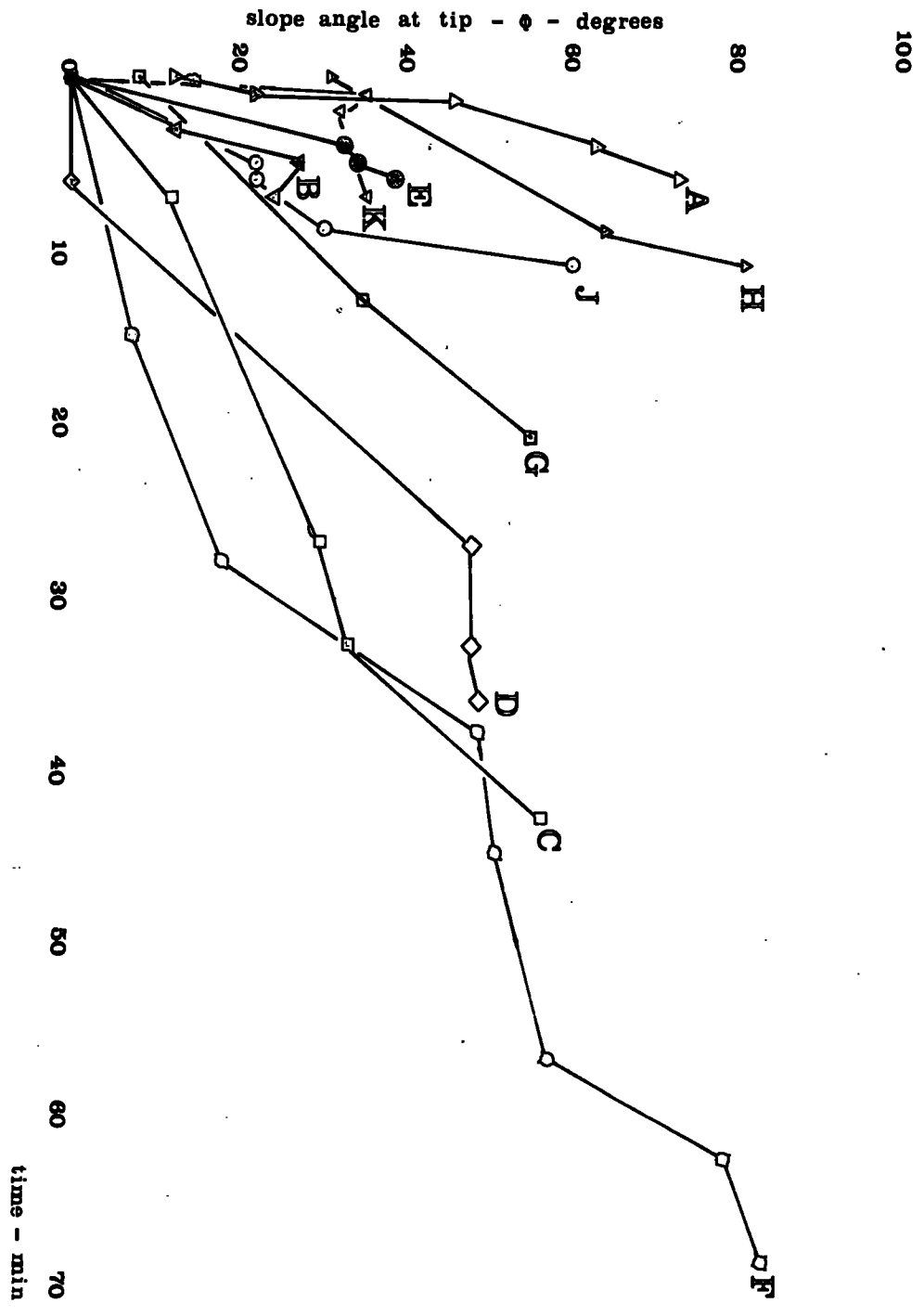


FIG 39  
 BENDING OF STAGE IV SPORANGIOPHORES IN AIR, IN RELATION TO TIME.



*P. moreaui*

The data relating to this species are shown in Tables 23-25 (G-K) and Figs. 36-39 (G-K). Downward migration of the bend is seen in sporangiophores G-J, coupled with upward migration of the bend of these sporangiophores. In one sporangiophore: K, recovery was associated with a change in the distribution of the slope angle. This species also shows no correlation between growth and bending.

TABLE 23

LOCATION OF PHOTOTROPIC BENDING OF STAGE IV SPORANGIOPHORE AT SUCCESSIVE TIME INTERVALS : SLOPE ANGLE ( $\phi$ ) ALONG SPORANGIOPHORE AT DISTANCE UPWARDS FROM A FIXED MARKER (Sy).

*P. anomala*

## SPORANGIOPHORE A

Time (min)	Height above fixed marker *: ( $\mu\text{m}$ )									
	136	272	408	544	680	816	952	1088	1224	
	Angle in degrees:									
0	0	4	11	9	12	12				
1	10	10	13	22	22	22	22			
1.5	14	14	16	24	46	46	46			
4	21	35	53	69	73	63	63			
6	30	37	55	68	68	73	73	73	73	

## SPORANGIOPHORE B

Time (min)	Height above fixed marker *: ( $\mu\text{m}$ )									
	167	334	501	668	835	1002	1169	1336	1503	
	Angle in degrees:									
0	0	0	0	0	0	0	0			
3	0	0	0	12	12	12	12			
5	0	0	12	20	27	27	27	27	27	
7	0	0	0	0	14	24	24	24	24	24

