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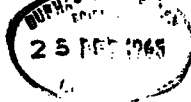
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Studies of the Growth and Tropisms of
Aspergillus giganteus and other fungi

A Thesis Submitted by
A.P.J. Trinci, B.Sc. (Dunelm)
for the Degree
Doctor of Philosophy in the University of Durham

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January 1965



GROWTH AND TROPISM OF ASPERGILLUS GIGANTEUS
AND OTHER FUNGI

A.P.J. Trinci

ABSTRACT

The tall conidiophores of Aspergillus giganteus are produced by large foot cells formed by the mycelium. They only continue to grow when exposed to light of wavelength below 530 m μ . The photochemical reaction involved in the inductive process is a 'low energy' reaction, i.e. light acts as a 'triggering' mechanism. The response to light is localised in the areas of the mycelium actually exposed to the radiation. The density of tall conidiophore production, but not their height, is influenced by the concentration of glucose in the medium.

The tall conidiophores have the usual type of growth curve; they reach their maximum rate of elongation when they are c. 3 mm tall. Elongation is confined to the terminal portion of the conidiophore. At low light intensities the conidiophores show regular oscillations along their length. The rate of conidiophore growth is influenced by light intensity; they do not show 'light growth reaction' of the kind found in Phycomyces

sporangiophores. The conidiophores only continue to elongate in light when the relative humidity is close to 100%. There is a rapid reduction in the rate of conidiophore growth during vesicle formation; no further growth takes place after the vesicle has been formed.

The optimum temperature for mycelial growth is c. 30°C, while the optimum temperature for maximum conidiophore height is 20 - 23°C.

The presence in the medium of the riboflavin inhibitors, 1-lyxoflavin and mepacrine, or the β -carotene inhibitor, diphenylamine, did not inhibit the photoinductive process. The presence of gaseous carbon dioxide is not essential either during the photoinductive process or for conidiophore growth. Free oxygen is not required during the actual period of photoinduction but is required for conidiophore growth. Photoinduction in the absence of gaseous oxygen is less effective in inducing carotenogenesis than photoinduction in the presence of oxygen.

The conidiophores are strongly positive phototropic but do ^{not} appear to be sensitive to the stimulus of gravity. There is a reversal of the normal phototropic response in ultra-violet radiation of 280 m μ and when the conidiophores are submerged in liquid paraffin.

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SECTION I.

ACKNOWLEDGMENTS, INTRODUCTION AND

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1. ACKNOWLEDGMENTS

I wish to record my sincere gratitude to Mr. G.H. Banbury for the support, encouragement and assistance he gave me throughout the course of this research. I would also like to thank Professor D.H. Valentine for allowing me to undertake the work in his Department, and the Botany Technicians, particularly Mr. Redhead and Mr. Swinhoe, for the assistance they gave me in the preparation of the photographs and figures. Finally, I would like to express my thanks to the Department for Scientific & Industrial Research for providing the grant on which the final two years of this work were completed.

2. INTRODUCTION

Light is known to influence many aspects of the growth and development of fungi. In some it is essential for the production of fruiting structures (Pilobolus kleinii; Page, 1956) while in others it increases the amount of fruiting (Diaporthe phaseolorum; Timmick et al., 1951). Light has also been shown to influence carotene production (Neurospora crassa; Zalokar, 1954), spore discharge (Sordaria fimicola; Ingold & Hadland, 1959) and rate of growth (Blastocladiella emersonii; Cantino, 1959). A large number of fungal sporophores show phototropic responses (the sporangiophores of Phycomyces and perithecial necks of Sordaria fimicola; Ingold & Hadland, 1959).

A few of these light influenced systems, e.g. the phototropic behaviour of Phycomyces sporangiophores, have been investigated intensively, but the majority have been subject to only superficial studies. It appeared from work conducted by Gardner (1950) that Aspergillus giganteus might prove to be a suitable organism to study the influence of light on fungal growth. It was hoped that an investigation into the growth and tropisms of this fungus might serve to illuminate some of the basic physiological processes involved in these phenomena.

3. REVIEW OF PREVIOUS WORK

Aspergillus giganteus was first described by Wehmer (1907-8). He compared A. giganteus with the closely related A. clavatus and concluded that they were distinct species; the principal characters which he used to distinguish between them were conidiophore height and vesicle size. Thom & Raper (1945) also separate the two species in their monograph. Monica White (1963), however, has recently challenged the view that A. giganteus is a 'good' species. She obtained a sector of A. clavatus which was morphologically and physiologically indistinguishable from A. giganteus. She concluded from her investigation that A. giganteus was either a mutant or diploid form of A. clavatus. A. giganteus has been isolated many times from nature and the strain used in the present work was perfectly stable. It may well prove not to be a 'good' species but it is undoubtedly a 'good' physiological organism to work with. If the genetic nature of A. giganteus, particularly its relationship to A. clavatus, can be determined, it will be of some considerable interest to relate this with the physiological properties of the organisms. For convenience, the strain used in this work will be referred to simply as A. giganteus.

Philpot et al. (1944) have investigated the

antibiotic capacity of A. giganteus, while three workers have made a physiological study of the organism. Webb (1942) made a brief study of the effects of temperature, light and composition of the medium on conidiophore elongation. Gardner (1950, 1955) and Zurzyka (1956) made more extensive investigations into the factors influencing conidiophore growth.

1. SOURCE OF STRAINS USED

- (a) Aspergillus giganteus. The strain was obtained from Professor Weston's collection at Harvard University and was the same as that used by Gardner (1950). The Harvard collection number is W-267.
- (b) Collybia velutipes. This species was obtained from Dr. Plunkett's collection at Birkbeck College.
- (c) Schizophyllum commune. Also obtained from Dr. Plunkett.
- (d) Coprinus bisporus. This species was obtained from Dr.P.G. Fothergill's collection at Newcastle University.

2. DEFINITION OF SOME OF THE TERMS USED

The following are definitions of some of the terms used in this thesis.

Tall or Giant Conidiophore. This term will be used to designate any conidiophore which is taller than the tallest conidiophore produced in darkness under the same culture conditions.

The Vesicle. The swelling, formed at the tip of the conidiophore, from which the sterigmata arise.

Conidiophore Head. The whole springing head formed at the tip of the conidiophore.

Induction Period. The time lag between inoculation and appearance of the tall conidiophores. Gardner (1955)

defined this term slightly differently; she used the word "elongation" instead of "appearance" of tall conidiophores. The reason for changing the definition is discussed on page 30 .

3. CULTURE MEDIA EMPLOYED

Three culture media were employed in the experimental work described in this thesis. Two were synthetic media; one devised by Gardner (1950) and the other by Zurzyka (1956). The composition of the media are as follows:-

Zurzyka's Medium

Glucose	50.0 grammes
Potassium dihydrogen phosphate KH_2PO_4	1.0 "
Ammonium nitrate NH_4NO_3	1.0 "
Magnesium sulphate $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1.0 "
Zinc sulphate $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.002 "
Ferrous sulphate $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.002 "
Distilled water	1000.0 ccs

Gardner's Medium

Maltose	30.0 grammes
Sodium nitrate NaNO_3	2.0 "
Potassium dihydrogen phosphate KH_2PO_4	1.0 "
Magnesium sulphate $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5 "
Potassium chloride KCl	0.5 "
Ferrous sulphate $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.01 "
Distilled water	1000.0 ccs

Malt Medium

Malt extract	20.0 grammes
Distilled water	1000.0 ccs.

The malt extract used was bought from Timothy White & Taylor Ltd. When solid media were required, 20 grammes of agar per litre of distilled water were included in the above formulae.

4. STERILIZATION AND INOCULATION PROCEDURES

The culture media and vessels were normally sterilized by autoclaving at a pressure of 15 lbs/sq. inch for 15 minutes. The 'rafts' described below (page 3) were sterilized by dipping them into vigorously boiling water for about 2 minutes.

Culture media were normally inoculated with drops of a spore suspension. The spore suspensions were obtained by flooding agar slopes with sterile distilled water and making up the volume to about 15 mls.

5. CULTURE VESSELS

(a) RAFT CULTURE METHOD. This culture technique was used when it was necessary to transfer mycelia between different culture media. The mycelium was supported on Terylene curtain net stretched over polythene rings. The rings had a diameter of $1\frac{1}{2}$ inches with a rim of approximately $\frac{1}{2}$ inch and the Terylene cloth was kept in position over the

rings by an elastic band which fitted into a groove in the outer rim. The rings were floated in culture media in either 50 or 100 ml squat beakers covered with aluminium foil caps. The rings floated on the surface of the liquid and for convenience these Terylene cloth covered polythene rings will be referred to simply as 'rafts'.

For one particular experiment a smaller type of raft was employed. This consisted of Terylene cloth stretched across a nylon curtain ring which had a diameter of 25 mm. These rafts will be called 'small rafts' in order to distinguish them from the larger type described above. Plate 1a is a photograph of a culture growing on one of the larger rafts.

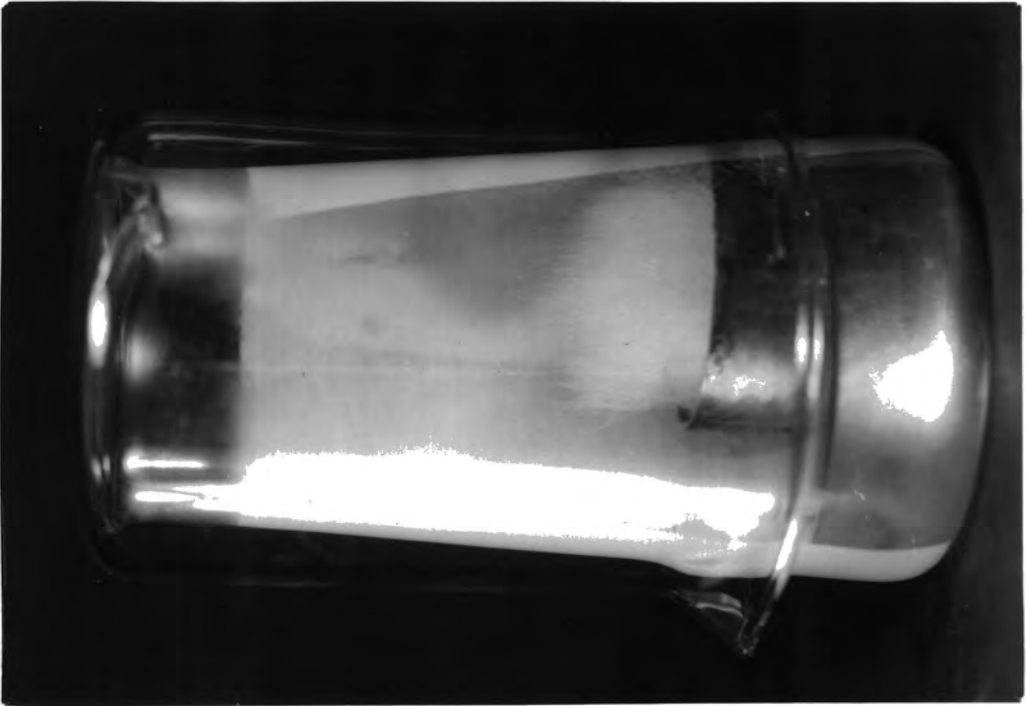
(b) ALUMINIUM OXOID CAP CULTURE METHOD. This technique was used in experiments which involved a determination of the height of conidiophores produced under various culture conditions. The method was used in preference to conventional vessels as it ensured that the conidiophore stalks elongated in an atmosphere saturated with water vapour. Observation of the type of growth produced in ordinary culture vessels appeared to indicate the presence of a humidity gradient above the medium.

Two sizes of caps were used:

1. Large oxoid caps with a diameter and depth of 3 cm which held 22 mls of culture medium when full.

PLATE 1b Oxoid cap culture method

PLATE 1a Raft culture method.



2. Medium oxoid caps with a diameter and depth of $2\frac{1}{2}$ cm which held 15 mls of culture medium.

The oxoid caps were placed in 125 ml conical beakers which were nearly completely lined with blotting paper. About 25 mls of distilled water was poured into each beaker and this ensured a high humidity inside the culture vessels. The conical beakers were capped with 150 ml squat beakers. Plate 1b is a photograph of a culture growing in one of these culture vessels.

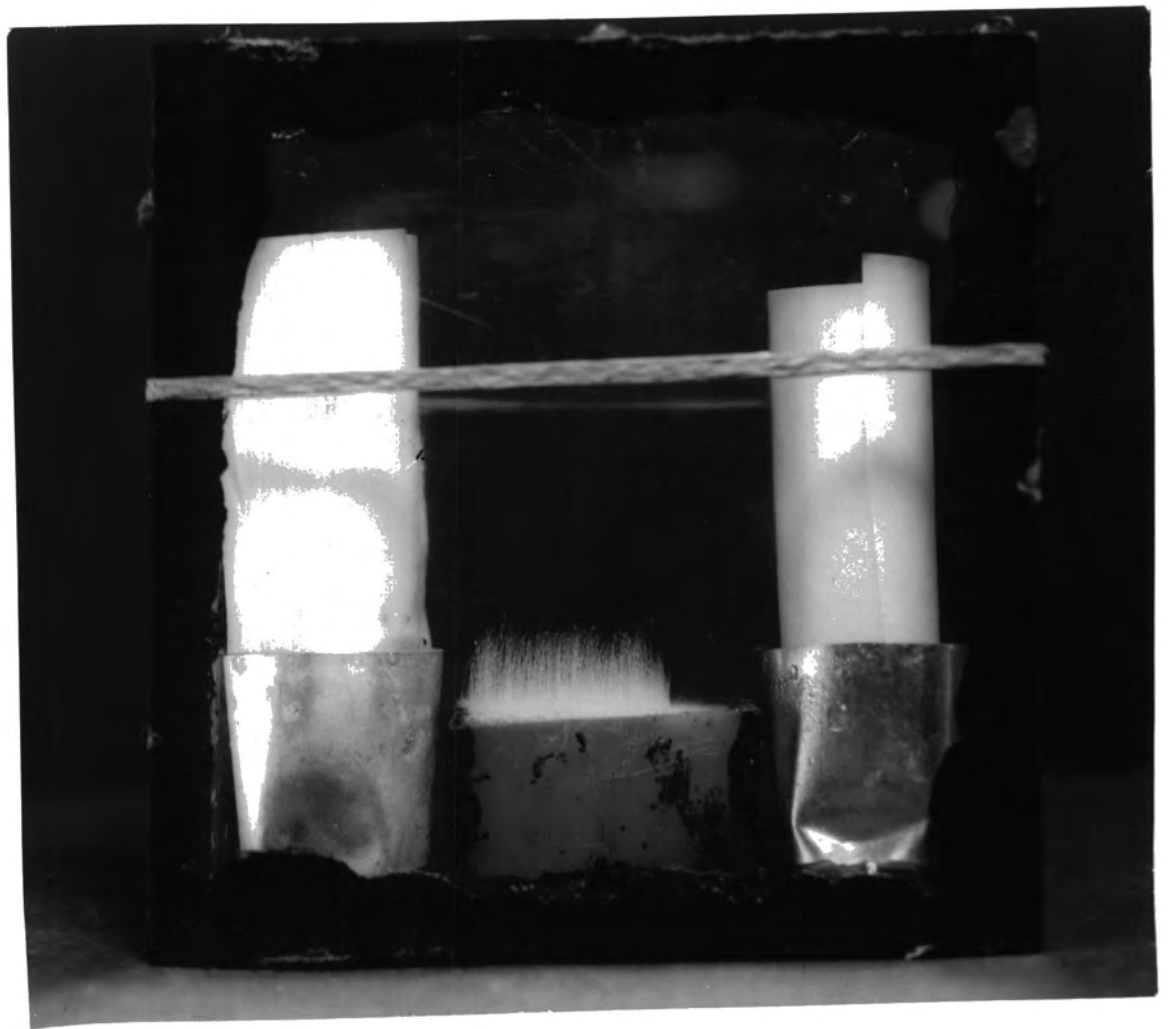
(c) VESSELS USED FOR MICROSCOPIC EXAMINATIONS:

(i) Culture cells. The cells which held the culture medium were made from strips of glass cut from microscope slides; black Bostik was used as the adhesive agent. These cells were 3.0 cm long, 1.5 cm deep and 1.0 cm wide; they held approximately 4.5 mls of culture medium. After inoculation they were placed into larger observation cells made from lantern slides. Each side of these cells consisted of a 8 cm x 8 cm lantern slide. Glass strips, 2 cm wide, were glued to one lantern slide to form a glass box. The cell was completed by placing a second lantern slide over the glass box; it was held in position by elastic bands.

A small oxoid cap in which stood a cylinder of blotting paper was placed on either side of the small cell. These caps served as reservoirs for water or salt solutions and their presence served to maintain the humidity in the large



PLATE 2 A culture growing in one of the observation cells used for microscopic examinations.



cell at a prescribed level. Plate 2 is a photograph of a culture growing in an observation cell.

(ii) Specimen tubes. In some instances 5 cm x 2.5 cm specimen tubes were used as culture vessels for microscopic work. The top of each tube was covered with a glass cover slip.

(d) CONICAL FLASKS. Two types of 100 ml Pyrex conical flasks were employed. One type had a neck diameter of 2 cm while the other had a neck diameter of 1.5 cm. The flasks were capped with glass or aluminium foil caps.

6. TECHNIQUES USED TO STUDY GROWTH OF THE FUNGUS UNDER CONTROLLED CONDITIONS.

(a) LIGHT BOXES. The light boxes were made from tea-chests which had their inner surface painted white. The base of these tea-chests measured 48 cm x 48 cm and they were 60 cm deep. A hardboard lid, which had a 16 cm x 16 cm square hole cut from its centre, was made for each box. This opening in the lid was covered by a square of 50 mm thick ground glass, upon which was placed a large beaker containing a 0.5% solution of copper sulphate (anhydrous); the depth of this solution in the beaker was 13 cm. A bulb was suspended above the beaker so that the light illuminating the box had to pass through the copper sulphate solution. A thin layer of paraffin oil was poured on to the surface of the copper sulphate solution, to prevent evaporation. The distance

from the base of the bulb to the bottom of the box was approximately 77 cm. Estimation with a spectroscope showed that no detectable light of wavelength longer than 630 m μ was transmitted by the copper sulphate solution. Due to this absorption of infra-red radiation the bulb did not have a heating effect upon the contents of the box. This was confirmed by comparing the temperature ranges recorded by maximum and minimum thermometers placed inside and outside the tea-chest. In order to maintain rigid light regimes the glass plate, beaker and bulb on top of each tea-chest were enclosed in a cardboard box, which had a hole cut in the bottom to coincide with the hole in the hardboard lid of the tea-chest.

Unless otherwise stated the bulbs used in experiments involving the light boxes were 60 watt 'Neta-bulbs' made by the Co-operative Society. These gave a light intensity on the bottom of the tea-chest of 8.5 foot candles or 91 metrcz candles. The atmosphere inside the boxes was kept humid by the presence of beakers of distilled water in which stood cylinders of blotting paper. Fig. 1 is a diagram of a section through a light box.

(b) ILLUMINATED INCUBATORS. Each incubator was illuminated by four 4-volt 0.3 amp Sunshine cycle lamp bulbs. These were chosen because their heating effect was quite small and they were fitted into sockets on

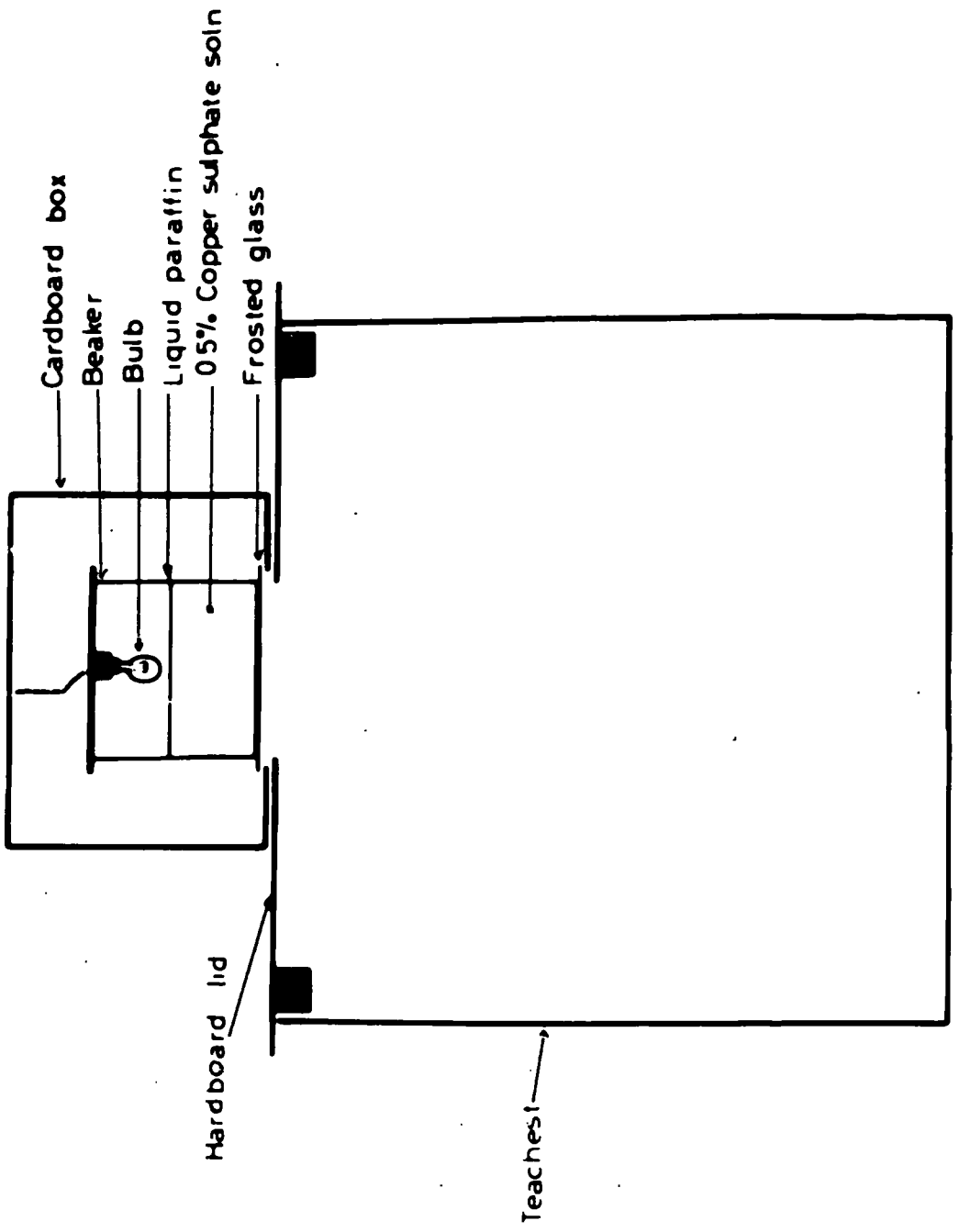


FIGURE 1 DIAGRAM OF LIGHT BOX

hardboard strips arranged so as to give as even an illumination as possible. The bulbs were run off a 4.5 volt D.C. current supplied by a transformer. The shelf on which the cultures were placed was situated 40 cm below the bulbs. The light intensity at the level of this shelf was 3.4 foot candles or 36 metrc candles. The atmosphere inside the incubators was kept humid by the presence of beakers of distilled water in which cylinders of blotting paper were stood.

(c) METHODS USED IN MICROSCOPIC EXAMINATIONS.

(i) Observation of elongating conidiophores. The horizontal microscope was housed in a 25 x 30 x 40 cm glass tank which had been painted inside and out with matt blackboard paint. The open side of the tank was covered with a sheet of polythene which had a hole cut in it to allow the eyepiece and part of the barrel of the microscope to pass through. The mirror had been removed from the microscope and the source of illumination for photographic work was a high intensity red lamp situated outside the tank. The bulb used in this lamp was a 12 volt 21 watt Osram car bulb; no light of wavelength less than 610 m μ was transmitted by the red cellophane filter. Additional filters were used to remove infra red radiation in order to ensure that there was no appreciable heating effect on the object illuminated. The filters consisted of a 3 cm thick solution of 2.5% copper sulphate and a similar thickness of distilled water. These solutions were contained in

medicine bottles, the former solution was located outside the tank, the latter inside. The source of white light used to illuminate the cultures under examination was a Baker 'Patholette' high intensity microscope lamp; a rheostat was used to vary the intensity of the light. The bulb of the lamp was approximately 45 cms above the culture on the microscope stage. In order to filter off infra red radiation from this light source the beam was passed through 20 cm of a 0.5% copper sulphate solution contained in a beaker placed on top of the tank; a layer of liquid paraffin on the surface of the solution prevented evaporation. Figure 2a is a diagram of a section through the apparatus.

Three beakers of water with cylinders of blotting paper standing in them were placed inside the tank to ensure a high internal humidity. A thermostat operating a heating unit was also placed inside the glass tank. A Vinten Mark I camera was used in the time lapse cine work. The whole apparatus was housed in a darkroom. Measurements of conidiophore elongation were obtained by projecting the film.