## SPORANGIOPHORE C

Time (min)	Height above fixed marker *: ( $\mu\text{m}$ )													
	150	300	450	600	750	900	1050	1200	1350	1500	1650	1800	1950	
	Angle in degrees:													
0	0	0	0	0	0									
7	0	5	11	14	13	12								
27	24	35	30	30	30	30								
33	26	26	29	33	33	33	33	33	33					
43	31	29	30	33	35	39	40	42	47	53	53	56	56	

## SPORANGIOPHORE D

Time (min)	Height above fixed marker *: ( $\mu\text{m}$ )									
	200	400	600	800	1000	1200	1400	1600	1800	
	Angle in degrees:									
0	-9	-10	-10	-10						
6	-9	-7	-9	-9	-9					
27	0	0	0	20	42	48	48	48		
33	0	0	0	30	41	48	48	48	48	48
36	0	0	9	30	42	49	49	49	49	49

TABLE 23 (continued)

*P. caucasica*

## SPORANGIOPHORE E

Time (min)	Height above fixed marker * : ( $\mu\text{m}$ )				
	182	364	546	728	910
	Angle in degrees:				
0	-11	-11	-11		
4	0	20	20	22	
5	0	20	20	23	23
6	0	20	20	28	28

## SPORANGIOPHORE F

Time (min)	Height above fixed marker * : ( $\mu\text{m}$ )					
	166	332	498	664	830	996
	Angle in degrees:					
0	0	0				
15	0	7	7			
28	0	16	18	18		
38	0	27	42	49		
45	0	30	39	51	51	
57	15	48	57	57	57	
63	17	46	67	74	79	79
69	14	52	69	84	83	83

TABLE 23 (continued)

*P. moreaui*

## SPORANGIOPHORE G

Height above fixed marker \* : (  $\mu\text{m}$  )  
 143 286 429 570 715 858 1001 1144 1287 1430

Time (min)	Angle in degrees:									
0	0	0	9	8	8					
13	0	10	22	28	32	35	35	35	35	
22	0	14	23	33	39	50	55	55	55	55

## SPORANGIOPHORE H

Height above fixed marker \* : (  $\mu\text{m}$  )  
 160 320 480 640 800 960 1120 1280 1440 1600 1760 1920

Time (min)	Angle in degrees:											
0	0	15	21	28	31	28	31	31				
9	28	37	42	48	58	69	64	64	64	64	64	
11	27	32	36	52	71	69	65	65	65	80	80	80

## SPORANGIOPHORE J

Height above fixed marker \* : (  $\mu\text{m}$  )  
 200 400 600 800 1000

Time (min)	Angle in degrees:					
0	0	0	0			
5	0	8	22	22		
6	15	15	22	22		
8	17	20	30	30		
11	17	33	45	60	60	

## SPORANGIOPHORE K

Height above fixed marker \* : (  $\mu\text{m}$  )  
 182 364 546 728 910 1092 1274

Time (min)	Angle in degrees:							
0	0	0	0	0				
1	5	18	28	31	35			
2	7	18	28	30	32			
7	10	16	23	23	26	26	35	

\* The points at which angles of deviation of sporangiophore from direction at the base were measured, represent points at regular fixed intervals above a fixed base point. The magnitude of the intervals is appropriate to the scale employed.

TABLE 24

## LOCATION OF LENGTH OF BENDING ZONE

*P. anomala*

Time (min)	* Lower Limit ( $\mu\text{m}$ )	o Upper Limit ( $\mu\text{m}$ )	Length of bending zone ( $\mu\text{m}$ )	Rate of growth of sporangiophore ( $\mu\text{m min}^{-1}$ )	† Rate of bending of sporangiophore (degrees $\text{min}^{-1}$ )
SPORANGIOPHORE A					
0	204	612	408	-	-
1	68	476	408	102	10
1.5	68	612	544	0	48
4	68	748	680	11	8
6	68	748	680	125	8
SPORANGIOPHORE B					
0	0	0	0	-	-
3	585	585	0	55	4
5	418	752	334	100	7.5
7	752	919	267	92	0
SPORANGIOPHORE C					
0	0	0	0	-	-
7	225	225	0	38	1.7
27	0	375	375	0	0.9
33	0	525	525	55	0.5
43	0	1425	1425	40	2.3
SPORANGIOPHORE D					
0	100	100	0	-	-
6	100	100	0	18	0.2
27	700	1100	400	20	2.7
33	700	1100	400	23	0
36	700	1100	400	47	0

TABLE 24 (continued)

*P. caucasica*

Time (min)	* Lower ( $\mu\text{m}$ )	o Upper ( $\mu\text{m}$ )	Length ( $\mu\text{m}$ )	Rate of growth ( $\mu\text{m min}^{-1}$ )	† Rate of bending (degrees $\text{min}^{-1}$ )
SPORANGIOPHORE E					
0	0	0	0	-	-
4	273	637	364	89	8.3
5	273	637	364	117	1.0
6	273	637	364	59	5.0
SPORANGIOPHORE F					
0	-	-	-	-	-
15	249	249	249	8	0.6
28	249	415	166	7	0.6
38	249	415	166	13	3.1
45	249	415	166	7	0.4
57	83	415	332	4	0.4
63	83	581	498	21	2.7
69	83	581	498	22	0.7
<i>P. moreuxi</i>					
SPORANGIOPHORE G					
0	358	358	0	-	-
13	215	642	427	44	2.1
22	215	787	572	19	2.1
SPORANGIOPHORE H					
0	240	560	320	-	-
9	80	720	640	47	3.7
11	80	720	640	160	10.0
SPORANGIOPHORE J					
0	-	-	-	-	-
5	300	500	200	40	4.4
6	100	500	400	40	0
8	100	500	400	60	4.0
11	100	700	600	10	10.0
SPORANGIOPHORE K					
0	-	-	-	-	-
1	91	637	546	72	35.0
2	91	637	546	93	0
7	91	455	364	69	0

\* Point midway between lowest point at which bending was apparent and point immediately below, at which bending was not apparent.

o Point midway between highest point at which bending was apparent and point immediately above, at which bending was not apparent.

† Increase in the angle between direction of axis at the tip and direction of axis at the base, in relation to unit time.

TABLE 25

DATA RELATING TO GROWTH AND BENDING OF STAGE IV SPORANGIOPHORES IN AIR

Sporangiophore	Average rate of growth <sub>1</sub> ( $\mu\text{m min}^{-1}$ )	Average rate of bending * <sub>1</sub> (degrees $\text{min}^{-1}$ )	Reaction Time † (min)	Total period of observation (min)
<i>Pilaira anomala</i>				
A	51	10	1	6
B	79	3	3	7
C	18	1	7	43
D	22	2	6	36
Mean	43	4	4	23
<i>P. caucasica</i>				
E	89	6.3	4	6
F	10	1.4	15	69
Mean	50	3.7	10	38
<i>P. moreaui</i>				
G	34	2.5	13	22
H	67	7.6	9	11
J	26	5.5	5	11
K	73	5	1	7
Mean	50	5.1	7	12

(\* and † as Table 22)

## NEGATIVE PHOTOTROPISM OF STAGE I SPORANGIOPHORES UNDER LIQUID PARAFFIN

The phenomenon has been observed in all three species of *Pilaira* studied here, negative curvature being recognised on plates filled with paraffin and illuminated unilaterally in an incubator. The effect was clearly marked in *P. anomala* (Fletcher, 1971) but only weakly developed in the other two species. A single sporangiophore of *P. anomala* is shown undergoing negative phototropism in Fig. 32 (G).

## NEGATIVE PHOTOTROPISM OF STAGE IV SPORANGIOPHORES UNDER LIQUID PARAFFIN

*Pilaira anomala*

The data relating to these sporangiophores are shown in Tables 26-28 (A-B) and Figs. 40-43 (A-B) similar to those of sporangiophores in air.

The apparently rapid increase in growth rate and bending in A between 5 and 6 min may be a consequence of a downward sagging of the sporangiophore over that time period; bringing a sporangiophore that was previously turned somewhat towards the observer, into a plane perpendicular to the optical axis of the microscope. The method of observation employed left open the possibility of occasional errors of this type.

The rate of bending of sporangiophore B between 0 and 3 min was about  $4.0 \text{ degrees min}^{-1}$ . After a further time interval of 12 min, the sporangiophore was recorded as having turned through  $90^\circ$  and having shown some compensatory balancing curvature. Because of the apparently abrupt increase in length at this stage, it seems likely that some sagging took place.