A camera lucida was made to use with the horizontal microscope. It consisted of a large cover slip glued to two pins fixed at an angle of 45° to the vertical on a cork collar. The way in which this collar fitted on to the microscope is shown in Figure 2b.

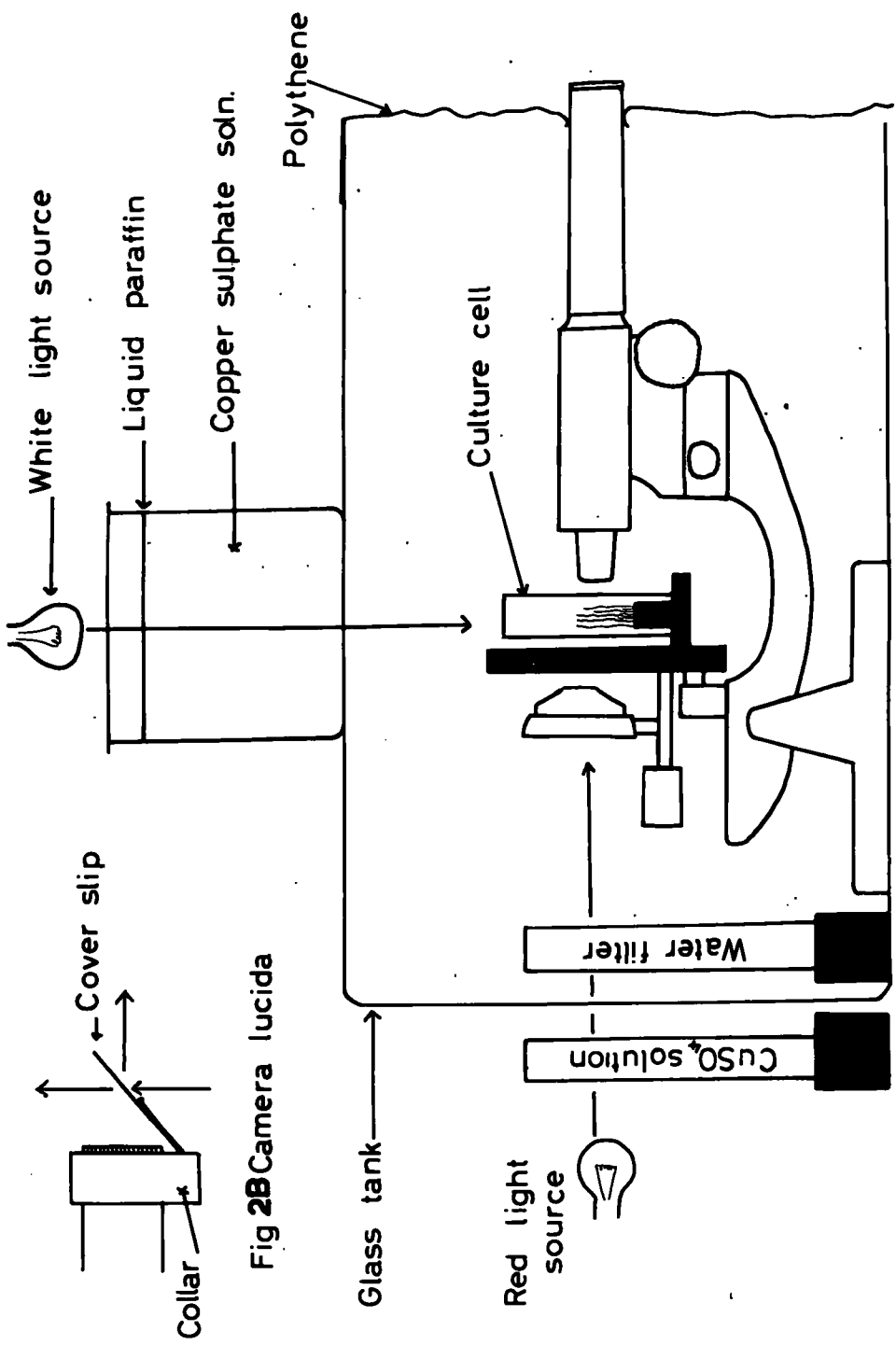


Fig 2B Camera lucida

Figure 2A APPARATUS FOR MICROSCOPIC EXAMINATION

A Baker micrometer eyepiece with a travelling graticule was used in other work involving measurement of conidiophore elongation.

(ii) Observation of mycelial growth. In order to examine mycelial growth microscope slides coated with a thin layer of culture medium were inoculated and placed in petri dishes containing a few ml's of sterile distilled water. The cultures were examined at intervals after inoculation.

A second technique employed was to inoculate strips of sterile cellophane placed on the surface of culture media in petri-dishes. At intervals the pieces of cellophane were stripped off, mounted on slides and examined.

(d) METHOD USED TO CULTURE THE FUNGUS IN LIGHT OF DIFFERENT WAVELENGTH RANGES.

Ifford filters 690-609 were used to obtain light of varying quality. The wavelength range of the radiation transmitted by each filter is recorded in Table 7 and their spectral transmissions are shown in Figure 3. A number of 3" x 3" x 3" tins were modified to form small growth chambers. The inner surfaces of the tins were painted with matt blackboard paint and the filters, which had a surface area of 51 sq. mm, were placed over slightly smaller apertures cut in the lids of the tins. The growth chambers were made light tight with an adhesive tape seal around the edges of the filters. The tins were arranged at distances from a 60 watt tungsten filament bulb which had been calculated to give approximately

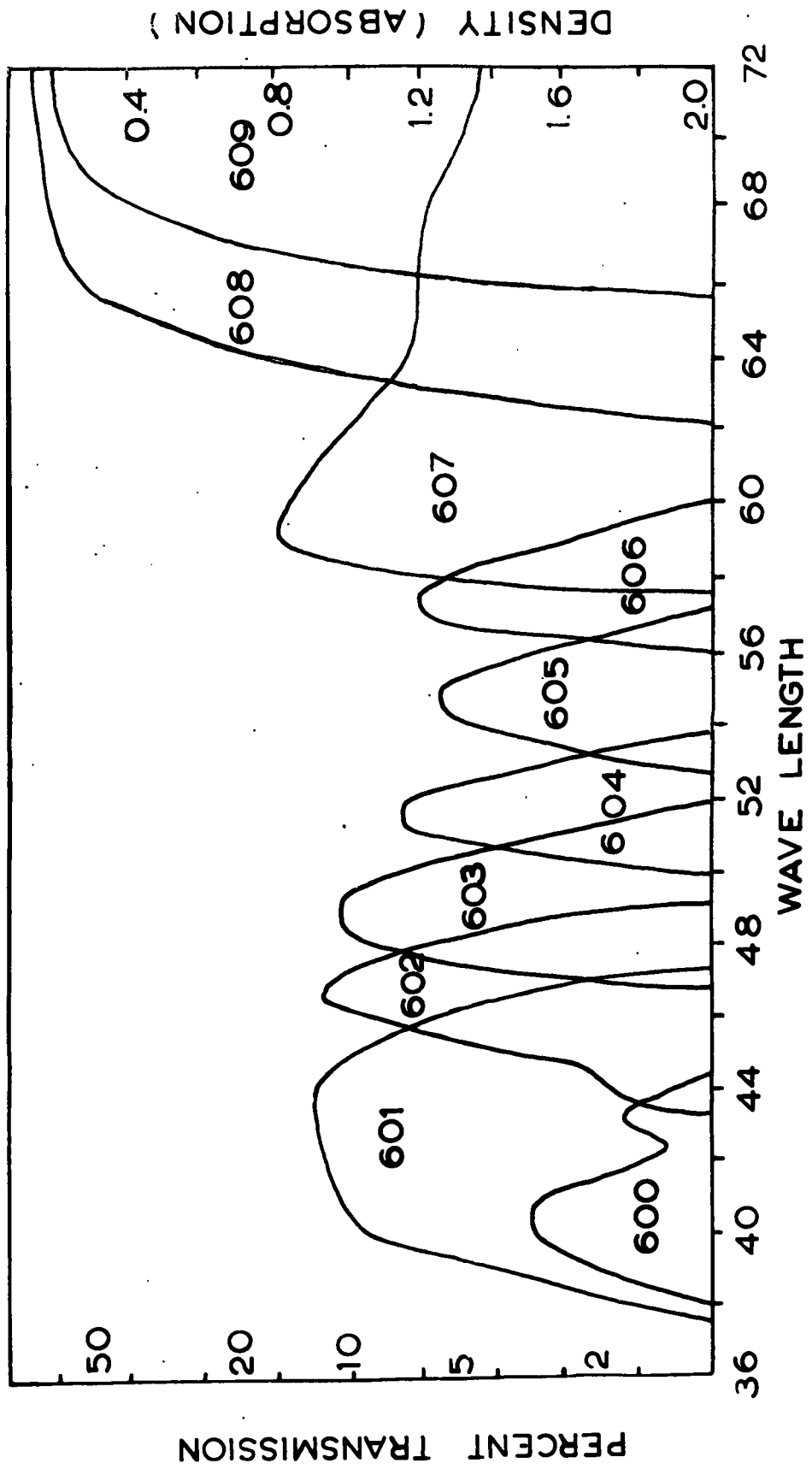


FIGURE 3 Spectral transmission of filters

the same radiant energy inside each tin. The electricity supply to the bulb was passed through a constant voltage transformer to minimise fluctuations in the filament temperature and hence in the spectral composition of the light emitted.

TABLE 7. Spectral transmissions of the Filters

Filter	Transmission in m μ
600 deep violet	380-540 (peak 406)
601 violet	380-470 (peak 415)
602 blue	440-490 (peak 468)
603 blue-green	470-520 (peak 490)
604 green	500-540 (peak 515)
605 yellow-green	530-570 (peak 550)
606 yellow	560-610 (peak 575)
608 red	620 onwards
609 deep red	650 onwards

(e) METHODS USED TO CULTURE THE FUNGUS IN AN ATMOSPHERE

FREE FROM OXYGEN.

Figure 4 is a diagram of the apparatus used in this method. The culture vessel was a 3 litre glass anaerobic jar manufactured by Gallenkamp. 'White spot' nitrogen was bubbled into a wash bottle containing a solution of sodium pyrogallate, through a second wash bottle containing sterile distilled water and

into the culture vessel at the rate of 50-60 litres/hour. The gas leaving the culture jar was passed through a third wash bottle which also contained a solution of sodium pyrogallate. In each experiment the culture vessel was flushed out with nitrogen for an hour and then sealed using the screw valves. The screw clips on the rubber tubing were tightened at the same time to prevent an inflow of air into the sodium pyrogallate solutions,

7. METHODS USED TO DETERMINE THE HEIGHT AND DRY WEIGHT OF AERIAL CONODIOPHORES AND TO ESTIMATE THEIR DENSITY.

All measurements of conidiophore height were made using a ruler held against the culture vessel. In most cases nearly all the conidiophores had attained a closely similar height at a given time, and this "average" height of the conidiophores was measured, but in a few instances the height of the taller conidiophores was also recorded. Other methods of measuring conidiophore height were tried but, although more accurate, they were found to be too laborious to employ.

The arbitrary method used by Gardner (1950) to estimate density of tall conidiophore growth was adopted. The scale 1 - 4 was used to represent conidiophore density, with 4 being the density of conidiophore growth formed by cultures grown in full light on the medium in question.

1 2 & 3 CONTAIN SODIUM PYROGALLATE

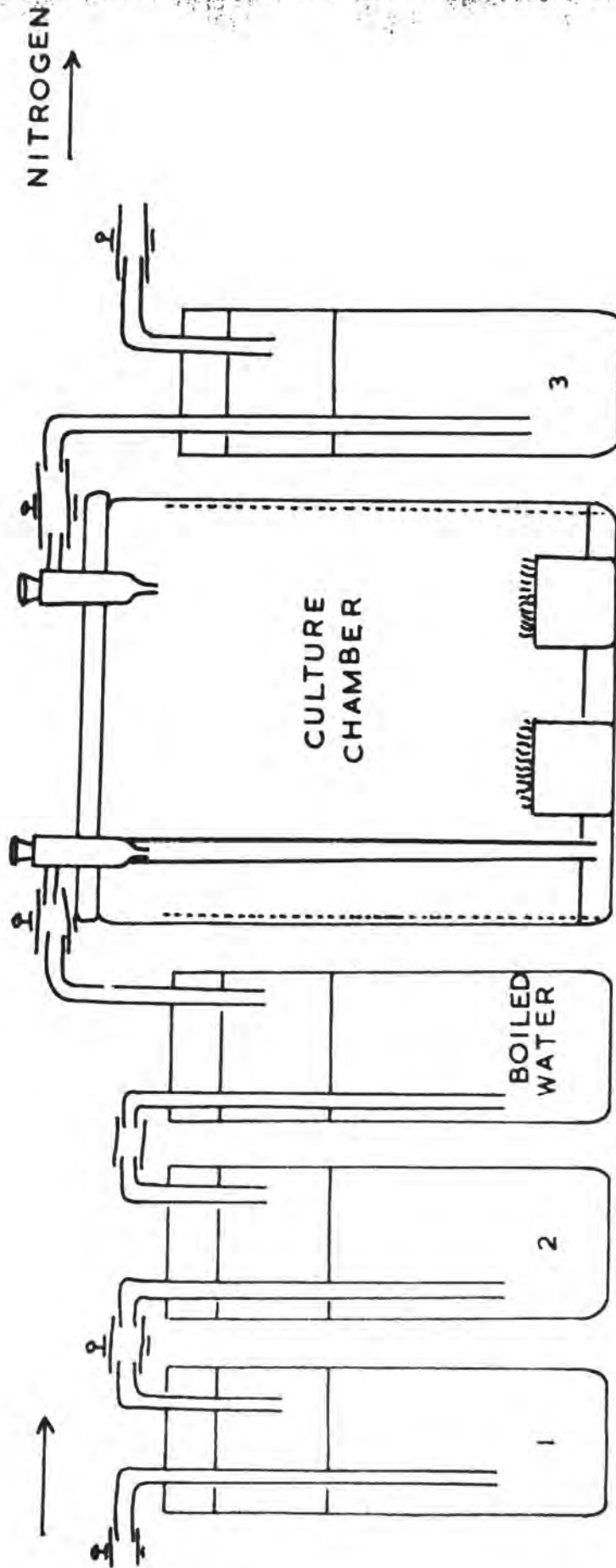


FIGURE 4 METHOD OF GROWING CULTURES IN NITROGEN

The dry weight of the conidiophores was determined on samples obtained by cutting them from the underlying mycelium with a razor blade. A good separation of the conidiophores from the mycelium could be obtained in this way.

8. METHODS USED IN QUANTITATIVE AND QUALITATIVE DETERMINATIONS OF CAROTENE CONTENT.

(a) Extraction procedures used in qualitative determinations.

The method used to extract carotenes from the fungus was as described by Goodwin (1955). The method is as follows:-

- (i) Fungal material ground to a fine powder with anhydrous sodium sulphate and acid washed silver sand.
- (ii) Powder transferred to a deep walled sintered glass crucible (GX4) and peroxide free ethyl ether added. Stirred.
- (iii) Ether filtered off. Repeated with successive portions of ether until all the pigment had been removed.

The extracts were then pooled.

- (iv) Solvent removed under reduced pressure.
- (v) Dry residue taken up in the appropriate solvent.

The concentrated pigment solution thus obtained was run on to the top of an alumina column and the component pigments separated by column chromatography as described.

(b) Quantitative determinations.

The method used for quantitative determinations of carotene content was one described by Hocking (1961).

It is a comparatively simple technique which does not involve chromatographic separation and subsequent treatment of individual carotene fractions. It can thus only be used effectively in instances where one particular carotene makes up most of the carotene content of a fungus. A quantitative determination of the individual carotenes present in Aspergillus giganteus has not been made but it is clear from visual examination of alumina columns that β -carotene is by far the most abundant carotene present. Maes et al. (1957) point out that it is a common practice in quantitative work to treat a mixture of carotene as if only one was present.

The various steps in the process are as follows:

- (i) Glass stoppered bottle weighed.
- (ii) Mycelium separated from the culture medium either by filtration or centrifugation.
- (iii) Mycelium washed with distilled water.
- (iv) Twenty-five mls of boiling distilled water poured on to the mycelium, left for about a minute, and then filtered off. This renders the protoplasm permeable and aids carotene extraction.
- (v) Mycelial pad transferred to the bottle and weighed.
- (vi) Ten, twenty or forty mls of acetone added and the bottle weighed again.
- (vii) Bottle tightly stoppered and placed overnight in a dark room at room temperature. Hocking found that there was no appreciable carotene degradation during

this period.

(viii) Aliquot of the acetone extract pipetted off and its optical density at 456 m μ determined using a spectrophotometer. One cm glass cells used with the blank containing acetone with 5% v/v distilled water.

(ix) Aliquot returned. Bottle placed in a vacuum oven and the acetone evaporated off using a water pump. Mycelium dried to constant weight at 80°C.

From the weights recorded the following can be calculated:

- I. Fresh weight of the mycelium.
- II. Dry weight of the mycelium, i.e. after the acetone has been evaporated off and the mycelium dried.
- III. Weight of water originally present.
- IV. Weight and hence volume of the solvent.

In the formula used to calculate the carotene content, the volume of acetone plus the volume of water present is used as the total volume of solvent. The formula is presented below.

Carotene content
expressed in $\mu\text{g}/\text{gram}$
dry weight of mycelium

$$\frac{\text{Optical density} \times \text{volume of extract} \times 10^6}{\text{Dry weight of mycelium} \times \text{Extinction Coefficient} \times 100 \text{ Mycelium}}$$

Jensen (1959) gives the extinction coefficient for β -carotene in acetone as 2490. Hocking found that saponification, or keeping the extract under nitrogen while left overnight in the dark, did not make a significant difference to the final result.

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SUMMARY

The tall conidiophores grow up from foot cells formed in the mycelium. The growth curve of the conidiophores is of the usual form; elongation is very slow at first but accelerates until the conidiophores reach their maximum rates of growth when they are c. 3 mm tall. Elongation is confined to the terminal portion of the conidiophore; the length of this zone in conidiophores taller than 3 mm is c. 200 μ . Conidiophores grown at low light intensities show oscillations along their length which probably result from the mutational nature of the growth of the tip; at high light intensities the conidiophores have a much straighter appearance. In some instances a slight clockwise twisting of the growing conidiophore tip was observed. There is a rapid reduction in the rate of conidiophore growth during vesicle formation; no further growth in length takes place after the vesicle has been formed. The rate of growth and the height of the conidiophores is influenced by light intensity. In suitable conditions conidiophores will continue to grow in darkness for many hours. The conidiophores do not show 'light growth reactions' of the kind found in Phycomyces sporangiophores.

The photochemical reaction involved in the light

induced growth of tall conidiophores is a 'low energy reaction', i.e. light acts as a 'triggering' stimulus. Only light of wavelengths below 530 m μ is effective in inducing conidiophore growth and the response is localised in those areas of the mycelium actually exposed to the radiation.

1. INTRODUCTION

Light is known to influence many aspects of the growth and development of fungi. Light sensitive processes in fungi which have been studied include:

(a) Biochemical differentiation. In all the systems described below, light must certainly induce biochemical differentiation, but in only a few cases have these metabolic changes been investigated. Cantino (1959) found that the light stimulated growth of Blastocladiella emersonii is associated with an increase in the organic phosphorus pool and a decrease in the inorganic pool. Light is known to influence carotenogenesis in many fungi. In some, e.g. Neurospora sitophila (Akagi, 1948) and Fusarium oxysporum (Carlile, 1956), light is essential for the induction of carotenogenesis. In others, light increases the amount of carotene produced, e.g. Phycomyces (Garton et al., 1951).

(b) Morphological differentiation. More work has been conducted on the influence that light has on morphological differentiation than on any other light induced process. Light influences the production of fruiting structures in a large number of species. In some fungi a period of illumination is essential for the formation of the fruiting body, e.g. in Pilobolus kleinii (Page, 1956) and

Sclerotinia sclerotiorum (Purdy, 1956). In others light increases the amount of fruiting, e.g. Diaporthe phaseolorum (Timnick et al., 1951).

(c) Changes in growth rate. The rate of growth of the sporangiophores of Phycomyces blakesleeanus and Thamnidium elegans (Lythgoe, 1961) is influenced by light, and Cantino (1959) found that the growth of Blastocladiella emersonii is greater in light than in darkness.

(d) Phototropism. A large number of fungi have structures which are phototropic, e.g. the sporangiophores of Phycomyces, the conidiophores of Conidiobolus villosus Martin, 1925) and the perithecial necks of Sordaria fimicola (Ingold & Hadland, 1959).

(e) Spore discharge. The discharge of spores may be influenced by light, e.g. light stimulates spore discharge in Sordaria fimicola (Ingold & Hadland, 1959) and inhibits spore discharge in Hypoxyylon coccineum (Ingold, 1953).

The investigation described below was undertaken to study the influence of light on the induction of conidiophore elongation and carotenogenesis in Aspergillus giganteus.

2. GROWTH IN LIGHT AND DARKNESS

(a) Types of growth produced in light and darkness

The type of growth produced by cultures of Aspergillus giganteus in light and darkness are strikingly different.

Nature of the growth produced by cultures in light. Cultures grown in light on 2% malt agar at 22-24°C produce tall conidiophores ranging in diameter from 35-50 μ . When cultured under suitable conditions these conidiophores continue to elongate for many days and may eventually attain a height of over 50 mm on 2% malt agar and over 80 mm on Zurzyka agar. Plate 3 is a photograph of a culture grown for three weeks in light on Zurzyka agar. It may be seen from this photograph that very few tall conidiophores have formed fruiting heads. Tall conidiophores produced by cultures grown on Zurzyka agar are narrower (c. 25-35 μ) than those produced on 2% malt agar, but the number of tall conidiophores produced per unit area of medium surface is much greater on Zurzyka agar than on 2% malt agar.

When a microscopic examination is made of the mycelium close to the medium the presence of a number of short conidiophores c. 1 mm in height are revealed. They have usually formed fruiting heads and their stalks are narrower (c. 8-15 μ) than the tall conidiophores. The density of these short conidiophores in cultures grown in light varies with environmental conditions and the inoculation technique employed. Plates 4a and 6 are side view photographs of cultures growing in light on 2% malt agar. Plate 4a shows

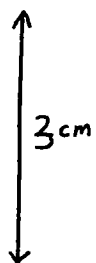


PLATE 3 Culture grown for three weeks in light
on Zurzyka medium.



the presence of short conidiophores with fruiting heads alongside stalks of tall conidiophores.

Nature of the growth produced by cultures in darkness. When cultures which have been grown in darkness for a week on 2% malt agar are examined it is found that a large number of short fruiting conidiophores c. 1 mm tall have been produced, but there is usually no visible sign of taller conidiophores.

Occasionally, however, a few tall conidiophore are produced by cultures grown in darkness. These conidiophore are never taller than c. 3 - 4 mm and they usually have not formed fruiting heads.

Microscopic examination of cultures revealed that a few tall conidiophore initials are usually present in cultures grown in darkness or red light but they fail to elongate. On Zurzyka agar quite a large number of tall conidiophore stalks are formed by cultures grown in darkness and they tend, eventually, to obscure the presence of the shorter fruiting conidiophores below them. A number of these tall conidiophore per unit surface area of the culture medium appear to be much less than that of cultures grown in light, and they never grow more than c. 6 - 7 mm tall. Plates 4b,c and d are photographs of cultures grown in darkness and red light.

(b) Mycelial growth

Experiment 1 to determine if light influences the rate of mycelial growth.

Experimental procedure. The fungus was cultured in plastic



PLATE 4a Cultured in light for forty hours on 2% malt agar.



PLATE 4b Cultured in darkness for three days on 2% malt agar.

100 μ



PLATE 4c Cultured in red light for five days on 2% malt agar



PLATE 4d Cultured in darkness for three days on 2% malt agar.

pre-sterilized petri dishes containing 20 mls of 2% malt agar. The media were inoculated with single drops of a spore suspension dispensed from a hypodermic syringe. Cultures were maintained in darkness by wrapping them in aluminium foil, while the cultures exposed to light were placed in transparent plastic bags. This ensured that both sets of cultures had approximately the same degree of aeration. There were five cultures in each set and the experiment was carried out in incubators maintained at 28°, 30° and 32°C. The light intensity at the level of the cultures was 3.4 foot candles or 36 metre candles.

Results. The mean diameter of the colonies in each set was calculated seven days after inoculation and the results are presented in Table 2.

TABLE 2

Influence of light on mycelial growth

Temp.	Colony diameter in mm.	
	Cultures grown in light	cultures grown in darkness
28°C	56, 56, 57, 55.5, 55.5 Mean 56.0	56, 55, 55.5, 56, 56. Mean 55.7
30°C	57, 57.5, 58, 58, 58. Mean 57.7	58, 57.5, 58.5, 58, 58. Mean 58.0
32°C	54, 55, 55, 55, 55. Mean 54.8	54, 55, 55, 54, 55.5. Mean 54.7

(c) Dry weight

The following experiment was conducted in order to determine if there is any difference in the dry weight of cultures grown in light and darkness.

Experiment 2 to determine if there is any difference in dry weight between cultures grown in light and darkness.

Experimental procedure. The cultures were grown in 100 ml flasks containing 15 mls of Zurzyka solution. They were divided into two sets with ten replicates in each. One set was cultured in light in a light box (light intensity at the level of the cultures was 8.5 foot candles or 91 metre-candles) while the other set was cultured in darkness in the same room. The experiment was carried out at $22 \pm 1^{\circ}\text{C}$.

Results. The dry weight of each culture was determined three weeks after inoculation and the mean value for each set calculated. The results are presented below in Table 3.

TABLE 3

Influence of light on dry weight

Set	Culture conditions	Dry weight in milligrams
1	Light	120, 125, 130, 129, 126. Mean 126
2	Darkness	120, 115, 114, 124, 112. Mean 117

Discussion of the results of Experiments 1 and 2.

Various workers have found that light influences the rate of mycelial growth of some fungi. Carlile, (personal communication), found that under optimal conditions the rate of growth of Sclerotinia fructigens is faster in darkness than in light, but under sub-optimal conditions the reverse is true. It is thus of some importance to consider the culture conditions when one is studying the influence of light growth. It was for this reason that experiment 1 was conducted at temperatures near the optimum for mycelial growth. It would appear from the results obtained that light has no influence on the rate of mycelial growth.

In experiment 2 it was found that when growth is determined on a dry weight basis it is slightly greater in cultures grown in light.

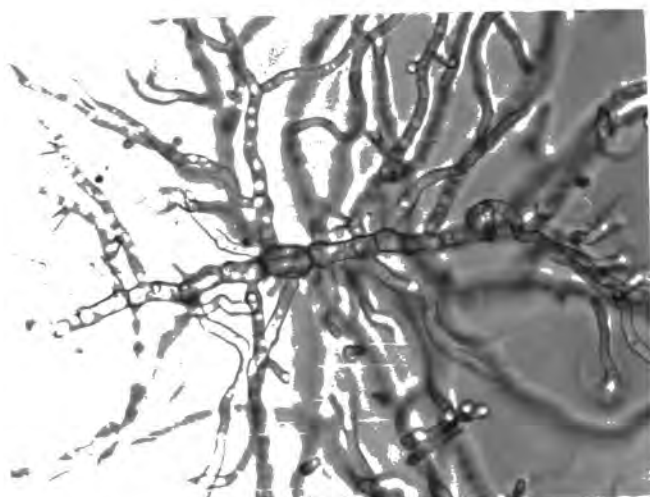
3. TALL CONIDIOPHORE GROWTH

Production and Elongation.

(a) INITIATION FROM FOOT CELLS

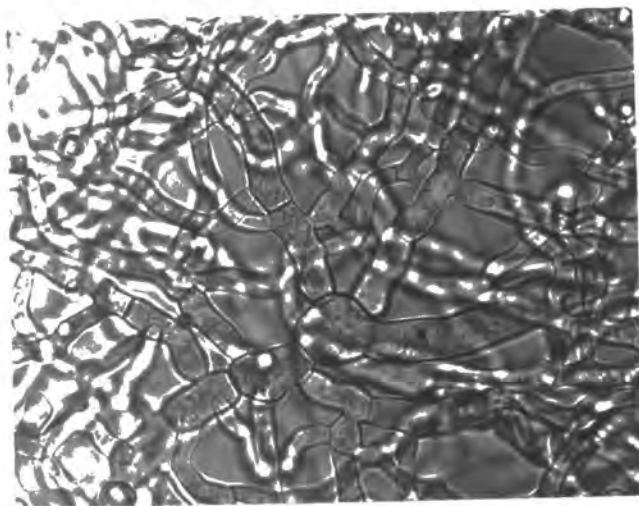
A feature of the Aspergilli which distinguishes them from the Penicillia is the presence of foot cells at the base of the conidiophores. Both the short and the tall conidiophores of Aspergillus giganteus arise from these special cells.

Plates 5a and 5b are photographs of foot cells formed on 2% malt agar at 32 - 32^oC. The photographs were taken 22 hours



Scale
100 μ

PLATE 5a



Scale
100 μ

PLATE 5b

Foot cells produced after 22 hours growth at 32 C
on 2% malt agar.

after inoculation. Plate 5a shows a chain of foot cells, while in Plate 5b the origin of a conidiophore from a foot cell can be detected. . . Soon after germination the hyphae which have grown out from the spores become septate; the divisions so formed swell to form foot cells. Attempts were made to determine if the tall and short conidiophores are produced by the same type of foot cell, but this point is still not clear. The development of the first tall conidiophores from the mycelium coincides with the location of the inocula. The photograph of Plate 6 shows tufts of tall conidiophores arising from small mounds which mark the position of groups of inoculating spores. Short conidiophores were also produced by small foot cells formed at the ends of aerial hyphae.

The foot cells develop a yellow pigment and become more deeply coloured with age; the surrounding mycelial strands are not nearly so deeply coloured. The pigment photoreceptor involved in conidiophore elongation is probably present in these cells.

Swollen cells. When cultures are grown on Zurzyka agar, chains of swollen cells can sometimes be detected in the mycelium. One can also find a few of these cells on 2% malt agar media. They arise from swellings behind the growing tips of the mycelial strands and do not appear to be foot cells. Plate 7 is a photograph of these cells developed on Zurzyka agar.

Scale 100 μ



PLATE 6 Tufts of tall conidiophores arising from inocula sites

Scale 100 μ
↔

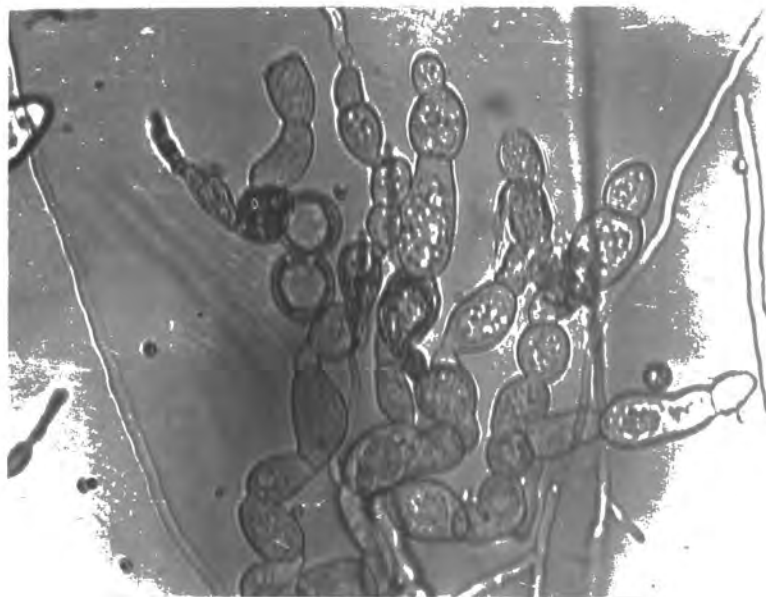


PLATE 7 Swollen cells formed on Zurzyka
medium.

(b) TYPE OF INOCULATION

Experiment 3 to study the type of growth resulting from different inoculation procedures.

Experimental procedure. Petri dishes containing 2% malt agar and Zurzyka agar were prepared. Half of each group was inoculated with small pieces of mycelium growing on nutrient agar while the other half was inoculated with drops of a spore suspension. Each group of cultures was further sub-divided into two sets, one of which was grown as an illuminated incubator at 22°C while the other was cultured in a similar incubator at 32°C. The light intensity at the level of the cultures was 3.4 foot-candles or 36 metre-candles.

Results. The cultures were examined five days after inoculation and the type of growth produced from each type of inoculum is recorded in Table 4. Short conidiophores were particularly abundant at the site of inoculation with a spore suspension. Growth produced from mycelium inocula or outside the sites of spore suspension inocula did not support as many short conidiophores.

Discussion of the results. The reason for the production of large numbers of short conidiophores from the region of inoculation with a spore suspension is not clear. A somewhat similar result may be found in

TABLE 4 Conidiophore production following different inoculation techniques

Type of inoculum	Type of conidiophore	2% Malt agar		Zurzyka agar	
		22° C	32° C	22° C	32° C
Small pieces of mycelium growing on agar	Tall	Present	Present	Present	Present
	Short	No visible sign	Very few	No visible sign	Present
Central drop of spore suspension	Tall	Present	Present	Present	Not many
	Short	Present, dense in area of inoculation	Present, dense in area of inoculation	Present but not abundant	Little growth

Phycomyces blakesleeianus. In this fungus the sporangiophores formed at the site of inoculation are more slender than those subsequently produced. It would be interesting to determine the variation in the density of short conidiophore production with the density of the spore suspension.

(c) CONIDIOPHORE GROWTH

Experiment 4 to determine the rate at which conidiophores of different heights elongate.

Experimental procedure. Small culture cells containing 2% malt agar were prepared and then placed in the observation cells described on page 5 . They were inoculated with a spore suspension and then placed in an illuminated incubator maintained at 22°C.

The cultures were transferred to the stage of the horizontal microscope 36 hours after inoculation. They were illuminated directly from above by a beam of white light with an intensity of 20 foot-candles. A cine record was made of the subsequent growth of the conidiophores; photographs were taken at fifteen minute intervals. The red light by which the photographs were taken was left on for the duration of the experiment, which was carried out at 22 - 23°C.

Results. Records of the growth of a number of conidiophores were obtained in this way, and from these results the rate of elongation of conidiophores of different heights was obtained. The mean rates of elongation were

calculated and the results are presented in graph form in Figure 5.

A growth curve of the tall conidiophores was also obtained from the cine record described above and this is presented in Figure 6.

Discussion of the results. The growth curve of tall conidiophores cultured at 22-23°C appears to be of the usual form. The growth rate is very slow at first but increases progressively until, when they are about 3 mm tall, it reaches a maximum of c. 9 μ /minute or c. 540 μ /hour. The conidiophores continue to grow at this rate for a number of hours.

Gardner (1955) states that "In cultures dark-conditioned two, three and four days, (tall conidiophore) elongation begins, in each case, after a (further) half day in light". She concludes that there is a time lag of c. 12 hours between illuminating the cultures and the onset of conidiophore elongation. It is difficult to understand why there should in fact be such a long time lag. The conidiophore growth curve presented in Figure 6 helps to clarify this point. There is probably very little time lag between illumination and the induction of conidiophore growth - certainly not one of 12 hours. Gardner presumably determined the beginning of conidiophore elongation from the time when conidiophore initials were first visible to the naked eye; there is

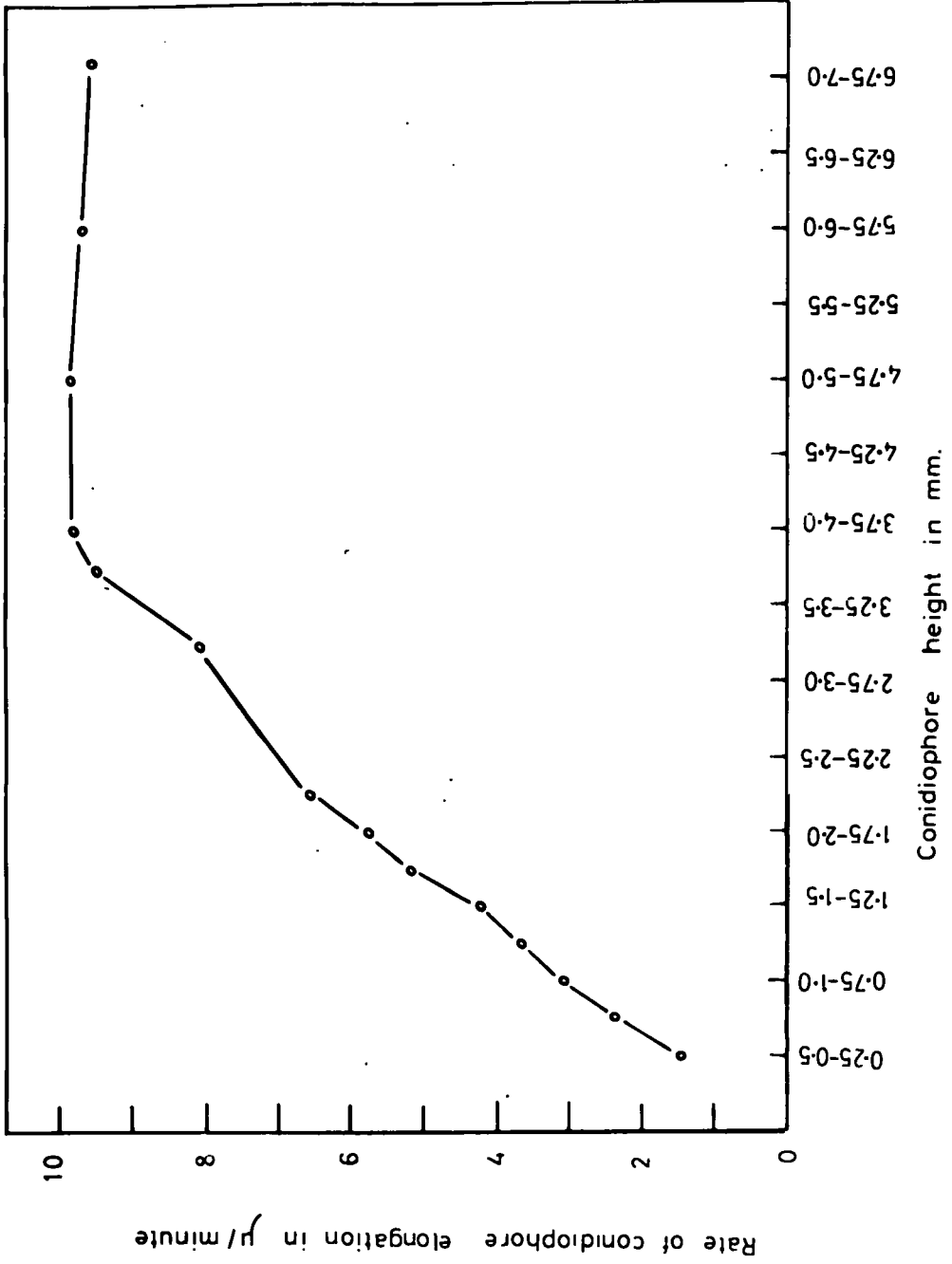


FIGURE 5 RATES AT WHICH CONIDIOPHORES OF DIFFERENT HEIGHTS ELONGATE

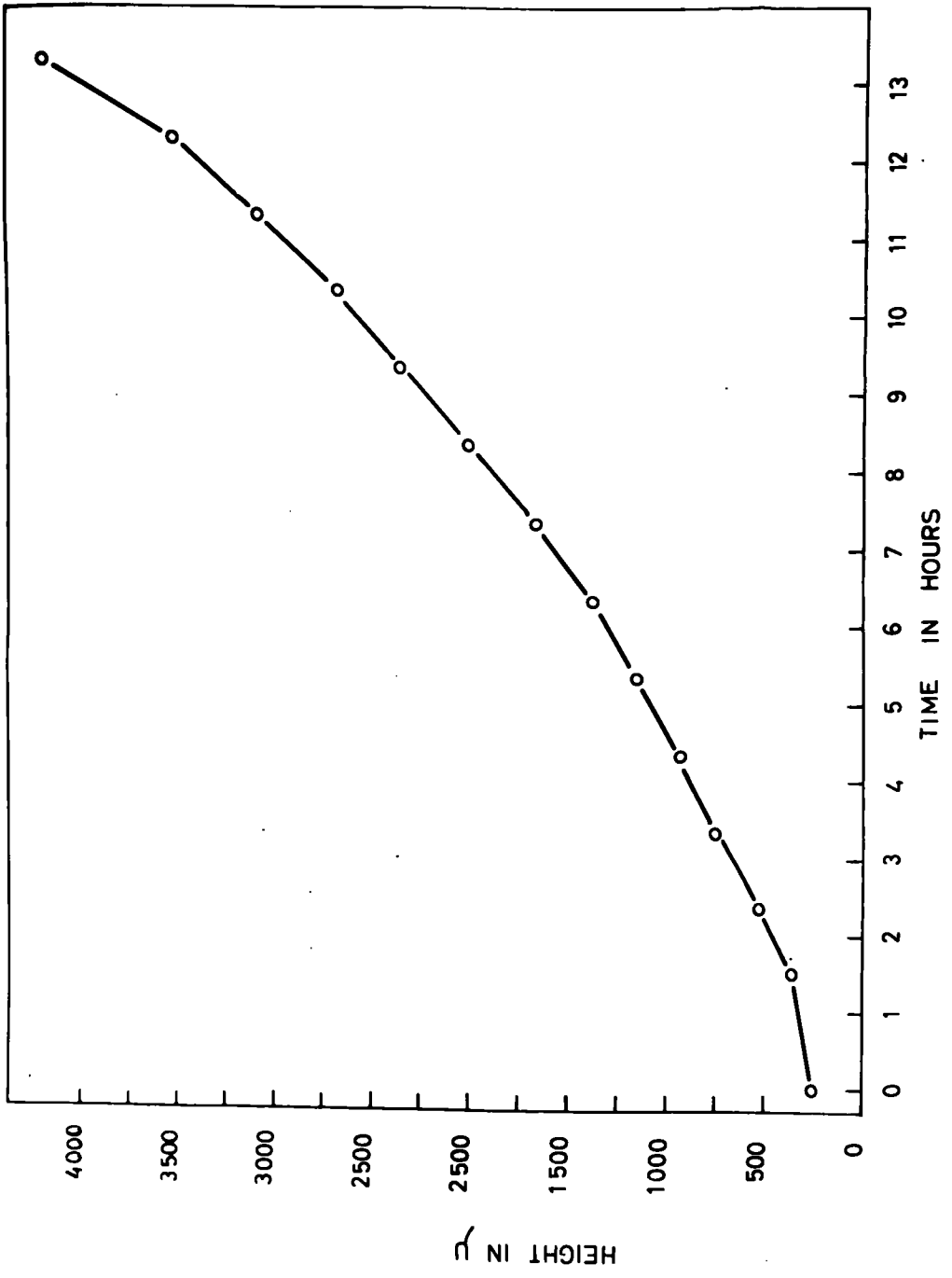


FIGURE 6 CONIDIOPHORE GROWTH IN HEIGHT

no record in her thesis of any microscopic examination of growth. During the first few hours after photoinduction of conidiophore elongation the rate of conidiophore growth would be very slow; it may be seen from Figure 6 that conidiophores 250 - 500 μ tall elongate at the rate of c. 90 μ /hour, shorter conidiophores would of course grow even slower. Hence it is not unlikely that it would take a conidiophore c. 12 hours growth after induction before it actually becomes visible to the naked eye and this might well explain why Gardner thought there was a long time lag after induction before the onset of conidiophore elongation. Because of this finding it was decided to change Gardner's definition of the induction period from "The time lag between inoculation and the beginning of conidiophore elongation" to "The time lag between inoculation and the appearance of tall conidiophores."

(d) ZONE OF CONIDIOPHORE ELONGATION

Experiment 5 to determine the length of the elongation zone of the tall conidiophores.

Experimental procedure. A number of 2" x 1" specimen tubes containing c. 5 mls of 2% malt agar were prepared and inoculated with a spore suspension. The cultures were cultured in light at a temperature of 21°C. The experiment was conducted with 3 - 5 mm tall conidiophores, i.e. conidiophores which had attained their maximum rate

of elongation. The conidiophores were marked by puffing starch over the specimen tube so that the grains drifted on to the conidiophores. A cover slip was then placed over the mouth of the specimen tube and the tall conidiophores were examined with a horizontal microscope. The cultures were illuminated from above.

A conidiophore suitably marked with starch grains was selected for observation; in order to determine the length of the elongation zone the conidiophore needed to be marked with a starch grain near its tip and have a second just below the first. The selected conidiophore was drawn using the camera lucida shown in Figure 2b, and further drawings were made at ten minute intervals until it was certain that both starch grains had passed out of the elongation zone.

The length of the elongation zone was calculated from these drawings. The distance between the two starch grains was measured on each drawing with a ruler; the distance between the starch grains increased while the upper starch grain was still in the elongation region. When it passed out of the elongation zone the distance between the markers remained constant. It was thus possible from the drawings to determine when the upper starch grain passed out of the region of elongation, and the length of the conidiophore tip above the marker that had ceased movement represented

the region of elongation. This region was measured on the drawings and its actual length was calculated. The maximum and minimum lengths of the elongating zone of twelve conidiophores were calculated in the manner described above and the mean of each value was determined. The results are presented in Table 5.

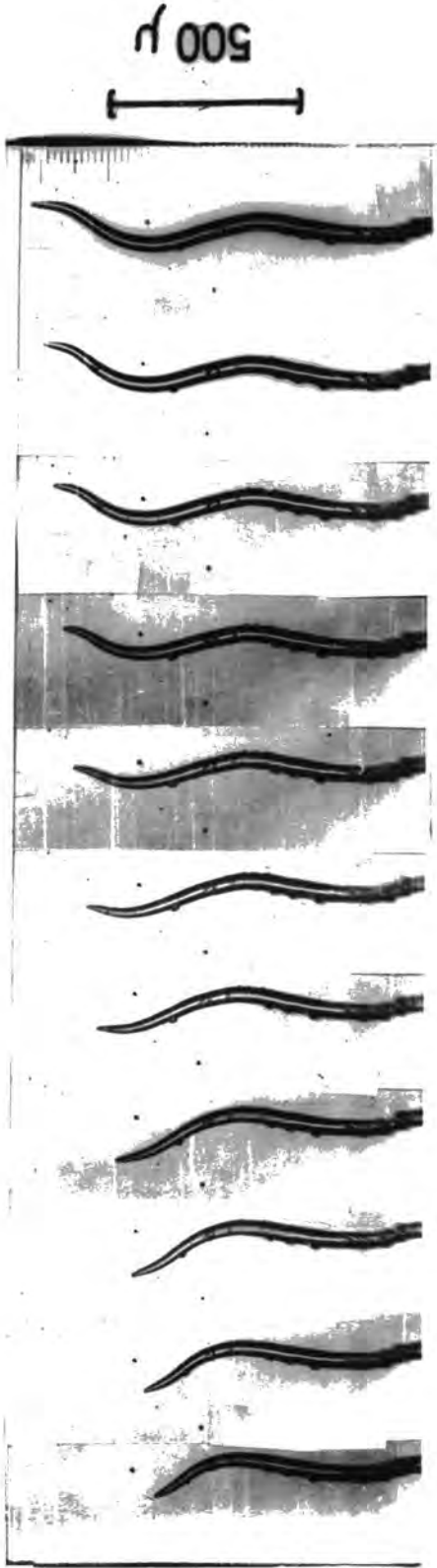
TABLE 5

Length of the elongation zone of the tall conidiophores

Minimum possible length of the elongation zone in μ	140, 110, 180, 150, 175, 140, 110, 170, 210, 160, 190, 180	Mean 160 μ
Maximum possible length of elongation zone in μ	200, 220, 225, 200, 250, 190, 160, 190, 275, 200, 210, 220	Mean 221 μ
Conidiophore width in μ	40, 40, 50, 55, 45, 50, 50, 55, 50, 45, 50, 40	Mean 47 μ

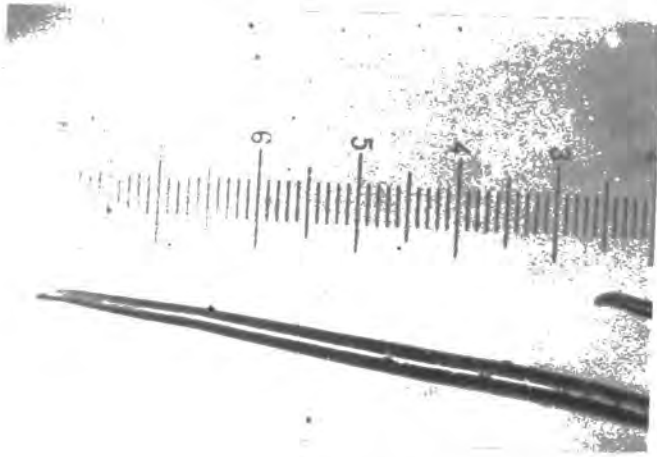
(e) OSCILLATORY AND SPIRAL GROWTH

(i) Oscillatory growth. Time-lapse photographs of conidiophores growing at different light intensities were taken. They revealed that conidiophores growing at low light intensities (2 foot-candles) exhibited slow sinusoidal oscillations about the vertical axis of growth. These oscillations had a fairly constant wavelength.



Photographs taken at intervals of 5 minutes

FIGURE 8 CONIDIOPORE GROWTH AT LOW LIGHT INTENSITY



Scale 100 μ

PLATE 9c Cultured in light of 20 foot-candles intensity



Scale 100 μ

PLATE 9b Cultured in light of 2 foot-candles intensity



Scale 100 μ

PLATE 9a Cultured in light of 2 foot-candles intensity.

Plate 8 is a series of photographs taken at five minute intervals of the growth of a single conidiophore. These photographs clearly show the snakelike side to side movements during conidiophore growth at low light intensities. Plates 9a and 9b are photographs of conidiophores grown at light intensities of 2 foot-candles and these also show the sinusoidal appearance of the conidiophores.