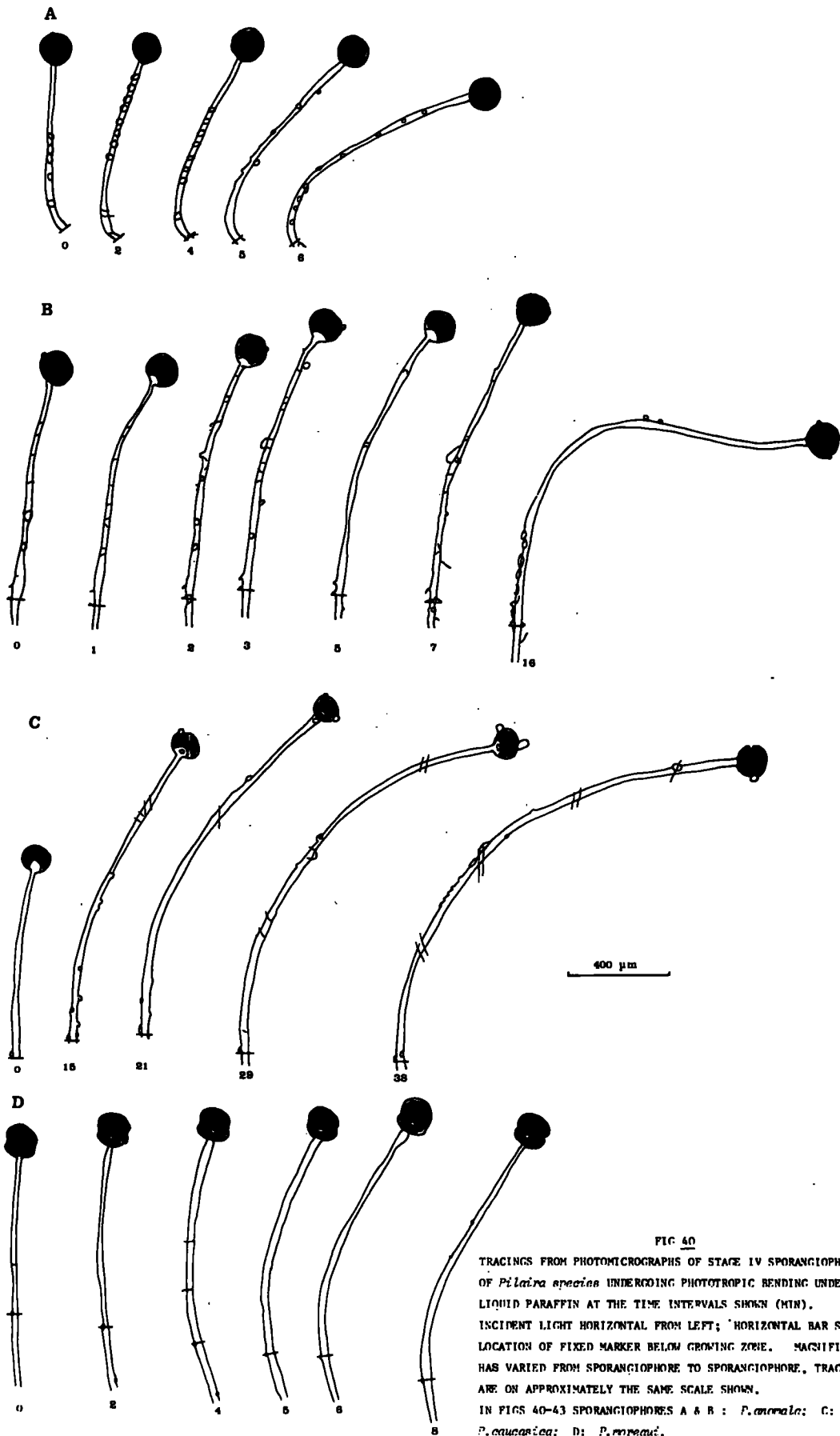
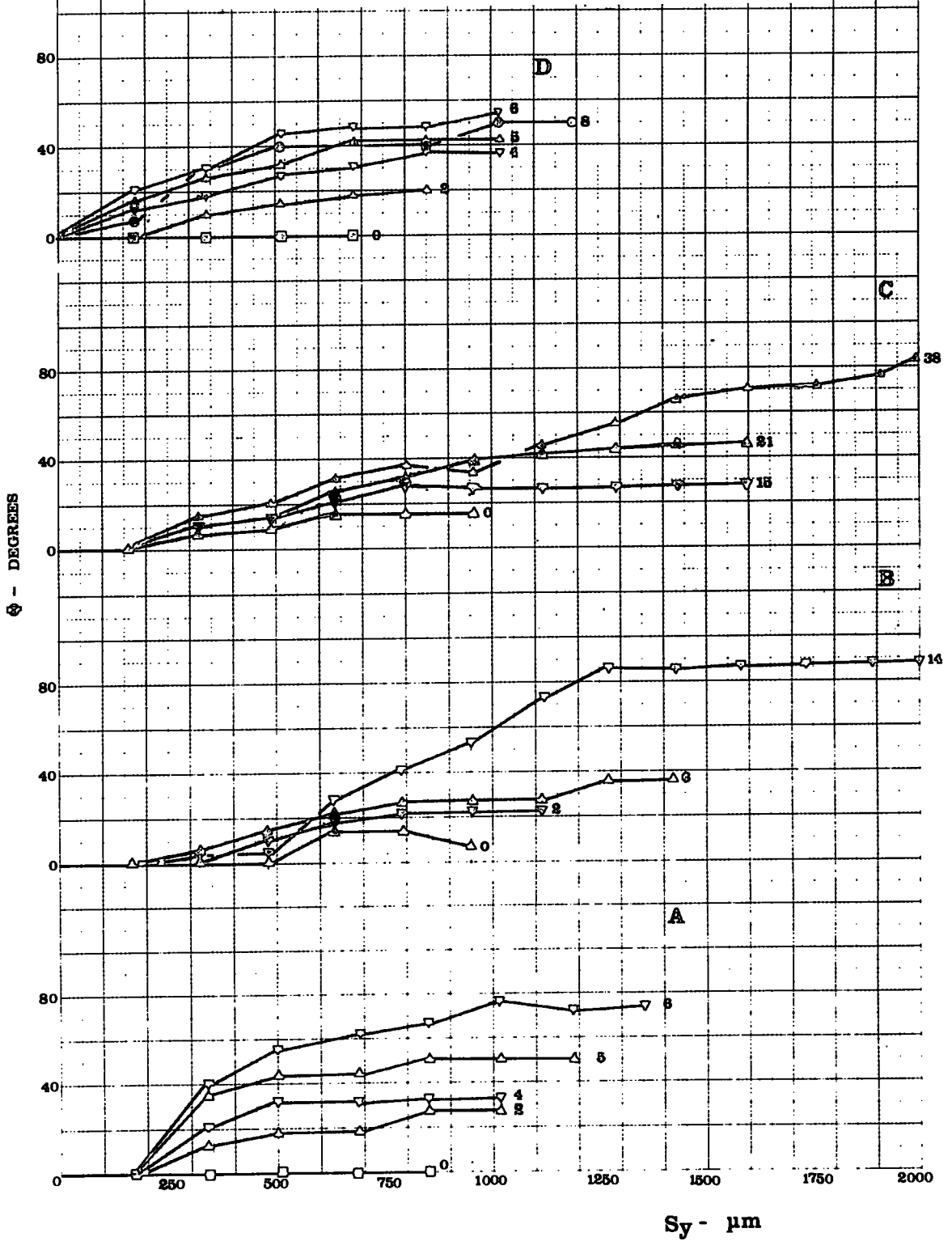


FIG 40  
 TRACINGS FROM PHOTOMICROGRAPHS OF STAGE IV SPORANGIOPHORES  
 OF *Pilaira species* UNDERGOING PHOTOTROPIC BENDING UNDER  
 LIQUID PARAFFIN AT THE TIME INTERVALS SHOWN (MIN).  
 INCIDENT LIGHT HORIZONTAL FROM LEFT; HORIZONTAL BAR SHOWS  
 LOCATION OF FIXED MARKER BELOW GROWING ZONE. MAGNIFICATION  
 HAS VARIED FROM SPORANGIOPHORE TO SPORANGIOPHORE. TRACINGS  
 ARE ON APPROXIMATELY THE SAME SCALE SHOWN.  
 IN FIGS 40-43 SPORANGIOPHORES A & B : *P. caucasica*; C :  
*P. caucasica*; D : *P. rorandii*.