When cultures are grown at light intensities of 20 foot-candles this snakelike growth is markedly reduced. Some conidiophores do show a slight side to side movement but most appear to grow in an almost straight line vertically upwards towards the overhead illuminating source. Conidiophores cultured at high light intensities have the straight appearance shown by the conidiophore in the photograph of Plate 9c.

(ii) Conidiophore coiling. When cultures grown in petri dishes are illuminated from above the tall conidiophores grow in coils against the upper glass dish of the petri dish. Conidiophores coiling upwards against the petri dish lid are shown in the photographs of Plates 10a and 10b.

(iii) Spiral growth.

Experiment 6 to determine if tall conidiophores grow spirally.

Experimental procedure. The conidiophores were cultured on 2% malt agar in the observation cells described on page

Scale

100 μ



PLATE 10a Conidiophores coiling against
the lid of a petri dish.

Scale

100 μ



PLATE 10b Conidiophores coiling against
the lid of a petri dish.

The light intensity was 20 foot-candles and the temperature was 22-24°C. The conidiophores were marked by puffing starch grains over them, and the cultures were then examined using a horizontal microscope. The conidiophores were examined and one which was suitable marked at its tip was selected for observation. The conidiophore was then drawn using the camera lucida shown in Figure 2b and further drawings were made at regular intervals. The treatment described above caused some of the conidiophores to stop growing, and often even when they continued to elongate it was clear that growth had been interfered with.

Results. Elongated conidiophores marked with starch grains are shown in the drawings of Figure 7a and in the series of photographs of Plate 11. The movement of starch markers around the tip of the conidiophore tip is shown in Figure 7b which is a diagram showing the position of the markers when views from above the conidiophore. It may be seen from this diagram that when viewed from this position the markers moved in a clockwise direction around the conidiophore tip. When conidiophores exhibited spiral growth it was always in a clockwise direction. No reversal of spiral growth prior to vesicle formation was observed; nothing comparable to the counterclockwise rotation of the sporangiophores of Phycomyces after sporangium formation, as first reported by Castle (1942), was detected.

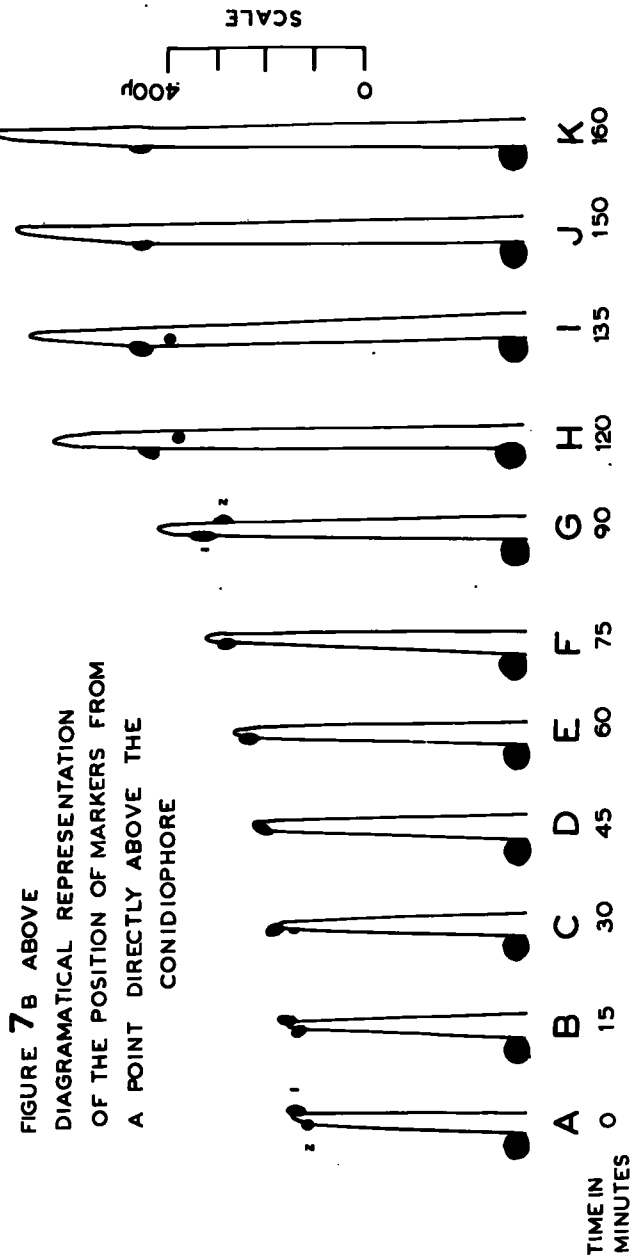
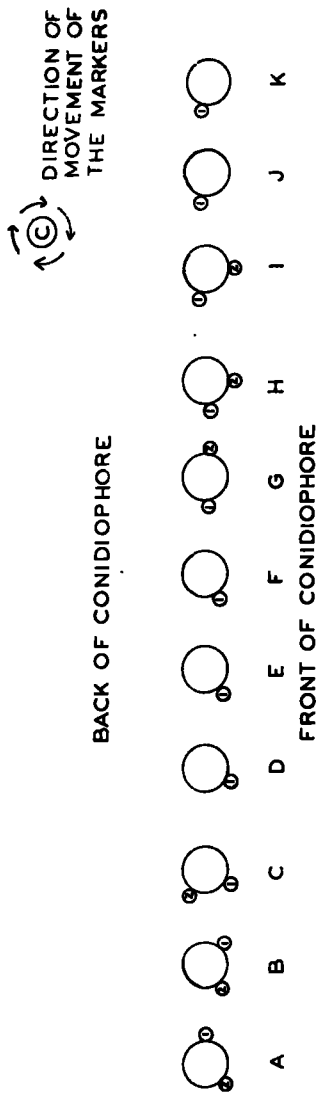
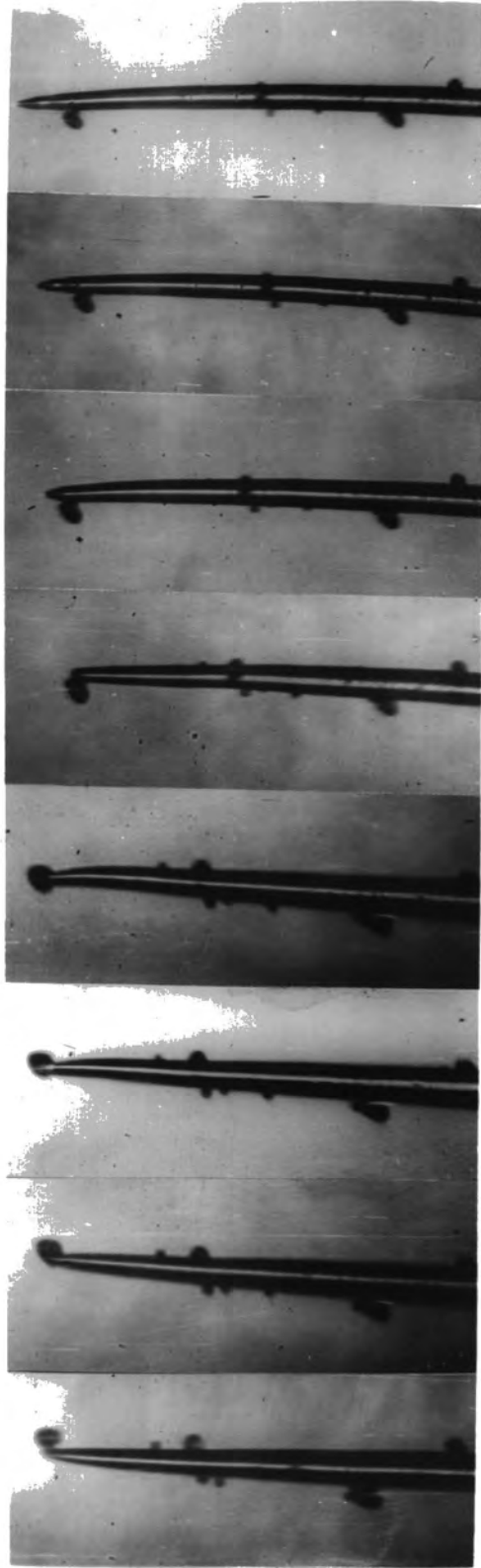


FIGURE 7A CAMERA LUCIDA DRAWINGS OF AN ELONGATING CONIDIOPHORE MARKED WITH STARCH GRAINS

100y
↔

**PLATE 11 Right and left sides of the conidiophore are reversed in
the photographs.**



This absence accords with the fact that there is no development of a growth zone below the vesicle after this swelling is formed, and, indeed, no elongation after vesicle formation.

Plate 11 shows a series of photographs taken during growth of a conidiophore marked with starch grains. The markers appear from the photographs to be rotating in an anticlockwise direction but as the sides are reversed in the photograph this was in fact not the case.

The maximum movement observed of any marker was about 180° . Some conidiophores which were suitably marked and continued to grow after marking did not show this spiral rotation.

(f) FORMATION OF EXUDATION DROPLETS BY THE CONIDIOPHORES

Conidiophores growing in the culture cells described on page were observed forming small exudation droplets behind the conidiophore tip. The droplets enlarge and often coalesce with each other in the older parts of the conidiophore. Occasionally an extra large droplet is formed and this causes the conidiophores to collapse. As the conidiophores grow older the exudation droplets become yellow, probably due to the secretion into them of carotenes. When conidiophores are exposed to air and the exudation droplets evaporate, their former locations are marked by the presence on the conidiophore wall of fibrous structures forming bulges.

That the droplets formed on the conidiophores are exudation and not condensation droplets is clearly demonstrated



Scale
↔
100 μ

PLATE 12 Exudation droplets
formed on a conidiophore

by the fact that they continue to be produced while the conidiophores are submerged in liquid paraffin (see page 191 and Plate 21). Plate 12 is a photograph of a conidiophore which has formed exudation droplets; it may be seen that small droplets have begun to form c. 1 mm below the tip.

(g) VESICLE FORMATION

Tall conidiophores elongate in light for a period of time, the duration of which depends upon such environmental factors as temperature, light intensity and relative humidity. Eventually the tip of the conidiophore swells, forms a vesicle and then a fruiting head.

Vesicle formation was observed by both the camera lucida and the time lapse photographic techniques. The cultures were grown on 2% malt agar in observation cells. The light intensity was 20 foot-candles and the temperature was $24 \pm 1^{\circ}\text{C}$.

The first indications that a conidiophore is about to produce a vesicle are that the rate of elongation decreases and the growing tip of the conidiophore becomes more rounded and club shaped. The rapid reduction in the rate of elongation during vesicle formation is shown in Figure 8. The graph was drawn from the mean of two sets of readings. It may be seen that the rate of conidiophore elongation fell from c. 12 μ /minute to 1 μ /minute in an hour.

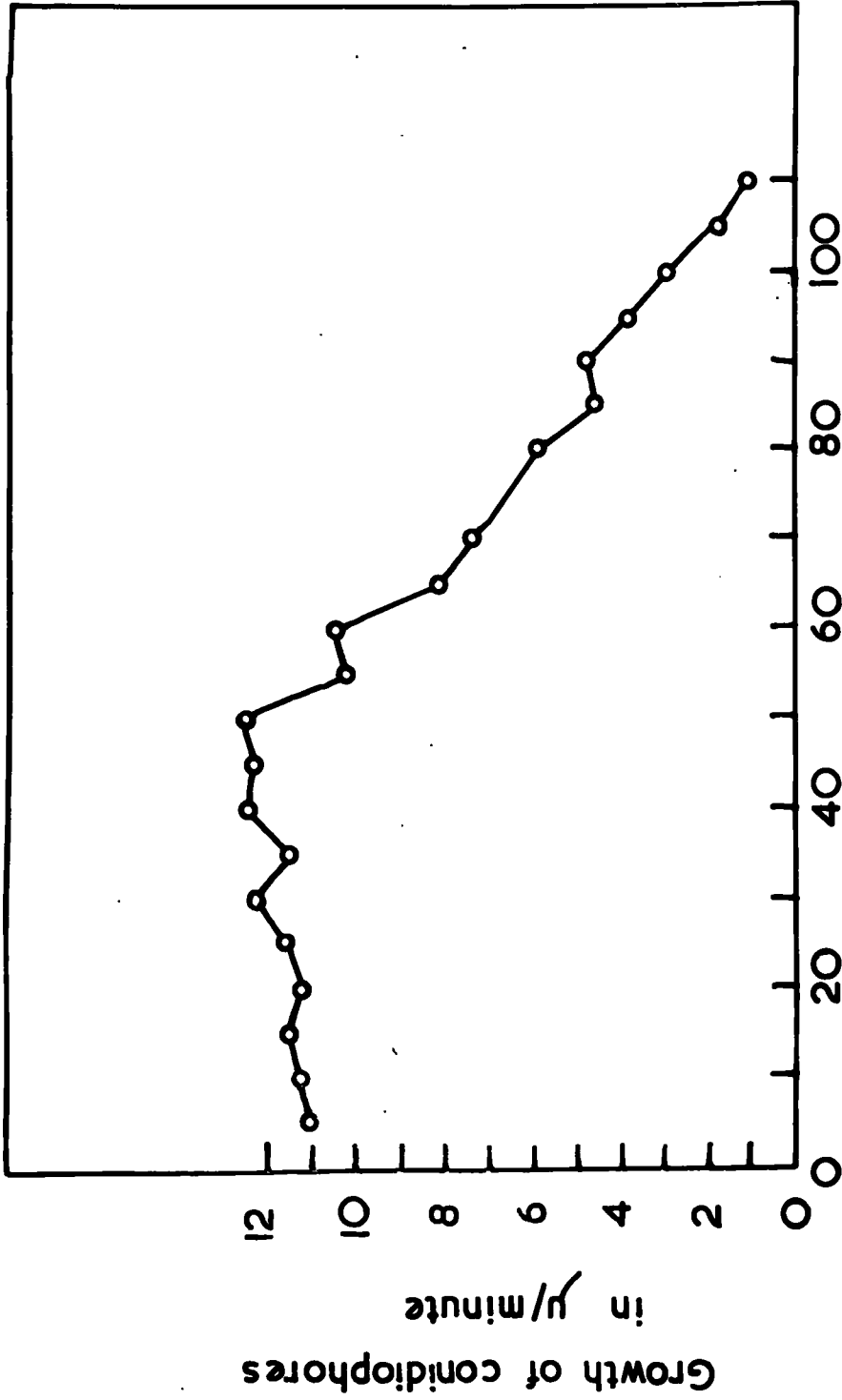


FIGURE 8 CONIDIOPHORE GROWTH DURING VESICLE FORMATION

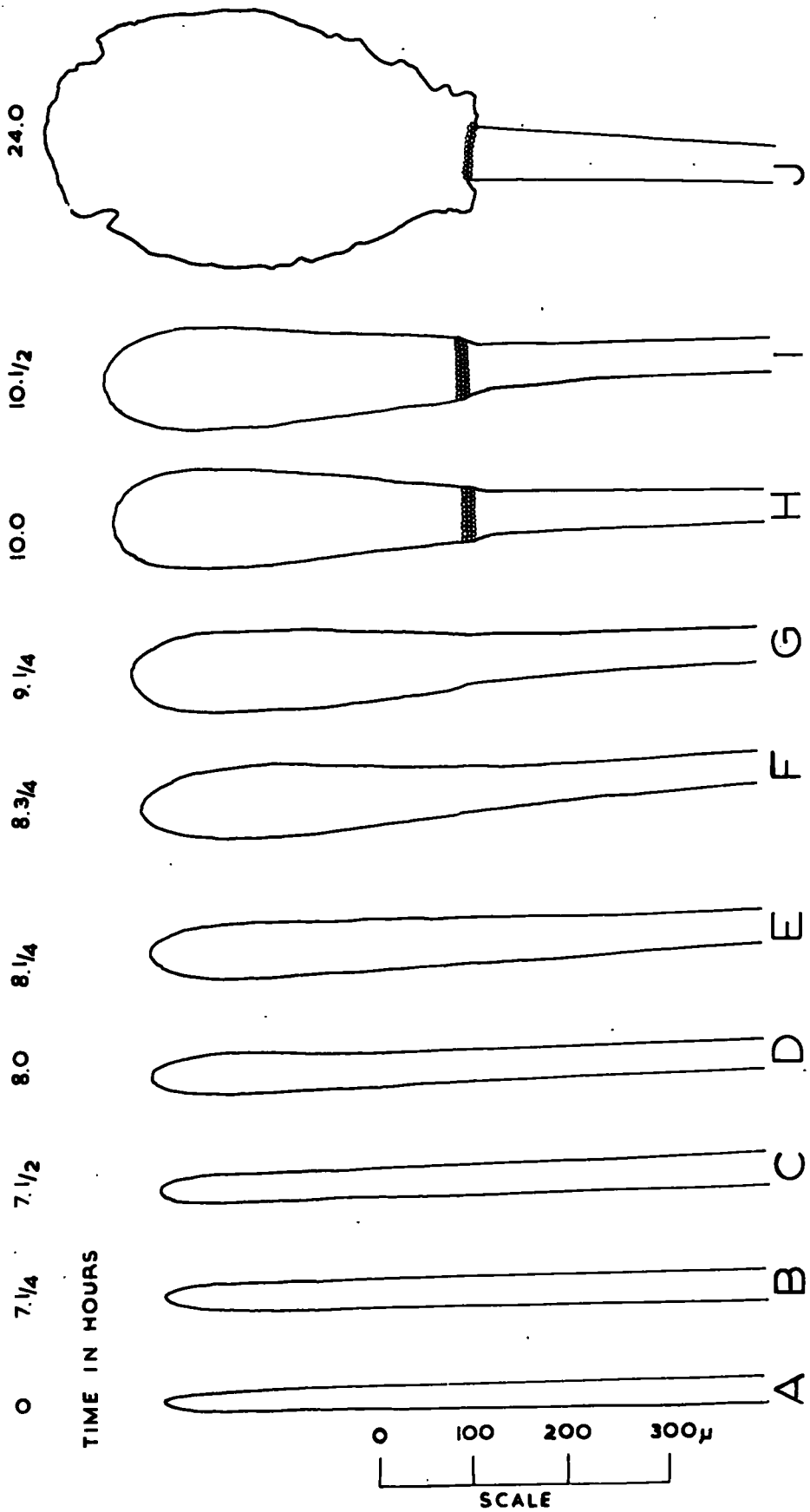


FIGURE 9

At the end of this period the conidiophores continue to elongate slowly due to the swelling at their tips as the vesicles are formed but once they are fully formed there is no further growth. Figure 9 is a set of camera lucida drawings showing swelling of the conidiophore tip during vesicle formation. It may be seen from these drawings that the fully formed vesicle (H) was formed c. 2.3/4ths hours after the conidiophore tip had started to swell. The vesicle does not become divided off from the conidiophore by a septum. Sterigmata formation starts at the vesicle base and the spores cut off from the eventually give the conidiophore head its characteristic shape.

(h) TYPES OF VESICLES PRODUCED

A detailed study was made to determine the variation in vesicle size and shape with conidiophore height. Fruiting heads of conidiophores of known height were mounted in cotton blue in lactophenol and the width and length of each vesicle was measured with a micrometer eyepiece. The width of the conidiophore below the vesicle was also measured. The results of this study are recorded in Table 6.

TABLE 6

Variation in vesicle size with conidiophore height



Conidio- phore height in mm.	Number examined	Vesicle dimensions in μ			Vesicle types			Conidio- phore width
		Length	Width	Ratio L/W	C.	I.	E.	
3 - 6	33	303 ⁺ 39	101 ⁺ 12	2.9	31	2	0	36 ⁺ 4
7 - 10	32	358 ⁺ 64	119 ⁺ 18	3.0	25	4	3	43 ⁺ 7
11 - 15	31	470 ⁺ 61	136 ⁺ 22	3.5	9	5	17	51 ⁺ 6
15 - 20	31	511 ⁺ 94	148 ⁺ 20	3.5	12	10	9	53 ⁺ 9
20 - 29	28	550 ⁺ 80	153 ⁺ 21	3.6	3	5	20	64 ⁺ 9

The vesicles could be broadly classified into three types, although variation in shape probably forms a continuous series.

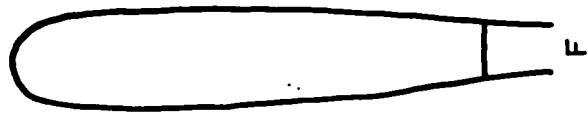
The three types are:

- (1) The clavate vesicle, examples of which are shown in drawings A, B and D in Figure 9a.
- (2) The intermediate vesicle, an example of which is shown in drawing E.
- (3) The elongate vesicle, an example of which is shown in drawing F.

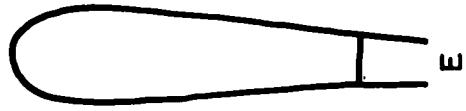
When the vesicles were measured they were classified into the above categories. The number in each group is recorded in Table 6 under the headings C, I and E. It may be seen from this Table that the shorter conidiophores generally have the clavate type of vesicle while the taller conidiophores have the elongate type. Thus the increase in spore bearing surfaces of the taller conidiophores is achieved by an increase in length rather than width of the vesicle.

Scale A  25 μ
 Scale B-F  200 μ

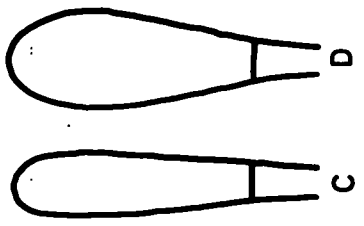
A
 Conidiophore height < 1 in mm.



30



20



10



6

FIGURE 9a. CAMERA LUCIDA DRAWINGS OF VESICLES

4. INFLUENCE OF VARIOUS LIGHT REGIMES

ON TALL CONIDIOPHORE GROWTH

(a) INFLUENCE OF CONTINUOUS ILLUMINATION OF DIFFERENT INTENSITIES.

Experiment 7 to determine the influence on tall conidiophore elongation and carotenogenesis of continuous illumination of different intensities.

Experimental procedure. Large oxoid caps containing Zurzyka agar were placed in 125 ml conical beakers capped with squat beakers. They were inoculated with a spore suspension and then divided into three sets with five replicates in each. Each set was placed into a separate light box and illuminated continuously with light of a particular intensity. A fan was placed near the bulb of the high intensity box to help to eliminate any heating effect that it might have had on the contents of the box. Maximum and minimum thermometers were placed inside the boxes which were in a room maintained at $23 \pm 1^{\circ}\text{C}$.

Results. The cultures were examined 15 days after inoculation. The mean height, dry weight and carotene content of the tall conidiophores in each set of cultures were determined and the results are presented in Table 7. In the cultures grown at the high light intensity, conidiophores with heads had been formed at two distinct levels. The mean height of the main group of conidiophores was 19 mm but a second sparser group was produced in each culture and the mean height of this group was 34.0 mm.

TABLE 7 Influence of light intensity on tall conidiophore production and carotenogenesis

Set	Light intensity	Tall conidiophores			Mean carotene content in μ gm/gm dry weight
		Height in mm.	Number of heads formed	Mean dry weight in milligrams	
1	1.0	55, 52, 55, 50, 53 Mean 53.0	none	181	265
2	8.9	50, 52, 54, 50, 54 Mean 52.0	considerable number	168	271
3	30.0	Main group at 19.0 but second group at 34.0	Nearly all the conidiophores had formed heads	157	362

Discussion of the results. It may be seen from the results presented in Table 7 that the carotene content, height and dry weight of the tall conidiophores is influenced by light intensity. In set 3, at the high light intensity, two layers of conidiophores with heads were produced; the main group was formed at c. 19 mm and a second group was produced at c. 34 mm. This second group of conidiophores was probably shaded by the first during its early growth.

Gardner (1955) found that there was no significant variation in conidiophore height over the light intensity range from 1 to 32 foot-candles, but she reported that she could not determine whether it was the light intensity or the temperature which resulted from it which depressed conidiophore growth at 135 foot-candles. The result of the present experiment, however, clearly indicates that high light intensities result in shorter conidiophores. The influence of light intensity on conidiophore height was confirmed during experiments in which conidiophore growth on 2% malt agar was studied in the observation cells. Conidiophores cultured at light intensities of 20 foot-candles were always shorter than those cultured at intensities of 2 foot-candles.

(b) SINGLE SHORT PERIODS OF ILLUMINATION

Experiment 8 to determine the influence on tall conidiophore elongation of periods of illumination given at different periods after inoculation.

Experimental procedure. Large oxoid caps containing 2% malt agar were placed in 125 ml conical beakers capped with 150 ml squat beakers. The cultures were inoculated with a spore suspension and then placed in darkness in a cupboard which was in a room maintained at a temperature of $22 \pm 1^{\circ}\text{C}$. At four hour intervals, from 34 - 62 hours after inoculation, a set of cultures was taken from the cupboard and illuminated in a light box. The light intensity at the level of the cultures was 8.5 foot-candles. At the end of each 4 hour period of illumination the set of cultures was returned to the cupboard.

Results. The cultures were examined two days after the last set had been illuminated. There were dense mats of very short fruiting conidiophores in the cultures of all the sets. In the majority of cultures there were also a few scattered tall conidiophores which ranged from 3 - 5 mm in height. Very few of these tall conidiophores had formed fruiting heads.

Experiments 9 and 10 to determine the influence on conidiophore elongation of single periods of illumination of different durations.

Experiment 9.

Experimental procedure. Large oxoid caps containing Zurzyka agar were placed in 125 ml conical beakers capped with 150 ml squat beakers. They were inoculated with a spore suspension and then placed in darkness in a room maintained at a temperature of $22 \pm 1^{\circ}\text{C}$.

Four days after inoculation the cultures were divided in dim red light into four sets with four replicates in each. Sets 2 - 4 were illuminated in a light box for the periods recorded in Table 8. The light intensity at the level of the cultures was 8.5 foot-candles or 91 metre-candles. After illumination the cultures were returned to darkness.


Results. The cultures were examined 3 days after illumination and the mean height of the tall conidiophores in each set was determined. The results are presented in Table 8. Plate 13 is a photograph of one culture from each set.

TABLE 8

Influence of single periods of illumination of varying duration on tall conidiophore production

Set	Duration of the illumination period (in minutes)	Mean height of the conidiophores (in mm)
1	0	4
2	1	7
3	10	8
4	100	8

3 cm



Illumination
period in
minutes -

0

1

10

100

PLATE 13 Influence of single periods of illumination on the
induction of conidiophore growth.



A considerable number of the tall conidiophores in the culture of sets 1 - 3 had formed heads; there appeared to be more heads in the cultures of set 1 than there were in sets 2 and 3. There was little sign of the presence of fruiting heads in all the cultures of set 4. Large numbers of short conidiophores with mature heads were revealed in all sets when the aerial growth of tall conidiophores and aerial mycelium was stripped from the mycelium.

Experiment 10

Experimental procedure. The cultures were grown on 2% malt agar in large oxoid caps placed in conical beakers capped with squat beakers. After inoculation the cultures were placed in darkness in a room maintained at $22 \pm 1^{\circ}\text{C}$.

Three days after inoculation the cultures were divided into four sets with five replicates in each. Sets 2 - 4 were then illuminated in a light box for the periods recorded in Table 9. The light intensity in the light box at the level of the culture was 8.5 foot-candles or 91 metre-candles. The cultures were returned to darkness after they had been illuminated.

Results. The cultures were examined three days after they had been illuminated and the mean height and density of the tall conidiophores in each set are recorded in Table 9.

TABLE 9

Set	Duration of the illumination period (in minutes)	Mean height of tall conidiophores (in mm)	Density of tall conidiophores
1	0	None produced	
2	1	8	1 +
3	10	7	1 -
4	100	7	1 -

Most of the surface area of the media was covered with a dense carpet of short conidiophores below 1 mm in height.

Discussion of the results of experiments, 8, 9 and 10. The results obtained in experiment 8 indicated that there is no definite period after inoculation which is particularly favourable for the induction of conidiophore elongation. It was shown in experiments 9 and 10 that the influence of a single period of illumination in inducing conidiophore elongation is not proportional to its duration; a period of illumination of one minute appears to be just as effective in inducing conidiophore elongation as a 100 minute period. The density of tall conidiophore production was not determined and it is possible that this is influenced by the duration of the exposure period.

The results obtained suggest that the light system involved in conidiophore growth is fully saturated by relatively short periods of illumination; light given in excess of this 'saturation minimum' does not increase the effectiveness of the exposure period.

(c) INTERMITTENT PERIODS OF ILLUMINATION

Experiment 11 to determine the influence on conidiophore elongation of daily periods of illumination of varying duration.

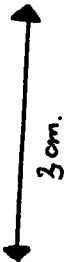
Experimental procedure. The cultures were grown on Zurzyka agar in large oxoid caps placed in 125 ml conical beakers capped with 150 ml squat beakers. They were inoculated with a spore suspension and then divided into 5 sets of five replicates. Three sets were placed into separate light-tight tins and these, together with a fourth set were placed in a light box. One of these sets was cultured continuously in darkness while each of the others received a daily period of illumination of 1, 10 or 100 minutes. A fifth set was placed in a separate light box and illuminated continuously. The light intensity at the level of the cultures was 8.5 foot-candles or 91 metre-candles. The light boxes were in a room maintained at a temperature of $23 \pm 1^{\circ}\text{C}$.

Results. The cultures were examined eighteen days after inoculation. The mean height, dry weight and carotene content of the conidiophores in each set was determined and the results are recorded in Table 10. Plate 13a is a photograph of the cultures.

Discussion of the results. In experiment 11 the height of the conidiophores above the medium in sets 2 - 4 was not directly proportional to their dry weights; the mean dry weight of the conidiophores in the set which received 100 minutes

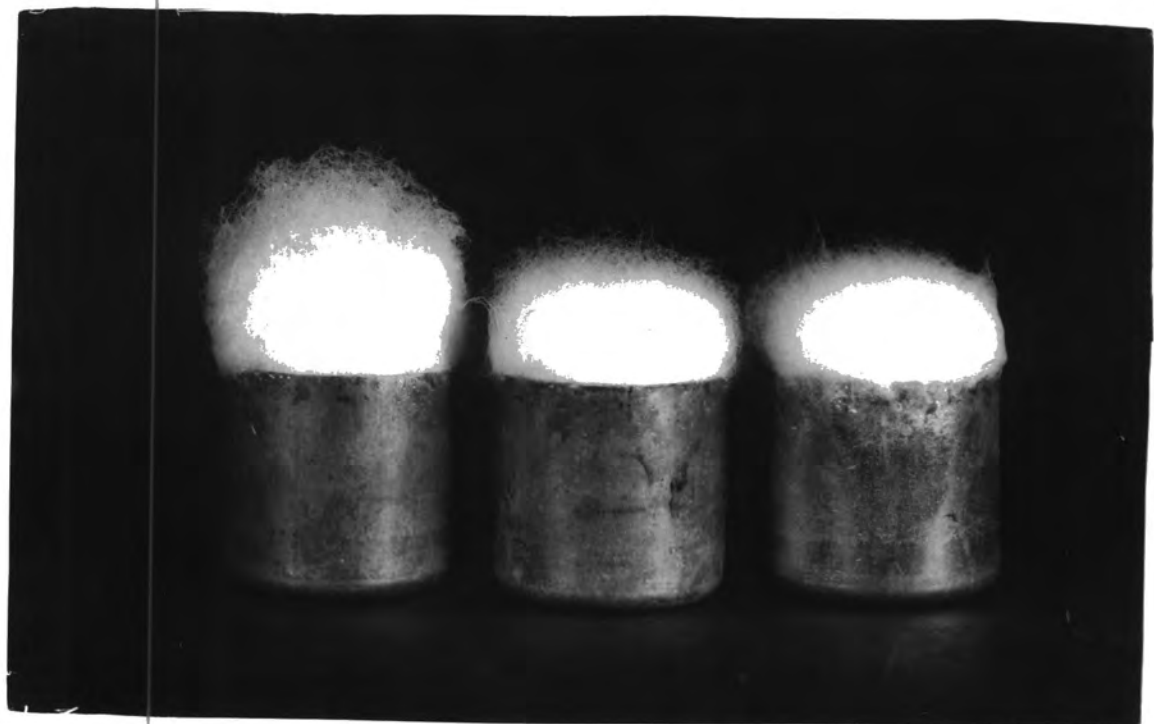
TABLE 10 Influence of daily periods of illumination of different durations on tall conidiophore growth and carotenogenesis

Set	Light Regime	Tall conidiophores						Carotene content as % of Set 1
		Height in mm. above medium	Mean dry weight in milligrams	Dry weight as % of Set 1	Mean carotene content in μ gm/gm dry weight			
1.	Cultured in continuous light	60, 55, 60, 55, 55. Mean 57.0	201	100%	230.0		100%	
2.	Cultures given 1-minute periods of light at 24 hour intervals	15, 15, 18, 16, 16. Mean 16.0	113	56.2%	54.0		23.7%	
3.	Cultures given 10-minute periods of light at 24 hour intervals	20, 22, 18, 18, 17. Mean 19.0	128	63.7%	103.0		44.8%	
4.	Cultures given 100-minute periods of light at 24 hour intervals	30, 28, 26, 26, 25. Mean 27.0	137	68.2%	222.0		96.5%	
5.	Cultured in darkness	Scattered tall conidiophores but all below 7 mm.						



Duration of daily illumination period in minutes	100	10	1
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PLATE 13a Influence on the induction of conidiophore growth of
daily periods of illumination of varying duration.



light per day was only about a fifth greater than that of the set which received 1 minute per day, although the height of the conidiophores in the former set was almost twice that of the latter. There is no gravitational orientation of conidiophore growth (see page 200), and it would appear from the results of this experiment that the extent of their vertical orientation varies with the duration of the illumination period. The greater height of the conidiophores above the medium in set 4 as compared to set 2 is probably due to the fact that there was greater vertical orientation of conidiophore growth in set 4. Dry weight determinations are thus a more reliable guide to the degree of induction of conidiophore growth than measurements of height.

It is clear from the results obtained that, within the limits of the experiment, conidiophore growth is not directly proportional to the duration of the daily illumination period. A light regime of one minute per day is sufficient to induce considerable conidiophore growth and increasing this to 100 minutes per day resulted in only a 21% increase in conidiophore dry weight.

In sets 2 and 3 there was approximately a doubling of the carotene concentration for each ten fold rise in the duration of the illumination period. Thus the influence of

the duration of the daily illumination period on conidiophore growth is different from its influence on carotenogenesis.

Experiments 12 and 13: to determine the influence on conidiophore elongation of periods of illumination of the same duration but different intensity.

Four separate experiments were conducted but the same procedure was employed for each.

Experimental procedure. Large oxoid caps containing Zurzyka agar were placed in 125 ml conical beakers capped with 150 ml squat beakers. They were inoculated with a spore suspension and then divided into 7 sets with 5 replicates in each. Two sets, one in a light-tight tin, were placed into each of the three light boxes. The cultures in the tins were illuminated for 1 minute per day; the other cultures in the light box were illuminated for 10 minutes per day. The light boxes were in a room maintained at a temperature of $22 \pm 1^{\circ}\text{C}$. (see discussion).

Results. The cultures were examined 18 days after inoculation. The mean height, dry weight and carotene content of the tall conidiophores in each set were determined and the results are presented in Tables 11a and 11b. There was no sign of orientation in the growth of the tall conidiophores in response to the direction of the light source under these conditions of short duration illumination. Only a very few conidiophores had formed fruiting heads.

TABLE 11/a Experiment 12

Light intensity in foot-candles	Set	Duration of daily illumination period	Tall Conidiophores			Mean carotene content in μ gm/gram dry weight
			Mean height in mm. above medium	Mean dry weight in milligrams		
8.9	7	1 minute	16.0	113	54.0	
	8	10 minutes	19.0	128	103.0	
2.0	9	1 minute	16.0	123	54.5	
	10	10 minutes	16.0	125	52.5	
1.0	11	1 minute	18.0	107	39.0	
	12	10 minutes	18.0	114	54.0	

TABLE 11b Experiment 13

Light intensity in foot-candles	Set	Duration of daily illumination period	Tall Conidiophores		
			Mean height in mm. above medium	Mean dry weight in milligrams	Mean carotene content in μ gm/gram dry weight
8.9	1	1 minute	16.0	84	43.0
	2	10 minutes	15.0	131	76.0
16.0	3	1 minute	16.0	85	53.0
	4	10 minutes	16.0	138	113.0
30.0	5	1 minute	12.5	97	44.0
	6	10 minutes	15.0	140	110.0

Discussion of the results of Experiments 12 and 13. A

comparison of Tables 11a and 11b shows that slight different results were obtained in the two experiments carried out at 8.9 foot-candles. Unfortunately during the second experiment the room temperature rose on occasions above 23°C. This probably accounts for the fact that the dry weight of the conidiophores illuminated for one minute per day in the first experiment was consistently lower than the results obtained in the second. Conidiophore growth, as determined by dry weight, varied only slightly over the range of light intensities studied. It is clear that, within the limits of the experiment, the induction of conidiophore growth is not greatly influenced by the light intensity of the daily illumination period. The results obtained of the influence of the intensity of the daily illumination period on carotenogenesis are somewhat difficult to interpret. It would appear that in some instances there was a marked reduction in carotenogenesis at the lower light intensities. At higher light intensities there was, in most instances, an approximate doubling of the carotene concentration for the tenfold rise in the duration of the period of illumination.

Experiment 14 to determine the influence on tall conidiophore elongation of illuminating cultures with light of the same intensity for the same total duration, but giving the light in different exposure periods.

Experimental procedure. The cultures were grown on Zurzyka agar in large oxoid caps placed in 125 ml conical beakers capped with 150 ml squat beakers. They were inoculated with drops of a spore suspension and then divided into four sets with five replicates in each. Each set was placed into a separate light box and grown under one of the light regimes listed in Table 12. The light intensity in the boxes at the level of the cultures was 8.5 foot-candles or 91 metre-candles. The experiment was carried out in a room maintained at a temperature of $21 \pm 1^{\circ}\text{C}$.

Results. The cultures were examined eighteen days after inoculation. The mean height, dry weight and carotene content of the conidiophores in each set were determined and the results are recorded in Table 12.

A few of the tall conidiophores in the cultures of set 1 had produced fruiting heads, but none had been formed by the conidiophores of sets 2 and 3. The conidiophores in the cultures of sets 1 and 3 grew vertically upwards from the medium, but those of set 2 exhibited no consistent directional orientation in their growth.

TABLE 12

Set	Light Regime	Height in mm. above medium	Tall Conidiophores				Carotene content as % of Set 1
			Mean dry weight in milligrams	Dry weight as % of Set 1	Mean carotene content in μ gm/ gm dry weight		
1.	Cultured in continuous light	55, 55, 55, 58, 52. Mean 55.0	198	100%	214	100%	
2.	Cultures given a single 25-minute period at 24 hour intervals	12, 16, 12, 16, 14 Mean 14.0	138	67.7%	95	44.4%	
3.	Cultures illuminated for 1 minute every hour	28, 26, 26, 26, 24 Mean 26.0	148	74.7	72	33.6%	
4.	Cultured in darkness		Some tall conidiophores but all below 6 mm.				

Experiment 15

Experimental procedure. The previous experiment was repeated but in this case the culture medium employed was 2% malt agar and the heights of the conidiophores were measured seven days after inoculation.

Results. The mean height of the conidiophores in each set was determined seven days after inoculation. The results are recorded in Table 13. A large number of the tall conidiophores in the cultures of set 1 had formed fruiting heads, but none had been formed in the cultures of sets 2 and 3. The conidiophores in the cultures of sets 1 and 3 grew vertically upwards from the surface of the medium while those of set 2 showed no consistent directional orientation.

TABLE 13

Set	Light regime	Mean height of tall conidiophores (in mm)	Abundance of tall conidiophores
1	Cultured in continuous light	30.0	4
2	Cultures given a single 25 minute period of light at 24 hour intervals	10.0	2 +
3	Cultures illuminated for 1 minute every hour	23.0	4
4	Cultured in darkness	Scattered conidiophores below 3 mm	
Medium 2% malt agar		Light intensity 8.9 foot-candles	

The tall conidiophores of each culture were plucked from the nutrient agar in order to expose any short conidiophores which might have been hidden beneath them. This treatment revealed in the cultures of set 1 the presence of a few short conidiophores below 1 mm in height; they had formed fruiting heads. In the cultures of sets 2 and 3 a considerable part of the surface of the medium was covered with a dense green carpet of mature short conidiophores. It seemed probable that the extent of this fruiting mass coincided with the original area of inoculation. The cultures of set 4 consisted of a mat of short mature conidiophores with only the occasional conidiophore above 2 mm in height.

Experiment 16

Experimental procedure. This experiment was conducted using the same method described in experiment 14 except that in this instance the periods of illumination were reduced from 25 to 12 minutes per day in set 2, and from 1 minute to 30 seconds per hour in set 3.

Results. The cultures were examined 18 days after inoculation. The mean height, dry weight and carotene content of the conidiophores in each set were determined and the results are recorded in Table 14. A few of the conidiophores in the cultures of set 1 had formed heads but none of the conidiophores in sets 2 and 3 had done so. The cultures in set 4 had formed a mass of short fruiting conidiophores; most of the taller conidiophores had not formed heads.

TABLE 14

Set	Light Regime	Height in mm. above medium	Tall conidiophores				Carotene as % of Set I
			Mean dry weight in milligrams	Dry weight as % of Set I	Mean carotene content in μ gm/ gm dry weight		
1.	Cultured in continuous light	60, 55, 60, 57, 54. Mean 57.0	203	100%	225.0	100%	
2.	Cultures given 12- minute periods of light at 24 hour intervals	15, 15, 13, 13, 14. Mean 14.0	118	58.1%	66.0	29.6%	
3.	Cultures illuminated for $\frac{1}{2}$ minute every hour	20, 18, 16, 18, 18. Mean 18.0	146	71.9%	45.0	20.0%	
4.	Cultured in darkness	Some tall conidiophores but all below 7 mm.					

Discussion of the results of experiments 14, 15 and 16.

It may be seen from the results presented in Tables 12 and 14 that the induction of conidiophore growth in sets which were illuminated for short periods at hourly intervals was greater than that in sets which received a single daily illumination period. The difference was more marked in the experiment in which the illumination period was 12 minutes per day. It would be interesting to repeat this experiment using even shorter and more frequent periods but with the timing devices available this was not possible. A further difference between conidiophore growth in the two light regimes was that splitting the illumination period into short hourly periods resulted in a greater vertical orientation of conidiophore growth. This latter point was particularly marked in experiment 14.

In both experiments the concentration of carotene in the conidiophores was higher in cultures which had received a single illumination period per day. The reason for this is not clear.

Thus the frequency of illumination influences conidiophore growth and carotenogenesis in different and opposite ways; conidiophore growth was greater when the illumination was given in short periods at frequent intervals than when it was given as a single daily exposure period; the opposite holds true for carotene concentration.

Discussion of the nature of the photochemical reaction
involved in the induction of conidiophore growth.

The results of experiments 11 and 15 show that only occasional light stimuli are required to maintain the growth of tall conidiophores. It is clear that the photochemical reaction involved in the inductive process is a 'low energy' reaction, i.e. light only acts as a 'trigger' to a chain reaction which maintains growth. The main characteristic of 'low energy' reactions is that the quantity of radiation energy required to sustain the process is only a fraction of the total energy required, i.e. the reaction as a whole is exergonic. This type of reaction contrasts with the photochemical reaction involved in photosynthesis, which is a 'high energy' reaction. In this case there is net gain of energy during the process, i.e. the reaction is endergonic. One would suspect that all the light induced processes in fungi involve 'low energy' reactions, and it would be interesting to determine if short light stimuli at regular intervals would also be sufficient to maintain these systems in operation.

5. MICROSCOPIC STUDIES OF THE INFLUENCE OF LIGHT

ON TALL CONIDIOPHORE GROWTH

(a) INFLUENCE ON THE RATE OF ELONGATION OF REDUCING THE LIGHT INTENSITY.

Experiment 16 to determine the influence on conidiophore elongation of lowering the light intensity.

Experimental procedure.

The cultures were grown on 2% malt agar in the 3.0 x 1.5 x 1.0 cm cells described on page 5 . The cells were inoculated with a spore suspension and then placed into the larger observation cells. The cultures were grown in an illuminated incubator at 22°C.

The observation cells containing the cultures were transferred to the stage of the horizontal microscope 2 - 3 days after inoculation; conidiophores, 1 - 2 mm tall, were present at this stage. The cultures were illuminated from above by a beam of light; the light intensity at the level of the cultures was 20 foot-candles or 205 metre-candles. The red light by which the photographs were subsequently taken was left on for the duration of the experiment. The experiment was carried out at a temperature of $23 \pm 0.5^{\circ}\text{C}$.

A time-lapse cine record of conidiophore growth was begun 20 - 26 hours after transfer from the incubator. A conidiophore about 6 - 8 mm tall was selected and

photographed at 5 minute intervals. The magnification of the eyepiece was x 6 and the lens had a focal length of 16 mm.

Conidiophore elongation under the conditions described above was recorded for 1 - 1½ hours. At the end of this period the intensity of the light illuminating the culture was reduced from 20.0 to 2.0¹ foot-candles and the subsequent growth of the conidiophore during the next 2 - 3 hours was recorded.

Results.

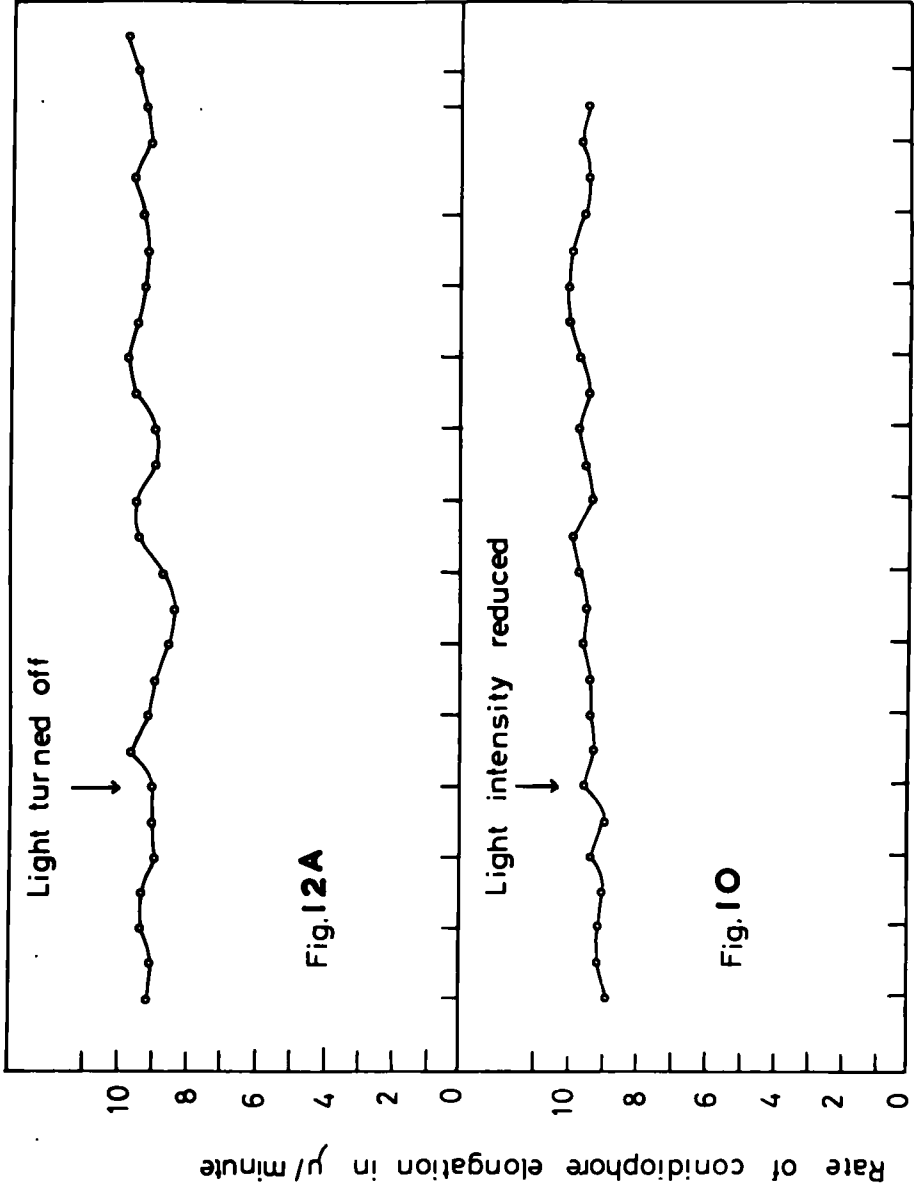
The experiment was repeated seven times using conidiophores which ranged from 6 - 8 mm in height. Figure 10 is a graph plotted from the mean of the results obtained in these experiments.

It can be seen from the graph that reducing the light intensity from 20 to 2 foot-candles had no observable influence on the subsequent rate of elongation during the following two hours. The conidiophores continued to elongate at the lower light intensity at the rate of c. 9 μ per minute or 540 μ per hour. Two conidiophores were kept under observation for 3 - 4 hours after lowing^{er} the light intensity and in neither case was there a reduction in the rate of elongation detectable by the measuring system employed.

Experiment 17

Experimental procedure.

The cultures were prepared in the same manner as



INFLUENCE OF A REDUCTION IN LIGHT INTENSITY ON CONIDIOPHORE ELONGATION

described in experiment 16; they were illuminated in the observation cells for c. 20 hours prior to the experiment with white light which had an intensity of 20 foot-candles or 205 metre-candles. The experiments were carried out at $23 \pm 1^{\circ}\text{C}$.

The rate of conidiophore elongation was measured with a micrometer eyepiece for 40 minutes prior to reducing the light intensity to 2 foot-candles and for 60 minutes afterwards. The readings were taken at 2 minute intervals.

Results.

The experiment was repeated 6 times using conidiophores which ranged in height from 5 - 8 mm. Figure 11 is a graph plotted from the mean of the results obtained. It may be seen that the rate of elongation after reducing the light intensity fell gradually from c. 9 μ per minute to c. 7.5 μ per minute in about 20 minutes. The mean rate of elongation after reducing the light intensity was c. 8 μ per minute.