FIG 41

POSITION OF CURVATURE ON SPORANGIOPHORE: SLOPE ANGLE ( $\phi$ ) PLOTTED AGAINST DISTANCE UPWARDS FROM A FIXED MARKER BELOW THE GROWING ZONE ( $S_y$ ), AT SUCCESSIVE TIME INTERVALS DURING THE BENDING PERIOD.



2800

FIG 42  
GROWTH CURVES OF STAGE IV SPORANGIOPHORES UNDERGOING PHOTOTROPISM  
UNDER LIQUID PARAFFIN.

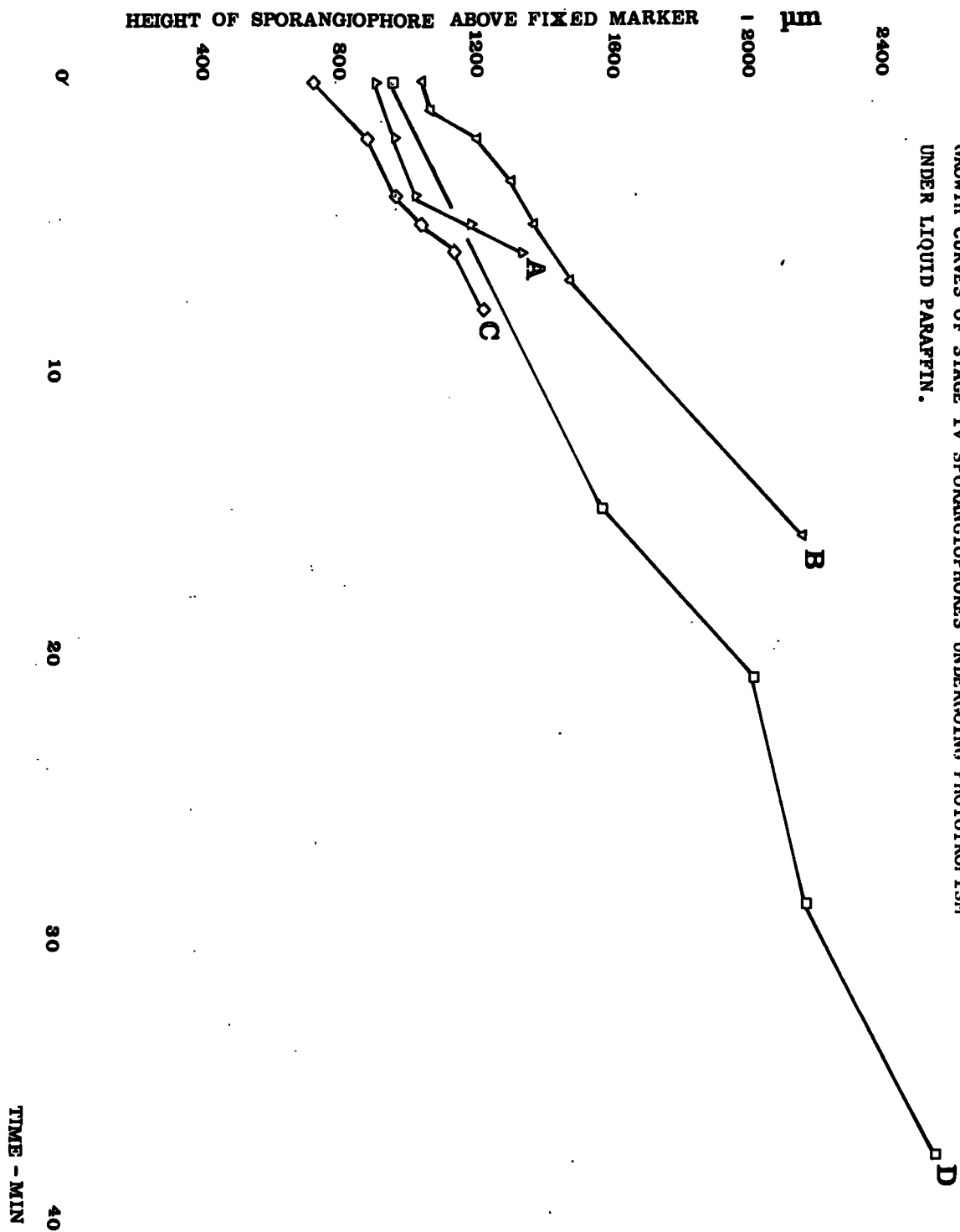
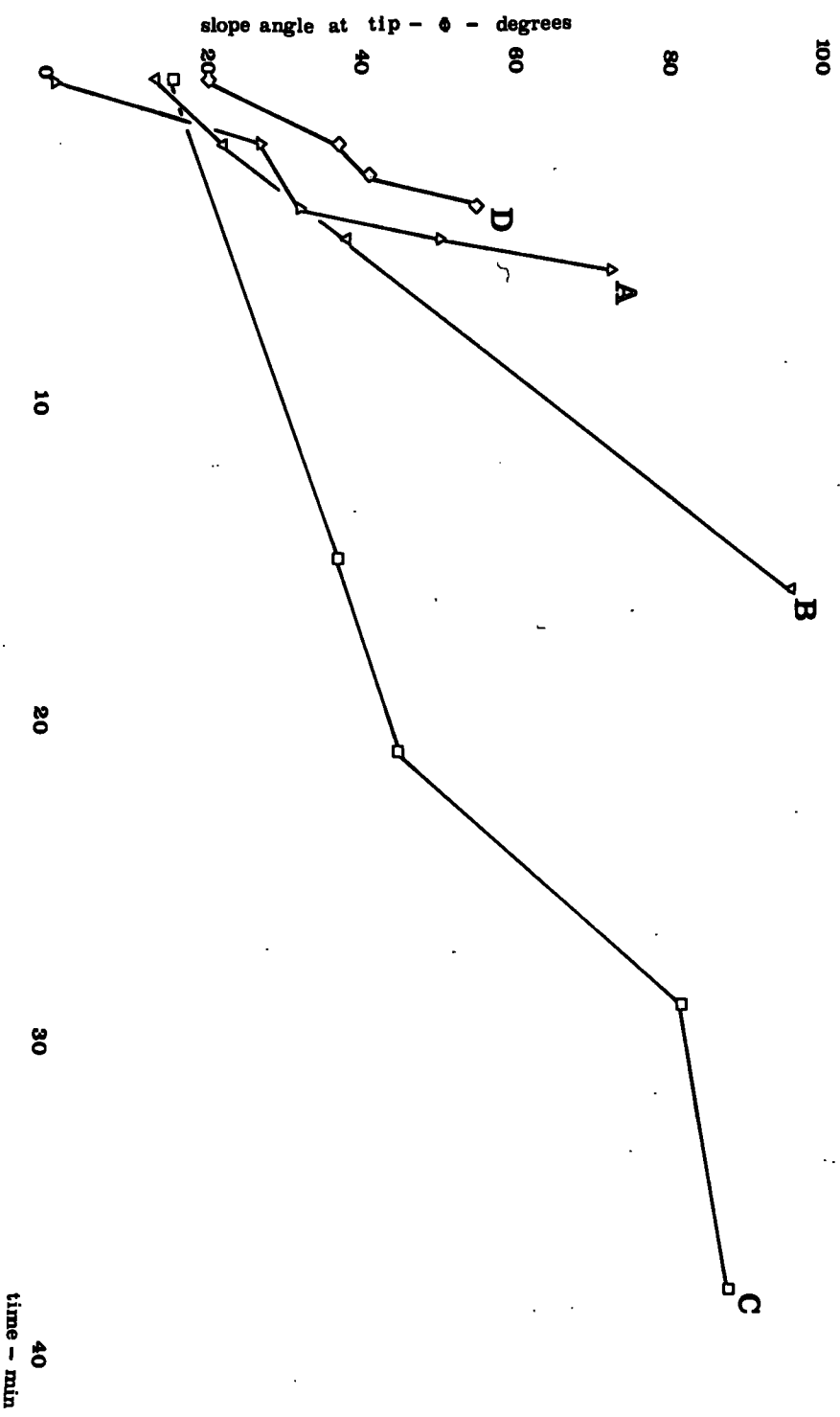


FIG 43  
BENDING OF STAGE IV SPORANGIOPHORES UNDER LIQUID PARAFFIN, IN  
RELATION TO TIME.



*P. caucasica*

The data relating to this sporangiophore are shown in Tables 26-28 (C) and Figs. 40-43 (C). It shows reversal and no obvious downward displacement of its bending zone, but it is a slower growing sporangiophore than A and B.

*P. moreaui*

The data relating to this sporangiophore are shown in Tables 26-28 (D) and Figs. 40-43 (D). This also follows a similar pattern to the other two species.

A lens effect has been found in Stage IV sporangiophores of these three species.

TABLE 26

LOCATION OF PHOTOTROPIC BENDING OF STAGE IV SPORANGIOPHORES OF *Pilaira* spp. UNDER LIQUID PARAFFIN, AT SUCCESSIVE TIME INTERVALS: SLOPE ANGLE ( $\phi$ ) ALONG SPORANGIOPHORE AT DISTANCE UPWARDS FROM A FIXED MARKER (Sy).

*P. anomala*

## SPORANGIOPHORE A

Time (min)	Height above fixed marker * : ( $\mu\text{m}$ )							
	170	340	510	680	850	1020	1190	1360
	Angle in degrees:							
0	0	0	0	0	0			
2	0	13	18	18	27	27		
4	0	21	32	32	32	32		
5	0	34	44	44	50	50	50	
6	0	39	56	63	67	76	72	72

## SPORANGIOPHORE B

Time (min)	Height above fixed marker * : ( $\mu\text{m}$ )												
	158	316	474	632	790	948	1106	1264	1422	1580	1738	1896	2054
	Angle in degrees:												
0	0	0	0	13	13	6							
2	0	0	9	15	22	22	22						
3	0	9	11	11	20	20	35	38					
5	0	6	12	18	25	27	27	36	36				
7	0	7	11	23	23	23	27	27	27				
16	0	4	4	27	40	54	73	96	96	96	96	96	96

*P. caucasica*

## SPORANGIOPHORE C

Time (min)	Height above fixed marker * : ( $\mu\text{m}$ )															
	160	320	480	640	800	960	1120	1280	1440	1600	1760	1920	2080	2240	2400	2560
	Angle in degrees:															
0	0	7	8	15	15	15										
15	0	10	14	20	28	27	27	27	27	27						
21	0	10	14	23	30	38	41	43	45	45	45	45				
29	0	11	19	29	32	36	39	49	56	63	71	75	82	82		
38	0	12	20	30	35	37	41	55	65	69	71	76	82	83	88	88

*P. moreaui*

## SPORANGIOPHORE D

Time (min)	Height above fixed marker * : ( $\mu\text{m}$ )							
	170	340	510	680	850	1020	1190	
	Angle in degrees:							
0	0	0	0	0				
2	0	10	14	18	20			
4	13	18	26	31	37	37		
5	15	26	31	41	41	41		
6	19	30	46	46	46	55		
8	7	30	40	40	40	50	50	

\* Points at which angles of deviation of sporangiophore from direction at the base were measured, represent points at regular fixed intervals above a fixed base point. The magnitude of the intervals is appropriate to the scale employed.