Experiment 18 to determine the influence on conidiophore elongation of turning off the source of white light.

Experimental procedure.

The cultures were prepared in the same manner as described in experiment 16. They were illuminated in the observation cell for 20 - 30 hours prior to the experiment

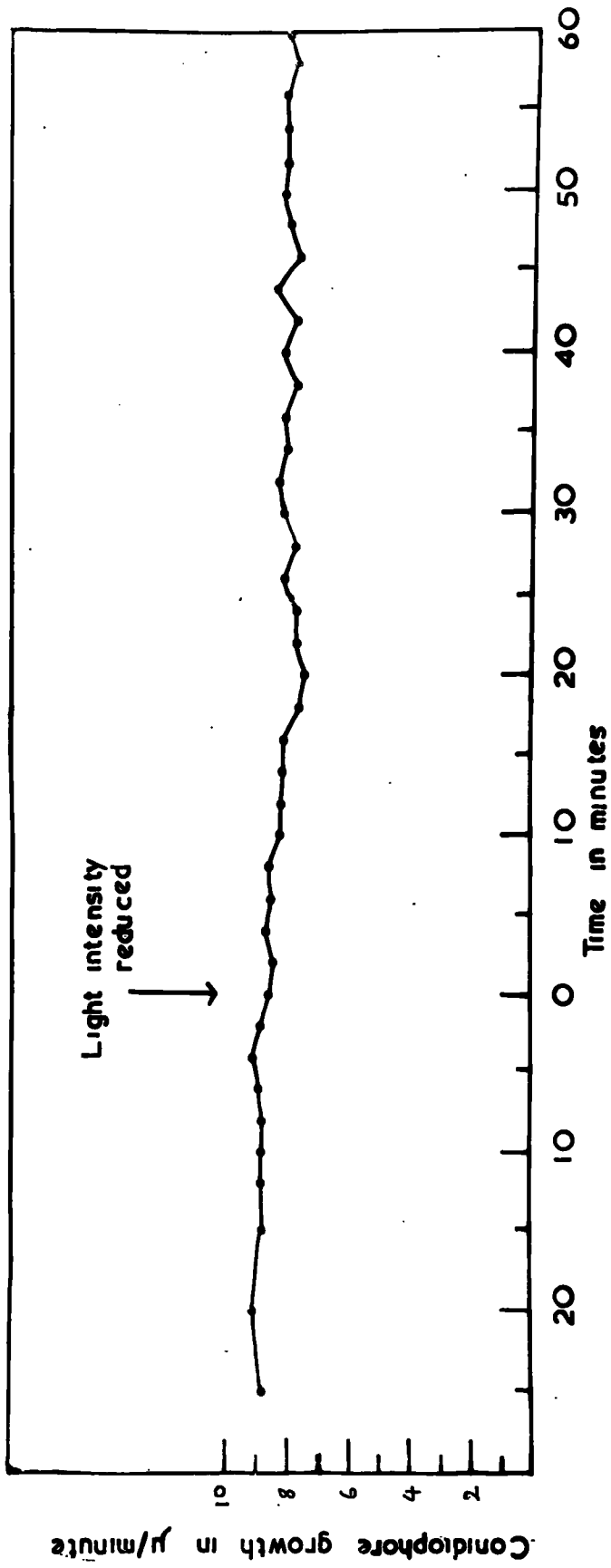


FIGURE 11

with a beam of white light which had an intensity of 20 foot-candles or 205 metre-candles. The red photographic light was left on for the duration of the experiment which was carried out at a temperature of $23 \pm 1^{\circ}\text{C}$.

The microscope was focused on a tall conidiophore which was photographed at 5 minute intervals for an hour. At the end of this period the light illuminating the culture was turned off and the subsequent growth of the conidiophore in red light during the following 2 - 5 hours was recorded.

Results.

The experiment was repeated five times using conidiophores which ranged from 3 - 7 mm in height. Figure 12a is a graph plotted from the mean of the results obtained.

The graph shows that the conidiophores, which were elongating at the rate of c. 9μ per minute or c. 540μ per hour, continued to grow at the same rate for at least two hours after the white light had been turned off, although, as can be seen from the graph, there was some oscillation in the growth rate. In two instances the conidiophores were kept under observation for over five hours after the light had been turned out. In neither case was there any reduction in the rate of growth. Figure 12b is a graph plotted from the results obtained in one of these two experiments.

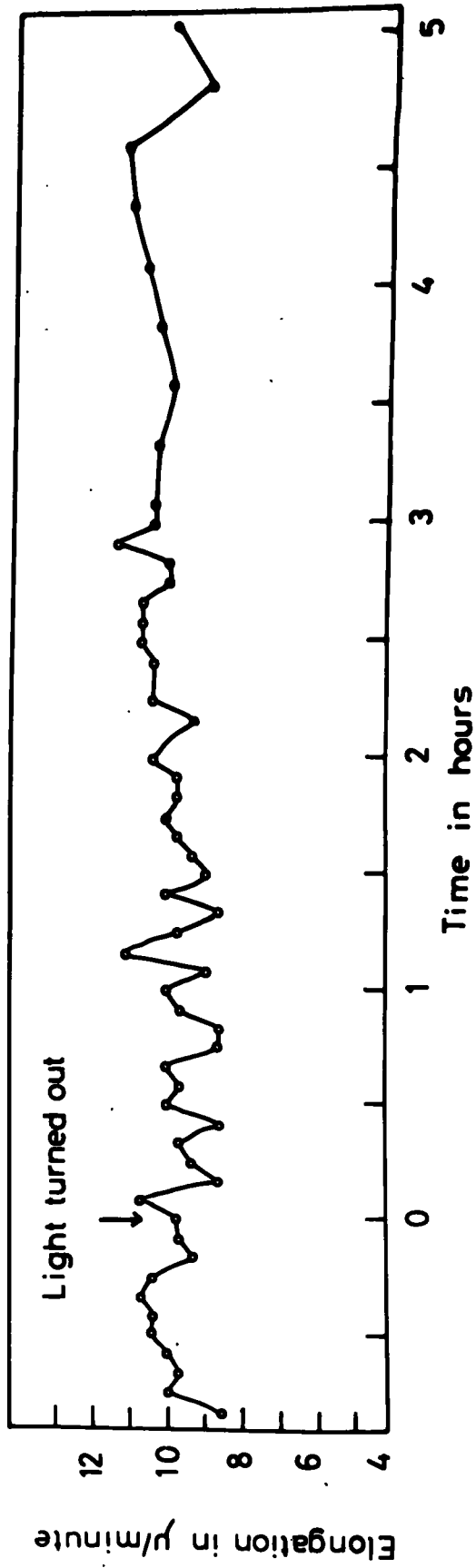


FIGURE 12b

(b) INFLUENCE ON THE RATE OF ELONGATION OF
INCREASING THE LIGHT INTENSITY

Experiment 19 to determine the influence of conidiophore elongation of increasing the light intensity.

Experimental procedure.

The cultures were prepared in the same manner as described in experiment 16; they were illuminated in observation cells for c. 24 hours prior to each experiment with white light of 2 foot-candles or 205 metre-candles intensity. The experiment was carried out at $23 \pm 1^{\circ}\text{C}$.

A tall conidiophore was photographed at five minute intervals for an hour. At the end of this period the light intensity was increased to 20 foot-candles and the subsequent growth of the conidiophore during the following two hours was recorded.

Results.

The experiment was repeated 10 times using conidiophores which ranged in height from 3 - 7 mm. Seven conidiophores showed only a slight increase in the rate of conidiophore elongation when the light intensity was increased. Figure 13a is a graph plotted from the results of these 7 experiments. It may be seen that the rate of elongation increased from c. 7μ per minute to c. 8μ per minute c. 25 minutes after raising the light intensity, falling slightly to c. 7.5μ per minute during the subsequent $1\frac{1}{2}$ hours.

The other three conidiophores showed a marked increase in the rate of elongation when the light intensity was increased. Figure 13b is a graph plotted from the results of these three experiments. The mean rate of elongation prior to changing the light intensity was c. 6 μ per minute; c. 35 minutes after increasing the light intensity there was an increase in the rate of elongation and after a further 20 minutes it was stabilized at c. 8.5 μ per minute.

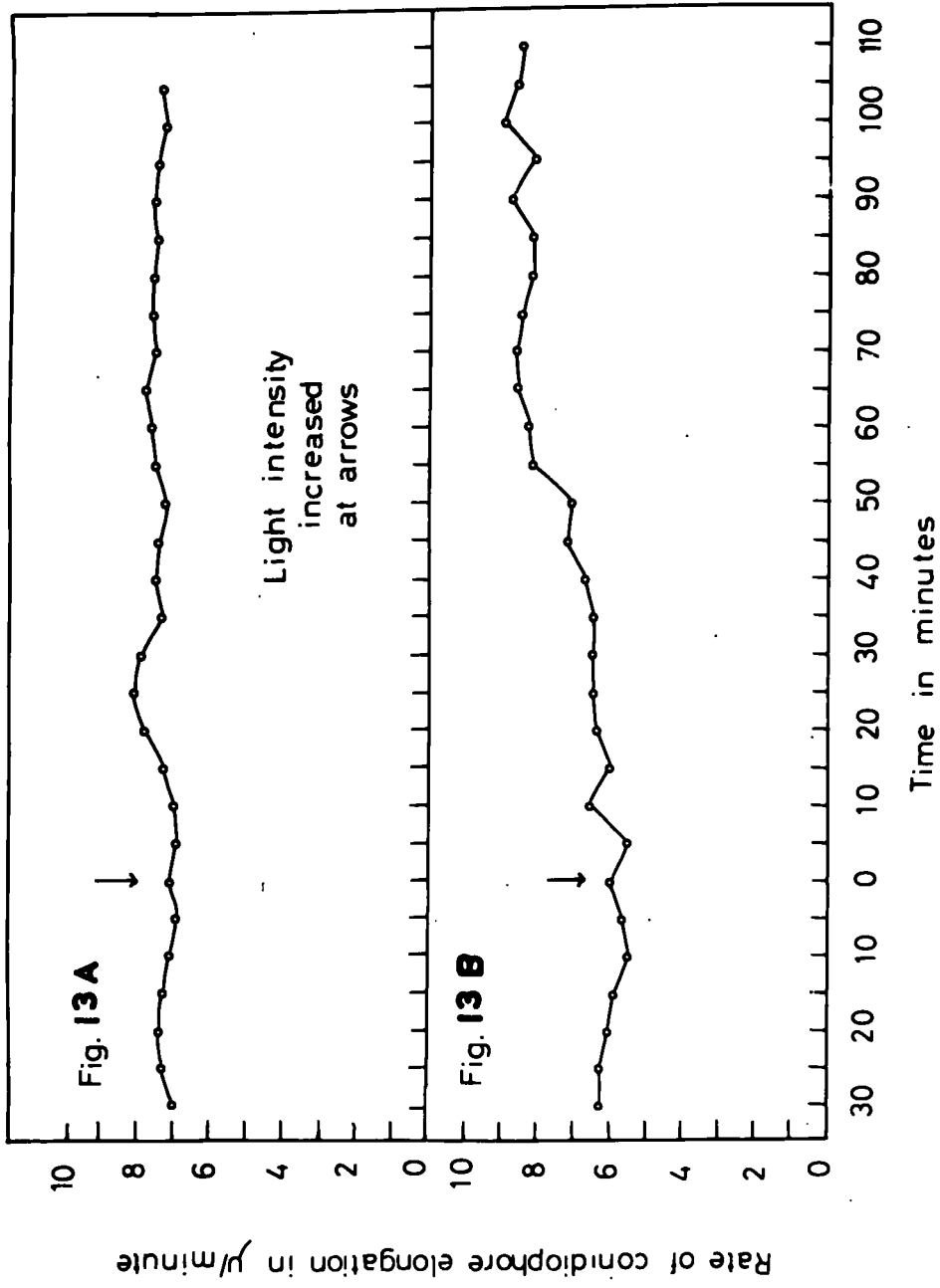
The results of all ten experiments are plotted in Figure 13c. It may be seen from this graph that there is an overall increase in the rate of conidiophore elongation from c. 7 μ per minute prior to increasing the light intensity, to c. 8 μ per minute after it had been raised.

Experiment 20

Experimental procedure.

The cultures were prepared in the same manner as described in experiment 16; they were illuminated in the observation cells for c. 20 hours prior to the experiment with white light of 2 foot-candles intensity. The experiment was carried out at $23 \pm 1^{\circ}\text{C}$.

The rate of conidiophore elongation was measured with a micrometer eyepiece for 40 minutes prior to increasing the light intensity to 20 foot-candles and for 60 minutes afterwards. The readings were taken at 2 minute intervals.



INFLUENCE OF AN INCREASE IN LIGHT INTENSITY ON CONIDIOPHORE ELONGATION

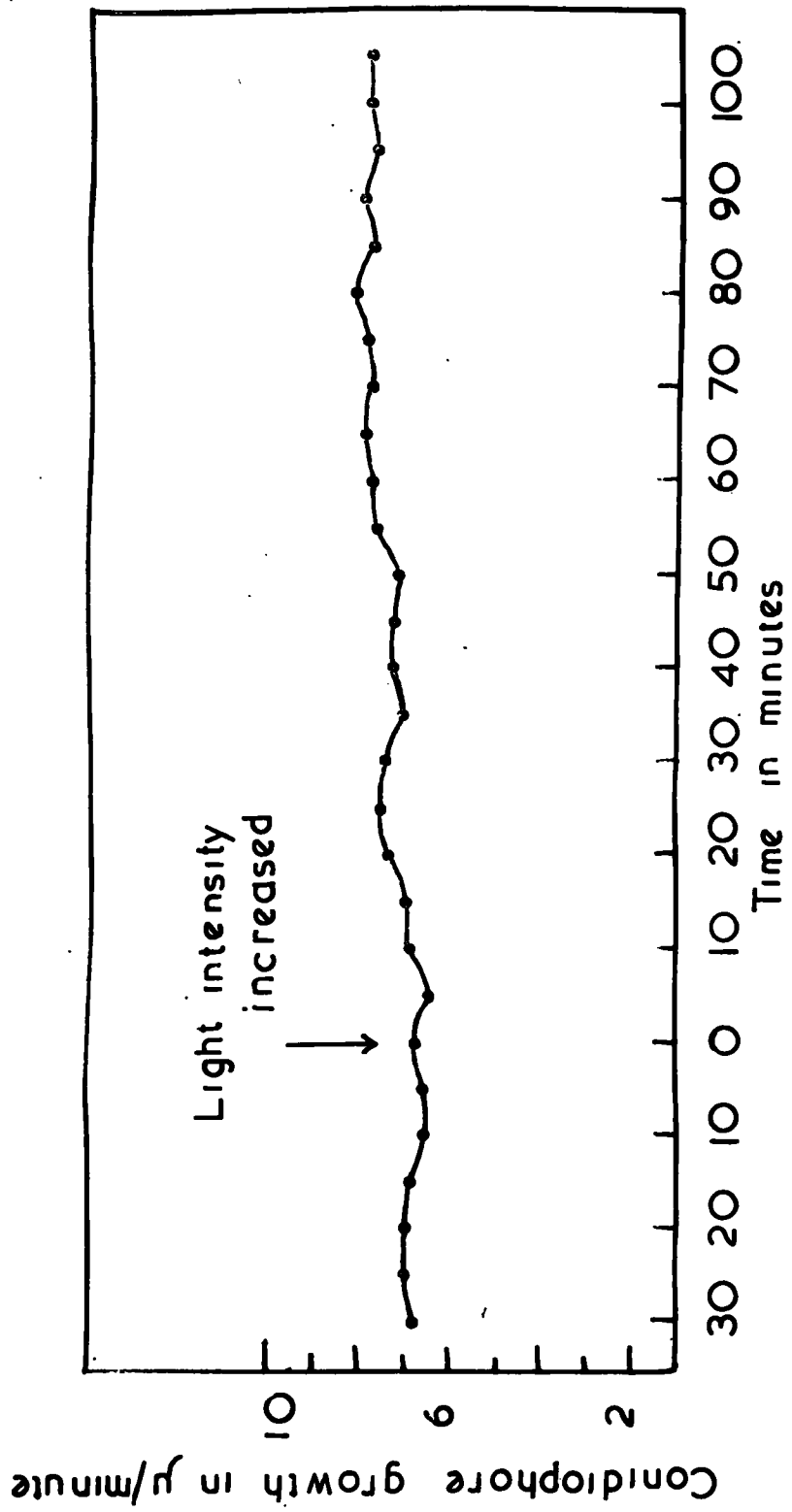


FIGURE 13c

Results.

The experiment was repeated 6 times with conidiophores which ranged in height from 5 - 8 mm. Figure 14 is a graph plotted from the mean of the results obtained. The rate of elongation prior to increasing the light intensity was c. 7 μ per minute and increased to c. 7.9 μ per minute 20 minutes after it had been raised.

Experiment 21 to determine the influence of white light on the rate of growth of conidiophores which had been kept in red light for 24 hours.

Experimental procedure.

The cultures were prepared in the same manner as described in experiment 16; they were kept in red light for 24 hours prior to each experiment which was carried out at $23 \pm 1^{\circ}\text{C}$.

The conidiophores were photographed in red light at five minute intervals for 25 minutes. At the end of this period the cultures were illuminated with white light which had an intensity of 20 foot-candles, and the subsequent growth of the conidiophores during the following $2\frac{1}{2}$ hours was recorded.

Results.

The experiment was repeated six times using conidiophores which ranged in height from 10 - 15 mm. Figure 15 is a graph plotted from the mean of the results obtained. It shows that the average rate of growth of the conidiophores after they had

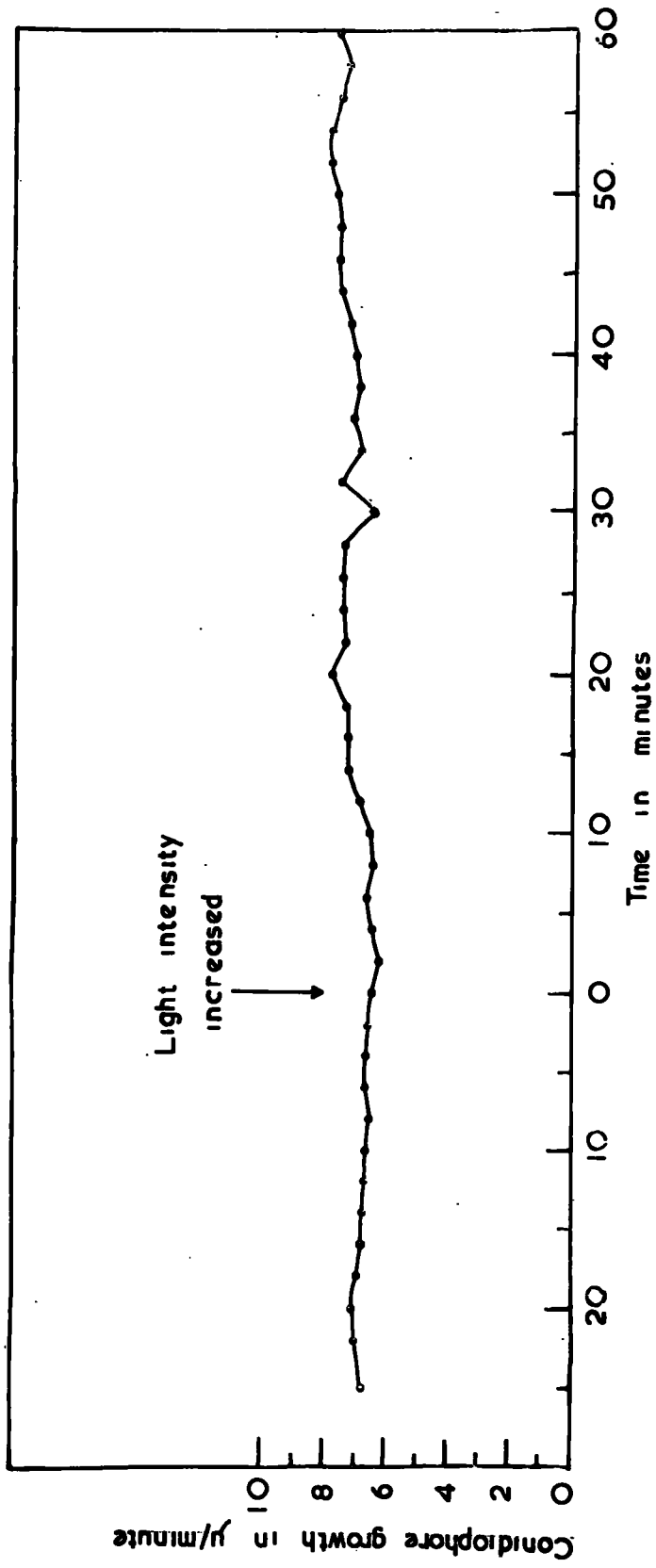


FIGURE 14

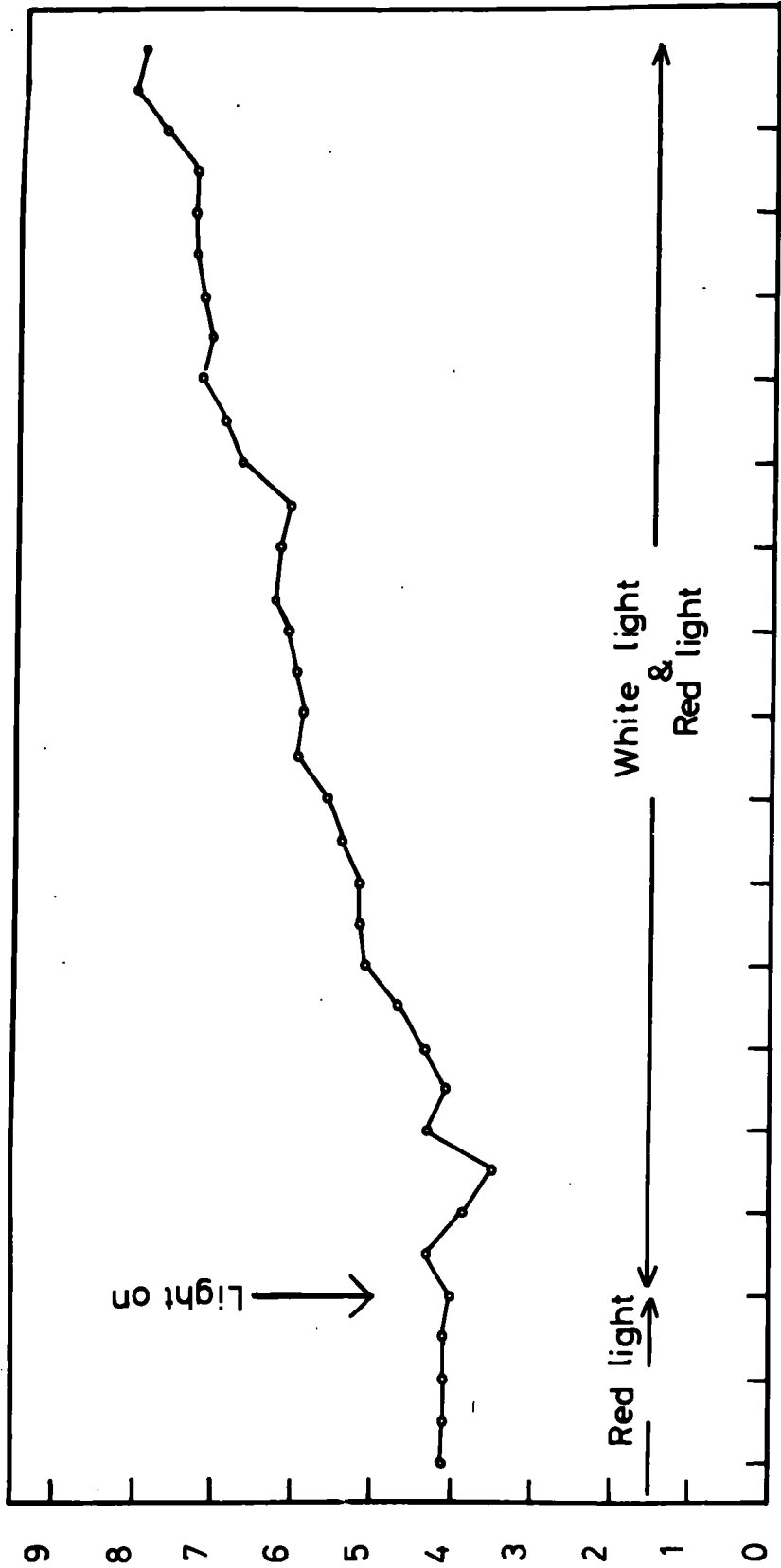


Figure 15 INFLUENCE OF CONTINUOUS WHITE LIGHT ON THE GROWTH RATE OF CONIDIOPHORES ELONGATING IN RED LIGHT

been in red light for 24 hours was c. 4μ per minute (240 μ per hour). The rate of growth started to increase c. 30 minutes after the white light had been turned on. This is also approximately the time taken for the appearance of a phototropic curvature in conidiophores which are illuminated unilaterally. The growth of the conidiophores continued to accelerate slowly over the following $2\frac{1}{2}$ hours when it had reached a rate of 8μ per minute (480 μ per hour).