TABLE 27:

## LOCATION AND LENGTH OF BENDING ZONE

Time (min)	* Lower Limit ( $\mu\text{m}$ )	o Upper Limit ( $\mu\text{m}$ )	Length of bending zone ( $\mu\text{m}$ )	Rate of growth of sporangiophore ( $\mu\text{m min}^{-1}$ )	† Rate of bending of sporangiophore (degrees $\text{min}^{-1}$ )
<i>P. anomala</i>					
SPORANGIOPHORE A					
0	-	-	-	-	-
2	85	425	340	38	13.5
4	85	425	340	38	2.5
5	85	425	340	47	20.0
6	85	765	680	218	20.0
SPORANGIOPHORE B					
0	553	553	0	-	
2	395	711	316	80	( average
3	237	711	474	100	about
5	237	711	474	31	
7	237	711	474	57	5.0 )
16	237	1185	848	75	
<i>P. caucasica</i>					
SPORANGIOPHORE C					
0	240	560	320	-	-
15	240	720	480	42	0.8
21	240	1040	800	75	3.0
29	240	1520	1280	20	4.6
38	240	1520	1280	42	0.7
<i>P. Moreaui</i>					
SPORANGIOPHORE D					
0	-	-	-	-	-
2	255	595	240	60	8.8
4	85	765	680	60	8.8
5	85	595	510	70	8.8
6	85	425	340	65	8.8
8	85	425	340	52	8.8

\* Point midway between lowest point at which bending was apparent and point immediately below, at which bending was not apparent.

o Point midway between highest point at which bending was apparent and point immediately above, at which bending was not apparent.

† Increase in the angle between direction of axis at the tip and direction of axis at the base, in relation to unit time.

TABLE 28

DATA RELATING TO GROWTH AND BENDING OF STAGE IV SPORANGIOPHORES UNDER LIQUID PARAFFIN

Sporangiophore	Average rate of growth <sub>1</sub> ( $\mu\text{m min}^{-1}$ )	Average rate of bending * (degrees $\text{min}^{-1}$ )	Reaction time † (min)	Total period of observation (min)
<i>Pilaira anomala</i>				
A	73	12	2	6
B	70	5	2	16
Mean	72	9	2	11
<i>P. caucasica</i>				
C	43	2.3	15	38
<i>P. moreaui</i>				
D	63	8.8	2	6

( \* and † as Table 22 )

## DIFFERENTIAL GROWTH OF SPORANGIOPHORE FLANKS DURING PHOTOTROPISM

In the course of phototropic curvature of *Phycomyces*, the rate of growth of the axis usually remains constant, whilst the rate of growth of the distal flank increases and that of the proximal decreases (Castle, 1961).

In order to test this observation on *Pilaira*, the rates of growth of axis and distal and proximal flanks were measured for a number of the Stage IV sporangiophores studied in this Section. Suitable sporangiophores were those where the growth was confined to the bending zone over the time intervals of observation and where straightening or reversal of the bend over that interval did not occur. The results for a typical sporangiophore, that of *P. moreaui* K from Table 23, are presented.

TABLE 29

LENGTHS OF THE AXIS AND PROXIMAL & DISTAL FLANKS ABOVE A FIXED MARKER, OF THE SPORANGIOPHORE OF *P. moreaui* K from Table 23. AT A MAGNIFICATION OF APPROXIMATELY X 110, TOGETHER WITH A DETERMINATION OF THE GROWTH RATES.

	Time (min) 0		1		Rate of growth ( $\mu\text{m min}^{-1}$ )
	(mm)	( $\mu\text{m}$ )	(mm)	( $\mu\text{m}$ )	
PROXIMAL	76	690	83	754	64
AXIS	76	690	84	764	74
DISTAL	76	690	85	774	84

The differential growth rate across this sporangiophore was found to be about 14% increase or decrease relative to the axis. This compares well with the value of 16% increase or decrease relative to the axis found by Castle (1961) in *Phycomyces*.

## DISCUSSION

The sporangiophores of *Pilaira* species at Stages I and IV resemble those of *Phycomyces* in that they respond rapidly to unilateral light by bending towards it until equilibrium is reached. A feature common to *P. anomala* and *Phycomyces* is that the bend migrates down the sporangiophore throughout the course of the bending process. This occurs in both Stage I and Stage IV sporangiophores. Bergman et al. (1969) point out that there is no clear explanation of the downward migration of the bending in *Phycomyces*, and this is also true for *Pilaira*. Positive phototropic bending involves differential growth across the sporangiophore, with both increased rate of growth on the distal flank and decreased rate of growth on the proximal flank, with respect to the rate of growth of the axis.

Castle (1966) and other reviewers usually discuss the process of phototropism in terms of light-growth reaction, although Castle (1961) points out that phototropism does not require a transient light-growth response, since phototropism is a steady-state process. The light-growth response involves adaptation which is absent in phototropism. However, the positive light-growth response shows that one general effect of an increase in illumination of *Phycomyces* is an acceleration of the extension growth of the sporangiophore. Since phototropism in air is positive, the light must be having a greater effect on the distal side rather than the proximal side with respect to illumination. This led to the concept of the lens effect, whereby the transparent sporangiophore acts as a cylindrical lens focussing the light on the distal side of the sporangiophore. It is usually considered that it is in the peripheral protoplasm and not in the wall itself, that the photoperception mechanism is located.

The lens effect can be demonstrated by immersing the sporangiophore in a liquid, usually liquid paraffin, of greater refractive index than the sporangiophore.

In the case of *Phycomyces*, this results in the reversal of phototropism, due to the divergence of the rays of light, rather than their convergence through the sporangiophore, such as occurs in air. Thus the sporangiophore receives maximum illumination on the proximal side under oil and bends away from the light source. This effect was confirmed by Banbury (1952) by a grazing beam of light causing a sporangiophore in air to bend almost perpendicularly away from the illuminated side. Page & Curry (1966) performed a similar experiment with Stage I sporangiophores of *Pilobolus*.

In an inconclusive preliminary survey some indications were found of a positive light-growth response and a negative dark-growth response in *P. anomala* at Stage IV. The data hitherto obtained are not sufficiently precise and extensive to justify inclusion in this thesis and a more detailed investigation will be required before firm conclusions can be drawn.

In *Pilaira* sporangiophores at Stage IV, there is a marked reversal of phototropism under liquid paraffin. At Stage I this is clearly demonstrable in *P. anomala* but is only weakly developed in the other two species. There is no evidence however, that the sporangiophores of *P. caucasica* or *P. moreauxi* have a higher refractive index than that of liquid paraffin, as is the case in some *Pilobolus* spp. (Page & Curry 1966). In *Pilaira*, the growth under liquid may have been insufficient for a clear result.

Thus the phototropism of *Pilaira* resembles more that of *Phycomyces* than of *Pilobolus*, although little work has been done on the responses of Stage I of *Phycomyces*.

## SOME GENERAL COMMENTS ON THIS WORK

Some aspects of *Pilaira* are well established in the literature, particularly the taxonomic characteristics that define the genus. Of the three available species studied here, there are marked differences of spore size, sporangial wall colour and thickness and nature of sporangial mucilage which are stable characters allowing for recognition of the species. *P. anomala* may be more adapted to the coprophilous habit than the other two species, in that the spores tend to remain sheltered by the sporangial wall and within a viscous drop of mucilage, although it is clearly not as efficient as *Pilobolus* in ensuring the effective dispersal of its spores.

This thesis sets out to contribute further knowledge on *Pilaira*, particularly in the comparison of various physiological activities in the three species. Only *P. anomala* shows the production of a conspicuous growth ring, following brief illumination. There is also no evidence for this phenomenon occurring in *Pilobolus* or *Phycomyces*. An absolute requirement for light for the maturation of sporangia on certain media is a feature of *P. anomala* only, but not in the other two species. A light requirement is also found in some *Pilobolus* species, but not apparently in *Phycomyces*.

The light-growth reaction in *P. anomala* is similar to that in *Phycomyces*, but not quite so marked. Rates of growth have been reported for Stage IV sporangiophores of *Pilaira* species similar to those found in *Phycomyces*. There are detailed differences in the phototropism of the three species of *Pilaira*, particularly in Stage I. All three species show positive phototropism in Stages I and IV in air and a reversal in liquid paraffin. This is similar to *Phycomyces*, and some other aspects of the responses of *Pilaira* resemble *Phycomyces* in detail. *Pilaira* also resembles some species of *Pilobolus* in its tropic behaviour.

A comparison has also been made of various morphological features of the three species.

With regard to the external and internal morphology of the sporangiophores, there appear to be no major differences, apart from those already mentioned. There are differences however, between the species, in the durations of each particular Stage of development. All three species show a marked clockwise twisting of the sporangium during Stage IV, but none of them show the preceding anticlockwise twisting, followed by reversal, the Stage IVa of *Phycomyces*. In the production of 'stolons' there are some specific differences. This phenomenon does not seem to occur in *Pilobolus* or *Phycomyces*.

Certain topics studied here require further elucidation. There is a need for an extensive survey of *Pilaira* species, attempting to re-isolate some of the other species that have been described previously. In the study of the growth ring, a knowledge of the precise response of individual hyphae to light is required as well as the responses on different media. There is the need for the analysis of pigments in *P. caucasica* and *P. moreaui* and a comparison with *P. anomala*. This still leaves the major uncertainty in all light responses; the nature of the photoreceptor.

Further work on sporangiophore initiation and development would need to involve more details of the morphological changes occurring, a precise measurement of the amount and wavelength of light required to induce the development and some investigation of the nutritional factors that 'replace' light on certain media.

An investigation into rotation of Stage IV sporangiophores of the three species correlated with the rate of growth would aid the understanding of that process. Further work is required to determine the factors controlling 'stolon' production.

A detailed study of the light-growth reaction in all three species is required. Similarly there should be more detailed studies of some aspects of tropism, particular the response of Stage I under liquid paraffin.



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### ACKNOWLEDGEMENTS

The author wishes to record his sincere gratitude to Mr. G.H. Banbury for his helpful advice and encouragement throughout the production of this thesis.

He also expresses his thanks to Mr. E.F. Middleton for the photographic printing.