Discussion of the results of experiments 16 to 21.

The results of these experiments indicate that the growth of conidiophores is influenced by light intensity. The mean rate of growth of conidiophores which have been cultured at light intensities of 2.0 foot-candles for several hours is c. 7.0μ per minute, while it is c. 9.0μ per minute for those cultured at 20.0 foot-candles. The nature of conidiophore growth also varies with light intensity; at the higher intensity the conidiophores are almost straight, but at the lower intensity they show more or less regular deviations from the vertical along their length (see page 33 and plate 9a). The "snakelike" pattern of growth, which is in fact helical in form, may have introduced an element of error in the measurement of conidiophore growth at low light intensities. It is unlikely, however, that the considerable difference between the growth rates at the

two levels of illumination can be attributed solely to this source of error. A second possibility is that the variation in growth rate may have been due to a difference in temperature rather than light intensity. No such temperature differences were detected, however, and there is no reason to suppose that the copper sulphate solution was not an efficient heat filter. It thus seems clear that there is a real difference in the rate of conidiophore growth at the levels of illumination employed, although it may not be as great as the above figures suggest.

It may be seen from the graphs in Figures 10 and 12a in experiments 16 and 18 that there was no marked decrease in the rate of conidiophore elongation when the light intensity was reduced. In experiment 17, however, there was a slight reduction in the growth rate from 0.9 μ per minute to 8 μ per minute. No evidence was obtained to indicate the presence of a marked negative light growth response and darkening similar to that shown by the sporangiophores of Phycomyces blakesleeanus. In this species the growth rate of the sporangiophores may fall to just over half the normal growth rate within ten minutes of lowering the light intensity (Delbruck & Reichardt, 1956).

The graphs presented in Figures 13a and 14 show that when the light intensity was increased there was an increase in the rate of growth of the conidiophores from c. 7 μ per minute to c. 8 μ per minute. The growth rate of some

conidiophores (Figure 13b) showed greater increases than others, but it should be noted that the original rate of elongation of these conidiophores was somewhat lower than the rest and they may be somewhat atypical. In Phycomyces the rate of sporangiophore growth begins to increase c. 2.5 minutes after increasing the light intensity and may reach a maximum which is over twice the normal growth rate. The sporangiophore returns to its original rate of growth c. 13 minutes after the light intensity increase.

Considering all the results together they would appear to suggest that the conidiophores of Aspergillus giganteus do in fact show small positive and negative light growth reactions, but the nature of these reactions differs from the large transient responses one obtains with Phycomyces sporangiophores. The conidiophores appear to respond to changes in light intensity by slight, but apparently prolonged, changes in their growth rates. Gardner (1955) reported that "upon return to darkness following light exposure, (conidiophore) elongation ceases at once and immature conidiophores fruit within a few hours." The results of the present investigation are clearly contrary to her findings. Tall conidiophore growth normally continues in darkness on 2% malt agar for more than 24 hours and on Zurzyka agar fruiting heads may not be produced until two or more days after cultures have been placed in darkness. After a number of hours in darkness the growth

rate of the conidiophores begins to decrease and they may eventually form fruiting heads.

(c) INFLUENCE OF SHORT PERIODS OF LIGHT ON

CONIDIOPHORE ELONGATION.

Experiment 22 to determine the influence of a short period of white light on the rate of growth of conidiophores which have been kept in red light for 24 hours.

Experimental procedure.

The cultures were prepared in the same manner as described in experiment 16; they were kept in red light for 24 hours prior to each experiment which was carried out at $23 \pm 1^{\circ}\text{C}$.

The conidiophores were photographed in red light at five minute intervals for 25 minutes. At the end of this period the cultures were illuminated for ten minutes with white light which had an intensity of 20 foot-candles. The subsequent growth of the conidiophores during the following three hours was recorded.

Results.

The experiment was repeated five times using conidiophores which ranged in height from 9 - 15 mm. Figure 16 is a graph plotted from the mean of the results obtained. The graph shows that the average rate of growth of the conidiophores after they had been in red light for 24 hours was a little over 3 μ per minute (180 μ per hour). The conidiophores continued to grow

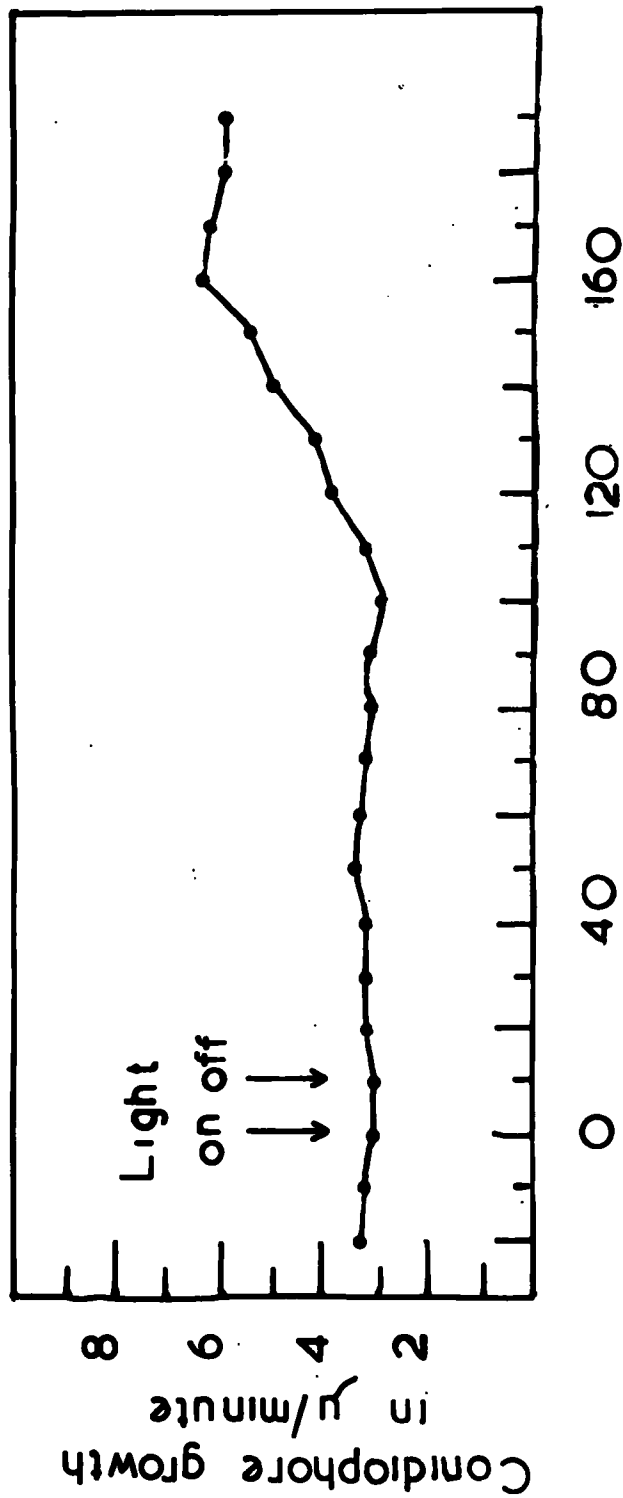


FIGURE 16 INFLUENCE OF A SHORT PERIOD OF ILLUMINATION ON THE GROWTH RATE OF CONIDIOPHORES GROWN IN RED LIGHT

at the same rate for c. 100 minutes after the beginning of the illumination period. At the end of this period there was a gradual acceleration in the rate of growth and after a further hour's growth it had doubled, to 6 μ per minute (360 μ per hour).

6. INFLUENCE OF LIGHT QUALITY ON TALL CONIDIOPHORE GROWTH

Only light of certain wavelengths will induce conidiophore elongation. Gardner (1955) found that elongation took place in radiation of wavelength 300 - 530 $m\mu$ with the near ultra violet below 400 $m\mu$ being particularly effective. The filters she used did not have very sharp cut-offs, e.g. the blue-green filter transmitted light of wavelength 360 - 550 $m\mu$. Also she apparently made no correction to insure that the cultures received equal energy doses of radiation. The following experiment was conducted to confirm Gardner's result.

Experiment 23 to determine the influence of light quality on conidiophore growth.

Experimental procedure.

Fifty specimen tubes containing 5 mls of 2% malt agar were prepared. They were inoculated with spores and five were placed into each of the light chambers described on page 10. The specimen tubes were so placed that the open mouths of the tubes were directed towards the window of the growth chamber.

Results.

Eight days after inoculation the average length of the

tall conidiophores in each culture was measured. The mean value for each set was calculated and the results are presented in Table 14.

TABLE 14

Influence of light quality on conidiophore height

Filter	Transmission in $m\mu$	Mean height of tall conidiophores (mm)
600 Deep Violet	380-450 (peak 406)	20.5
601 Violet	380-470 (peak 415)	24.5
602 Blue	440-490 (peak 468)	26.0
603 Blue-Green	470-520 (peak 490)	25.5
604 Green	500-540 (peak 515)	5.0
605 Yellow-Green	530-570 (peak 550)	none
606 Yellow	560-610 (peak 575)	none
608 Red	620 onwards	none
609 Deep Red	650 onwards	none

Discussion of the results

The results presented in Table 14 show that violet, blue and blue-green light of wavelength 380-500 $m\mu$ is most effective in inducing conidiophore growth, while light between 500-530 $m\mu$ is less effective. Light above 530 $m\mu$ does not induce conidiophore growth.

A number of other light sensitive processes in fungi appear to respond to light of a wavelength range similar to that effective in inducing conidiophore growth. These fungi are listed in Table 15. The similarity in the action spectra for the various processes may mean that a similar fundamental process is involved in each case.

TABLE 15

Fungus	Influence of light	Wavelength of light in mμ Effective	light in mμ Inéffective
1. <u>Coprinus lagopus</u> (Madelin, 1956)	Induces fruiting	450-495	520-660
2. <u>Pyronema confluens</u> (Robinson, 1926)	Induces fruiting	400-550	550-660
3. <u>Trichotherium</u> <u>roseum</u> (Sagromsky, 1956)	Increases conidia production	350-550	550-730
4. <u>Sordaria fimicola</u> (Ingold & Dring, 1957)	Induces spore discharge	400-550	550-720

7. INVESTIGATION TO DETERMINE IF THE LIGHT STIMULUS
INFLUENCES THE MYCELIUM NEAR TO BUT NOT WITHIN THE
ILLUMINATED ZONE

Experiment 24 to determine if tall conidiophores are produced
by mycelium formed immediately after transferring cultures to
darkness.

Experimental procedure

Petri-dishes containing Zurzyka medium were inoculated centrally with a spore suspension and then cultured in an illuminated incubator maintained at $22 \pm 0.5^{\circ}\text{C}$.

Ten days after inoculation a central circle of orange tall conidiophores, surrounded by a narrow ring of mycelium, had been formed in the cultures. The extent of the mycelial growth in each culture was marked on the bottom of the petri dish with a chinograph pencil. The petri dishes were then placed in darkness in a cupboard maintained at $22 \pm 1^{\circ}\text{C}$.

Results.

The cultures were examined after a further ten days growth in darkness. In no instance were the conidiophores formed by the mycelium subsequently grown in darkness taller than conidiophores produced in cultures grown continuously in darkness from the time of inoculation. The mycelium formed after transfer of the culture to darkness was white in colour and contrasted with the deep orange of the central circle of tall conidiophores.

Experiment 25 to determine if the light stimulus influences the mycelium near to but not within the illuminated zone.

Experimental procedure.

The apparatus involved in this experiment is shown in Figure 17. The glass tube was completely covered with

aluminium foil; it had a diameter of 1.5 cm and was 25 cm long. The light bulb was a 4.0 V, 0.3 amp Sunshine cycle bulb; it was run off a 4.5 volt transformer. The razor blade was sealed to one end of the glass tube with bostick.

Petri dishes containing thin layers of Zurzyka agar poured directly on to squares of black paper were prepared. The presence of the paper helped to reduce light reflection during the experiment. The petri dishes were inoculated centrally with a spore suspension and then cultured in darkness at a temperature of $22 \pm 1^{\circ}\text{C}$.

Five days after inoculation two of the cultures were removed in dim red light. The razor blade at the end of the glass tube was placed over part of the mycelium in one petri dish as illustrated in Figure 17. The light was then switched on. The lid of the second petri dish was removed and replaced by a square of aluminium foil covered cardboard. Both cultures were then grown in darkness at a temperature of $22 \pm 1^{\circ}\text{C}$.

Results.

The cultures were examined five days later. Plate 14 is a photograph of the culture illuminated through the base of the razor blade. Conidiophores, 20 mm tall, had been produced by the parts of the mycelium which had been directly illuminated. There was no sign of any light-induced conidiophore elongation in the mycelium outside the area

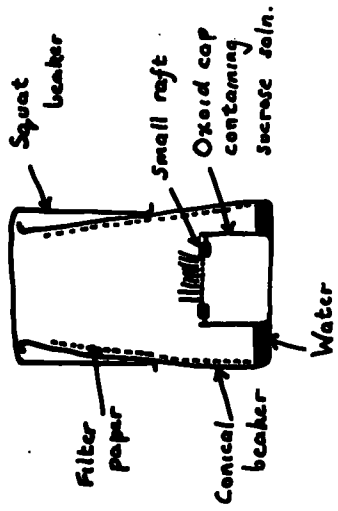


Figure 19 Method of culturing conidiophores on sucrose slns.

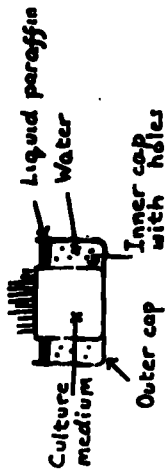


Figure 18 Method to prevent the medium from shrinking

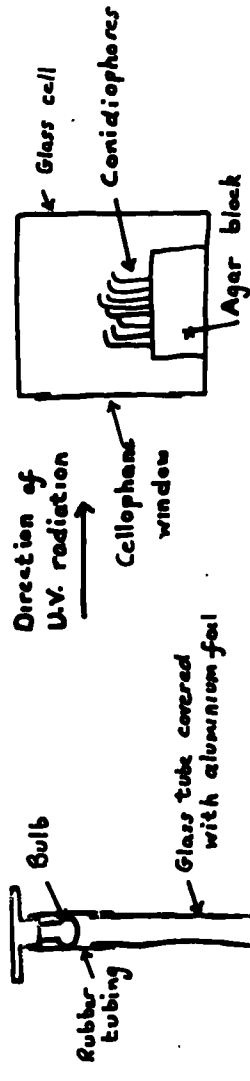


Figure 20 Culture cell used to irradiate conidiophores unilaterally with U.V. radiation.

Figure 17 Method of illuminating only part of the mycelium

covered by the blade. Immediately around the edge of the razor blade it appeared that most of the conidiophores had formed heads. The tall conidiophores produced by areas of the mycelium which had been illuminated were orange coloured. This is shown in the photograph of Plate 14. There was no visible sign of light induced pigment production in the parts of the mycelium either covered by the razor blade or in the mycelium around it.

Not as many conidiophores in the control culture had produced heads. In a similar experiment the razor blade was placed upon the mycelium of a culture grown in darkness which was not subsequently illuminated. It was found that this treatment also tended to increase the production of fruiting heads on conidiophores around the edge of the blade.

Experiment 26: Oxoid Cap experiment.

Experimental procedure.

The experiment was repeated but in this instance the end of the glass tube was covered with a small aluminium oxoid cap and not a razor blade. The cap had a diameter of 16 mm and had three small holes, each with a diameter of 2mm, drilled in its base. The circumferences of these holes were 2 mm from the edge of the cap at their nearest points.

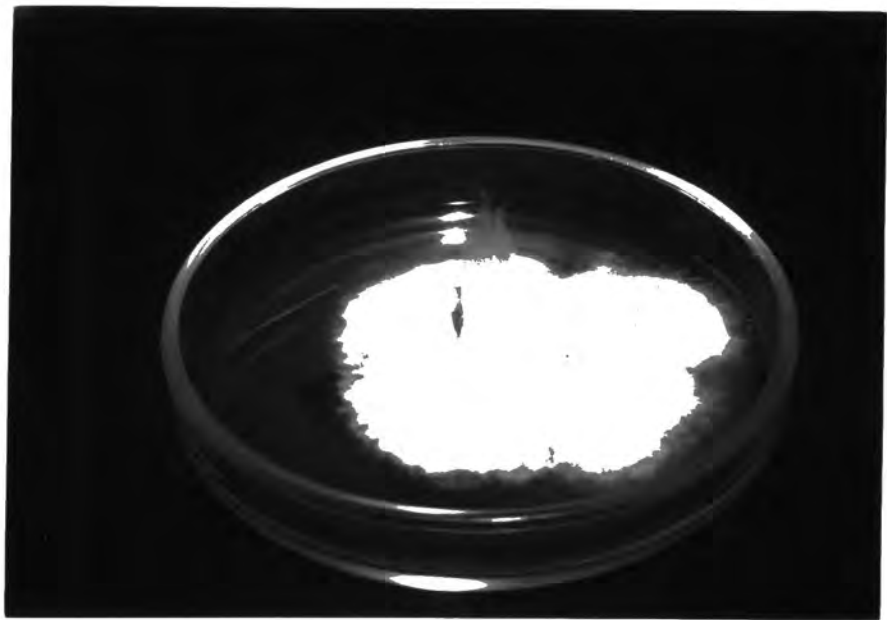


PLATE 14 Narrow area of the mycelium illuminated.

The experiment was conducted in the same manner as that described for the previous experiment; the perforated disc of the cap rested lightly on the surface of the culture, the tube being supported by a stand.

Results.

The cultures were examined four days after inoculation. The glass tube was carefully withdrawn from the culture to reveal the presence of three stands of tall conidiophores. These conidiophores had been produced by those areas of the mycelium which had been directly illuminated. Plate 15a is a photograph of this culture. There was no visible evidence to suggest that conidiophore elongation had been initiated in parts of the mycelium which had not been illuminated. Most of the conidiophores around the edge of the cap had formed heads. Plate 15b is a photograph of a culture which had not been illuminated.

The tall conidiophores were coloured orange; there was no sign of light induced pigment production in the parts of the mycelium not directly illuminated. In this experiment the illuminated 2 mm diameter zones were separated from the free surface of the culture by a covered region varying in extent but diminishing to not more than 2 mm wide at the outermost edge of each hole.

~~PLATE 15a~~ Narrow areas of the mycelium illuminated.

~~PLATE 15b~~ Culture not illuminated.



Discussion of the results of experiments 24, 25 and 26.

The results of this series of experiments indicate that the influence of the light stimulus in inducing tall conidiophore elongation and carotenogenesis cannot be translocated from illuminated parts of the mycelium to parts which have not been, or are not being, illuminated. A number of workers have found that in other fungi the light stimulus which induces a particular process cannot be translocated from mycelium grown in light to mycelium later produced in darkness. They include the stimulation by light of fruiting structures in Fusarium solani (Snyder & Hansen, 1941), Physalospora obtusa (Fulkerson, 1955), Pyronema confluens (Robinson, 1926) and Coprinus lagopus (Madelin, 1956). Robbins & Hervey (1960) have recently reported, however, that the stimulus of light in inducing spore production in Poria ambigua can be translocated from mycelium grown in light to mycelium developed later in darkness. Apart from this last instance, however, it seems general that if light is to be effective in inducing a particular process it must fall directly upon the mycelium involved. This would seem to imply that the effect which light has on the mycelium is very localised and does not involve the production of a diffusible hormone.

In experiments 25 and 26 narrow areas of mycelium were illuminated under conditions in which one would expect that

translocation to parts of the same mycelium not illuminated was still possible. Even under these conditions the influence of light in inducing carotenogenesis and conidiophore growth was restricted to the areas of the mycelium actually illuminated.

General discussion of the Nature of the Photoinductive Process.

Gardner (1950) found that the typical two day induction period can be divided into a non-light requiring part (c. the first 36 hours after inoculation) and a light requiring part. It seems clear that during the non-light requiring period foot cells are formed in the mycelium and that the duration of this part of the induction period is probably correlated with the production and maturation of these cells. The foot cells are active sites of pigment production and it is likely that the pigment photoreceptor involved in conidiophore growth is produced by them. Although light is not required for the production of foot cells it is required for the continued growth of the tall conidiophore stalks which they support, and only light of wavelength below 530 m μ is effective in inducing this growth. The essential changes involved in this photoinductive process occur in the mycelium and light must fall directly upon it if the radiation is to be effective in inducing conidiophore elongation. The chemical nature of the pigment photoreceptor is unknown but

it is presumably present both in the foot cells and the tall conidiophores.

The photochemical reaction involved in the induction of conidiophore elongation is a 'low energy' or 'trigger' reaction. Light must induce some kind of biochemical differentiation in illuminated cultures. Two ways in which the onset of this differentiation may be triggered by light are presented below.

Production hypothesis. Light may stimulate the production of a substance (or of course substances) which is essential in some way for conidiophore growth. In continuous light this substance is being produced all the time but when cultures are transferred to darkness its concentration in the cultures falls and this eventually results in a decrease in the rate of conidiophore growth followed finally by the production of fruiting heads. The substance induced by light is non-diffusible. Light could in fact be involved in the induction of bound enzyme systems.

Destruction hypothesis. An alternative hypothesis to that outlined above is that light may be involved in the photo-destruction of some substance (or, again, substances) which is required for the production of fruiting heads. Light would prevent the accumulation of this 'fruiting' substance and the conidiophores would continue to elongate. When cultures are transferred to darkness, this 'fruiting'

substance would accumulate and when it reached a critical concentration vesicles would be produced.

Nearly all the experimental evidence can be explained equally well using either hypothesis. There is, however, one experimental result which it is difficult to fit into either hypothesis, viz. the influence of light intensity on conidiophore height. It would appear that at certain intensities light actually has an inhibitory influence on conidiophore height. This type of situation is not unique. Barnett & Lilly (1950) found that while low light intensities (1 foot-candle) induce the production of conidia in Choanephora cucurbitarum, high light intensities (65 foot-candles) did not. This is thus another instance in which light is essential for the induction of a particular process but where high light intensities actually inhibit the process.

SECTION IV

INFLUENCE OF RELATIVE HUMIDITY AND TEMPERATURE

ON TALL CONIDIOPHORE GROWTH

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2. INFLUENCE OF TEMPERATURE ON MYCELIAL GROWTH AND CONIDIOPHORE HEIGHT	92
3. INFLUENCE OF TRANSFERRING CULTURES TO DIFFERENT TEMPERATURE CONDITIONS	98
4. INVESTIGATION TO DETERMINE IF LIGHT GIVEN AT HIGH TEMPERATURES IS EFFECTIVE IN INDUCING CONIDIOPHORE ELONGATION	101

SUMMARY

Relative humidity has a marked influence on the height of the tall conidiophores; very tall conidiophores are only produced at relative humidities approaching 100%. Exposure to low relative humidity is the most effective way of inducing conidiophores to form fruiting heads.

The optimum temperature for mycelial growth is c. 30°C, while the optimum temperature for maximum conidiophore height is 20 - 23°C. High temperatures do not inhibit the actual photoinductive reaction but only some later stage in the process which leads to conidiophore elongation.

1. INFLUENCE OF RELATIVE HUMIDITY ON TALL CONIDIOPHORE GROWTH

In the preliminary experiments it was noticed that there was a marked difference in the height of tall conidiophores grown under the same culture conditions but in different vessels; conidiophores cultured in $3\frac{1}{2} \times 1\frac{1}{2}$ specimen tubes were much shorter than those in conical flasks. This difference is recorded below in Table 16.

TABLE 16

Influence of culture vessel on conidiophore height

Type of vessel	Mean average height of tall conidiophores
150 ml conical flasks	35.0 mm
specimen tubes	9.0 mm

A difference in relative humidity or carbon dioxide concentration of the internal atmosphere of the two types of vessel could possibly account for this result.

An investigation into the influence of relative humidity on aerial fungal growth presents considerable problems. The solutions listed below have been widely used to obtain a range of relative humidities.

- 1) Solutions of sulphuric acid
- 2) Solutions of glycerol
- 3) Saturated solutions of mineral salts.

When the first two sets of solutions are used their concentration - and hence the relative humidities they provide - change during the progress of the experiment due to exchange of water vapour between the organism, the medium and the solution. Little change occurs when salt solutions are employed provided that an excess of the solid is used. Zwolfer (1932) pointed out that sulphur dioxide is given off from sulphuric acid solutions particularly if the solution is contaminated with organic particles. One disadvantage in using salt solutions is that they are rather sensitive to temperature changes, as also are sulphuric acid solutions. On balance it was decided to use saturated mineral salt solutions to obtain a range of relative humidities. The following is a list of the saturated salt solutions used together with the relative humidities they give at 20°C.

<u>Solution</u>	<u>Relative humidity</u>
Magnesium chloride	32%
Sodium bromide*	58%
Ammonium chloride*	77%
Potassium chloride*	85%
Sodium carbonate	92%
Lead nitrate	98%

The relative humidities of the solutions marked * were taken from Teitell (1958) while the others were taken from McLean & Cook (1952).

In preliminary experiments it was found that there was considerable contraction of the medium during the process of the experiment. This shrinkage changes the culture conditions in two ways: (i) The nutrient medium in effect becomes more concentrated and (ii) the space above the sunken medium probably has a higher relative humidity due to the sides of the container acting as a barrier to diffusion. One method employed to prevent this shrinkage was to supply water to the nutrient medium from below. The way in which this was achieved is shown in Figure 18 on page 76 . It was later considered that water evaporating from the surface of the medium probably altered the local humidity directly above the medium, precisely where the conidiophores grow, and because of this it was decided not to persist with this method. Experiment 27 to determine what influence relative humidity has on conidiophore elongation.

Experimental procedure.

An alternative method of limiting medium shrinkage is to reduce to a minimum the surface area exposed to air. In order to prevent possible staling effects this was done in the following manner. Small oxoid caps containing the medium, 2% malt agar, were covered with aluminium foil except for half a centimetre strip cut across the top to expose the surface to air. The exposed strips were inoculated with a

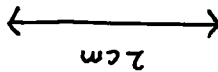
spore suspension and the caps were placed into 100 ml beakers which were lined with blotting paper and contained distilled water. Under these conditions there would be no shrinkage of the medium. The beakers were placed into an incubator maintained at 20 - 21°C and with a light intensity of 3.4 foot-candles (36 meter-candles).

Forty hours after inoculation the cultures were divided into seven sets with four replicates in each. Each set was placed into a 100 ml beaker lined with blotting paper and containing 40 mls of a saturated salt solution. The cultures were then returned to the incubator.

Results.

Four days later the height of the conidiophores in each culture was measured. The conidiophores in the cultures of all sets except No. 7 had mature fruiting heads, i.e. they had reached their maximum height. Only a few conidiophores in the cultures of set 7 had formed mature heads and they continued to elongate after the conclusion of the experiment, finally reaching a height of c. 35 mm. In some cultures, particularly those of set 6, a few conidiophore stalks had elongated above the general level at which others had formed mature heads; these may be seen in the photograph of Plate 1b. These conidiophore stalks were ignored when measurements were made because it was felt that they had been formed after the

2 cm



Relative
Humidity

58%

77%

86%

98%

100%

PLATE 16 Influence of relative humidity on condliophore height.



main crop had been produced. This would mean that they grew up through an established stand of conidiophores and thus grew in a 'shielded' atmosphere which was probably fully saturated. There was very little shrinkage of medium during the course of the experiment. The mean height of the conidiophores in each set are recorded below in Table 17.

TABLE 17

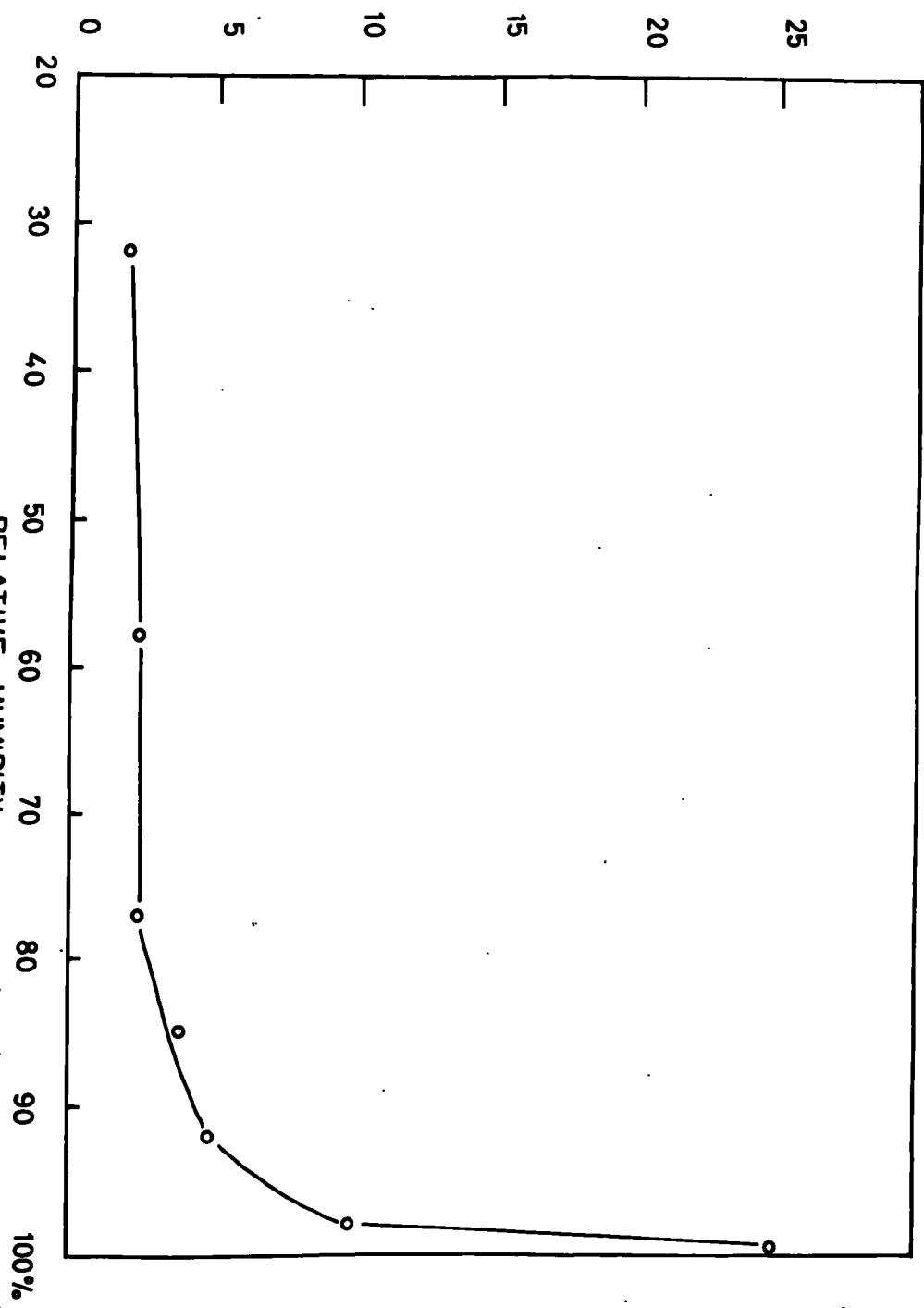
Influence of relative humidity on conidiophore height

Set	Relative Humidity	Mean height of the conidiophores (mm)
1	32%	2.0
2	58%	2.5
3	77%	2.5
4	85%	4.5
5	92%	5.5
6	98%	11.0
7	100%	25.0

The results are presented graphically in Figure 21.

The following experiment was conducted in order to confirm that it is differences in relative humidity rather than carbon dioxide concentration which causes the variation in conidiophore height in different culture vessels.

CONIDIPORE HEIGHT IN MM.



Experiment 28 to determine if the presence of carbon dioxide influences conidiophore elongation.

Experimental procedure.

The experiment described on the preceding page was repeated in duplicate. To each beaker of one treatment was added a 2" x 1" specimen tube containing a 10% solution of caustic soda in which stood a cylinder of blotting paper. The concentration of carbon dioxide in this treatment was thus kept at a low level.

Results.

There was no significant difference between the results obtained in the two treatments. Thus the removal of carbon dioxide from the atmosphere had no influence on conidiophore elongation.

Discussion of the Results of Experiments 27 and 28.

From the results of the experiments described above it seems fairly clear that the variation in the height of conidiophores produced in cultures grown under the same conditions but in different vessels could be explained in terms of differences in relative humidity between the internal atmospheres of the vessels. Conical flasks have a smaller area of aperture to volume of vessel ratio than specimen tubes and this probably results in their having a higher internal humidity. This influence of relative humidity on conidiophore elongation had not previously been recorded by other workers who have used the fungus.

It is clear that in experimental work on Aspergillus giganteus it is important to remember the influence which relative humidity has on growth, particularly when liquid culture media are used. This point may be illustrated by comparing the height of conidiophores produced on liquid Zurzyka solution in 100 ml flasks and on solid Zurzyka medium in large oxoid caps placed in the conical beakers described on page 4. When grown under the same cultural conditions it was found that the conidiophores in flasks only reached a height of c. 20 - 30 mm before forming heads, while on the solid medium the conidiophores often grew above 60 mm. This difference in the height attained by the conidiophores may be explained in terms of different relative humidities in the two types of vessel. The composition of the liquid medium influences the relative humidity in the conical flasks and no provision is made to prevent the establishment of a humidity gradient. The wet blotting paper (see plate 1b) in the conical beakers in which the oxoid caps were contained ensured that the atmosphere in these vessels was saturated.

Tall conidiophores only continue to elongate at relative humidities approaching 100%; at lower humidities growth soon stops and fruiting heads are formed. At low relative humidity is the most effective way of inducing tall conidiophores to produce sporing heads. Conidiophore

elongation is dependent upon the uptake of water from the medium and any factor which limits water supply would tend to exert a direct influence on conidiophore growth. The influence of relative humidity on elongation probably acts through its influence on the evaporation of water from the surface of the conidiophore. That the conidiophore wall is in fact permeable to water is shown by the formation of exudation droplets under conditions of high relative humidity. Under conditions of low relative humidity the rate of evaporation from the conidiophores would tend to be high and this would have the effect of reducing the turgor of the conidiophores and inhibiting their growth. High relative humidities result in lower rates of evaporation and this favours elongation.

It is an established fact that the growth of Phycomyces sporangiophores is not greatly influenced by differences in relative humidities. The tolerance of the sporangiophores to low relative humidities is due to the thick, relatively water-impermeable nature of their walls. The walls of the conidiophores of Aspergillus giganteus are thin and this results in their having a low tolerance of relative humidities below 100%.

2. INFLUENCE OF TEMPERATURE ON MYCELIAL GROWTH AND CONIDIOPHORE HEIGHT

The measurement of linear growth on a solid medium is a method commonly used to determine the growth of a fungus. Several authorities have suggested that this is not an accurate way of measuring growth, but Broncato & Golding (1953), after experimental investigation, found that the method was a reliable way of determining growth rates and comparing the effect of environmental factors on a fungus cultured on the same medium.

Experiment 29 to determine the optimum temperature for mycelial growth.

Experimental Procedure.

Three incubators were available for this work and the results presented below are from three separate experiments. In each experiment one incubator was maintained at 30°C and the cultures in this set served as a standard when comparing results between different experiments. The light intensity at the level of the cultures in the incubators was 3.4 foot-candles or 36 metæe-candles.

Plastic pre-sterilized petri dishes containing 20 mls of a 2% malt agar culture medium were used in each experiment. The media were inoculated with single drops of a spore suspension dispensed from a fine hypodermic needle. There

were five replicates in each set of cultures. The diameter of each colony was measured at regular intervals, two readings being taken at right angles to each other.

Results.

The mean colony diameter for each set seven days after inoculation was calculated and the results are presented below in Table 18 and in the form of a histogram in Figure 22. Thus the optimum temperature for mycelial growth under the culture conditions described above lies in the region of 30°C.

TABLE 18

Influence of temperature on mycelial growth

Temperature in °C	Diameter of each culture seven days after inoculation in mm					Mean
14-18	21.0	21.5	20.5	21.0	21.0	21.0
20-23	45.0	46.5	46.5	46.0	46.0	46.0
26-27	53.0	52.0	53.0	53.5	53.5	53.0
28	58.0	58.0	57.0	57.0	57.5	57.5
30	59.0	58.5	59.5	59.0	59.0	59.0
32	56.5	57.0	56.0	56.0	57.0	56.5
33-34	45.5	47.0	47.5	47.5	47.5	47.0

Gardner (1955) found that the optimum temperature for conidiophore elongation was in the region of 20 - 22°C. She used liquid culture media in her experiments and no special provision was made to maintain a high relative humidity around the elongating conidiophores. The

technique which Gardner used to cap the conical flasks that she employed, viz. coverslips partially sealed on with vaseline, probably ensured a very high internal relative humidity.

It was, however, decided to repeat the experiment because it was just possible that differences in relative humidity may have been responsible for the low temperature optimum found for conidiophore elongation.

Experiment 30 to determine the optimum temperature for maximum conidiophore height.

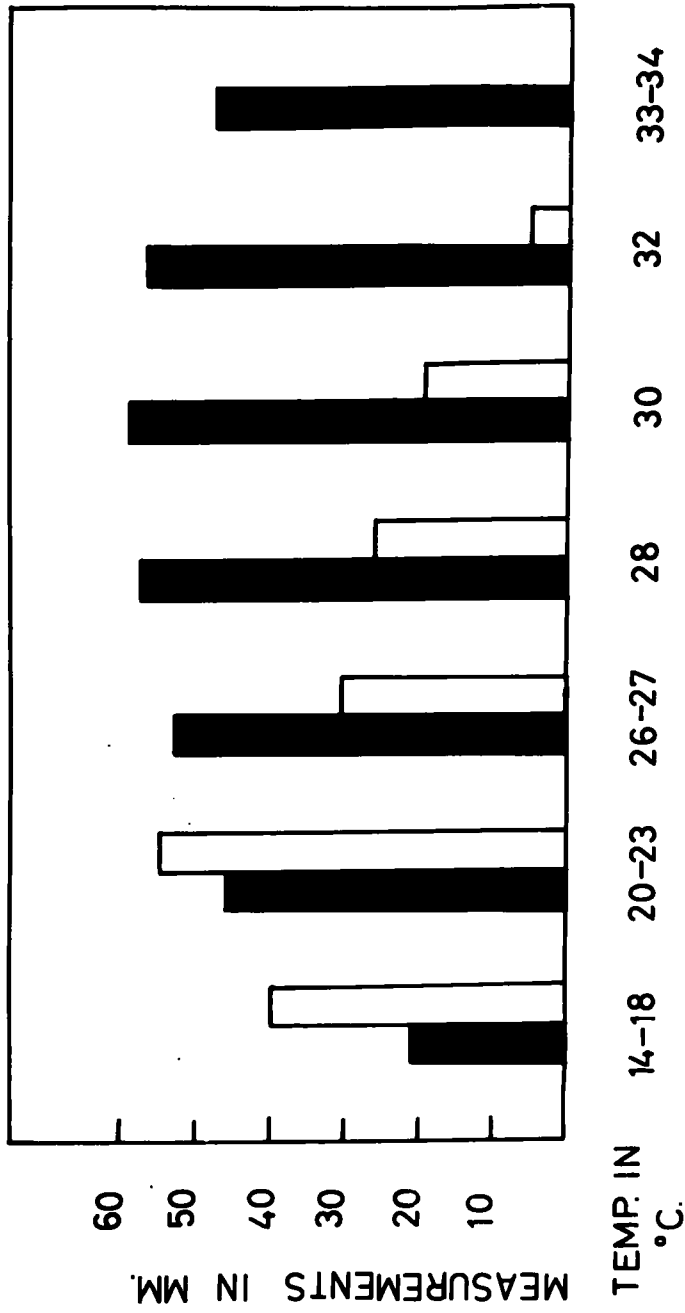
Experimental Procedure.

Small oxoid caps were filled with 2% malt agar were placed in 9 cm x 3 cm specimen tubes lined with wet blotting paper. Each oxoid cap was inoculated with two drops of a spore suspension; four specimen tubes were placed into each of a number of 250 ml beakers which were also lined with wet blotting paper. A petri dish lid was placed over each beaker. One of these beakers constituted a set. This culture method ensured that the conidiophores elongated in an atmosphere fully saturated with water vapour. The light intensity at the level of the cultures as 3.4 foot-candles or 36 metex-candles.

The height of the conidiophores in each specimen tube was measured at regular intervals and the mean value for the set was calculated.

Results.

The results of the experiment are presented graphically



■ Diameter of colony after 7 days
 □ Final height of the conidiophores

FIGURE 22. INFLUENCE OF TEMPERATURE ON MYCELIAL GROWTH AND CONIDIOPHORE HEIGHT

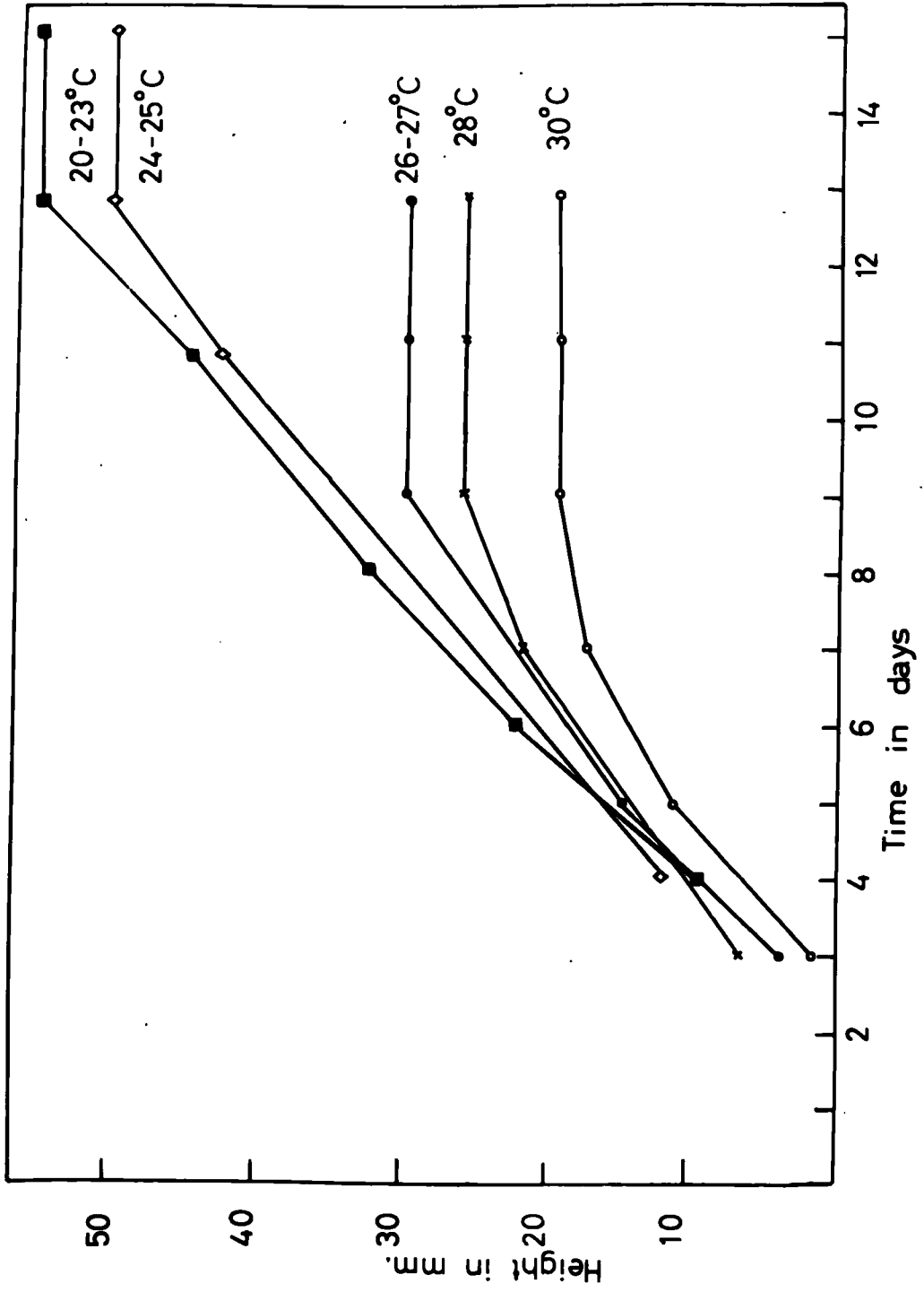


FIGURE 2-3 INFLUENCE OF TEMPERATURE ON CONIDIOPHORE GROWTH

in Figure 23. The maximum height of the conidiophores at each temperature is recorded below in Table 19 and in the form of a histogram in Figure 22. Thus the experiment confirmed Gardner's finding that the optimum temperature for maximum conidiophore height is 20 - 22°C.

TABLE 19

Influence of temperature on conidiophore height

Temperature in °C	Final height of the conidiophores in mm.					Mean
14 - 18	43	41	39	39	39	40.0
20 - 23	55	57	52	56	54	55.0
26 - 27	29	30	28	32	30	30.0
28	25	24	26	27	28	26.0
30	20	18	21	21	20	20.0
32	5	5	5	4	6	5.0
33 - 34	1.0 mm					

Experiment 31 to determine the influence of temperature on dry weight.

Experimental Procedure

The fungus was cultured in 100 ml flasks containing 20 ml of Zurzyka solution. Five flasks were placed into each illuminated incubator; the light intensity was 3.4 foot-candles.

Results.

Three weeks after inoculation the dry weight and carotene content of the mycelium in each flask were determined. The mean values for each set were calculated and the results are presented in Table 20.

TABLE 20Influence of temperature on dry weight and carotene production

Set	Temperature in °C	Mean dry weight in milligrams	Mean carotene content in $\mu\text{g}/\text{gm}/\text{gm}$ dry weight
1	20 - 23	187	271
2	25 - 27	191	369
3	30	147	424

Discussion of the results of Experiments 29, 30 and 31.

The results of experiment 30 confirm Gardner's (1956) finding that the optimum temperature for maximum conidiophore height is in the 20 - 23°C temperature range. She also reported that temperatures above 30°C completely suppress elongation, but it was found in experiment 30 that tall conidiophores are in fact produced at 32°C. A point which should be mentioned is that the tall conidiophores produced at temperatures of 30 - 32°C did not form fruiting heads. Comparing the results of experiments 29 and 30 one finds that there is a marked difference between the optimal temperatures for conidiophore height (20 - 23°C) and mycelial growth (30°C). A point which should perhaps be stressed is that 20 - 23°C is not the optimum temperature for the rate of conidiophore growth but the range in which the tallest conidiophores are produced. The rate of elongation over a range of temperatures was not determined but it was found that conidiophores grow faster at 25°C than at 23°C. It would be interesting to find the

optimum temperature for the rate of conidiophore elongation. One would rather expect it to be closer to 30°C than to 20°C.

Dry weight production for a complete range of temperatures was not determined but the results of experiment 31 would appear to indicate that the optimum temperature for dry weight production lies in the 25 - 27°C range. It should be noted that the concentration of carotene was highest at 30°C.

The difference in the temperature optima for mycelial growth and conidiophore height is very interesting. Low temperatures (20 - 23°C) favour the production of very tall conidiophores and depress mycelial growth. A possible explanation of this correlation is that the high temperatures which favour high rates of growth and metabolism also lead to a more rapid staling of the culture medium. This in turn might well reduce the duration of the period during which tall conidiophores supported by the mycelium can continue to elongate. It is intended in the future to conduct further experiments to investigate this hypothesis. One such experiment would be to culture the fungus at a high temperature on a culture solution which is changed at regular intervals in order to reduce the accumulation of staling products. It is possible that the accumulation of staling substances in the medium is not the only factor which inhibits conidiophore growth at high temperatures. It would be interesting to

determine what biochemical differences exist between cultures grown at high and low temperatures. An investigation of this nature would help in the interpretation of the causes of the morphological differences of cultures grown at different temperatures.

3. INFLUENCE OF TRANSFERRING CULTURES TO DIFFERENT TEMPERATURE CONDITIONS
Experiment 32 to determine the influence on conidiophore production and elongation of transferring cultures at intervals after inoculation to different temperature conditions.

Experimental Procedure.

Small oxoid caps containing 2% malt agar were prepared. Three of these were placed into each of a number of 400 ml beakers lined with damp blotting paper and capped with a petri dish lid. The cultures were inoculated with a spore suspension and then divided into two sets; one was placed in an illuminated incubator at 23°C while the other was placed in a similar incubator at 32°C.

Forty-eight and seventy-two hours after inoculation two beakers from the incubators were exchanged with each other.

Results.

The mean height of the tall conidiophores in each set of cultures was determined at intervals and the results are recorded in Tables 21a and 21b. The extent and time of short conidiophore production are also recorded.

TABLE 21a Influence on conidiophore growth of transferring cultures to a lower temperature

Time after inoculation in days	T r e a t m e n t					
	Set 1 cultured at 23°C		Set 2 cultured at 32°C for 2 days, then transferred to 23°C		Set 3 cultured at 32° for 3 days, then transferred to 23°C.	
2	Short conidio- phores	Height of tall conidiophores in mm.	Short conidio- phores	Height of tall conidiophores in mm.	Short conidio- phores	Height of tall conidio- phores in mm.
3	No visible sign of conidiophores	8, 8, 9, 10, 8. Mean 8.0	Surface mat with heads	None visible	Surface mat with heads	None visible
4	Only a few	12, 13, 12, 13, 12.5. Mean 12.5	"	4, 6, 4, 3, 3, Mean 4.0	"	1.5, 1.5, 1.5, 1.5, 1.5 Mean 1.5
6	"	30, 32, 30, 30, 28. Mean 30.0	"	8, 9, 9, 9, 10. Mean 9.0	"	6, 7, 6, 6, 5 Mean 6.0
9	"	50, 50, 48, 50, 52. Mean 50.0	"	20, 18, 22, 20, 20. Mean 20.0	"	15, 15, 16, 15, 14. Mean 15.0
				33, 30, 33, 34, 35. Mean 33.0	"	20, 22, 23, 22, 23. Mean 22.0

TABLE 21b Influence on conidiophore growth of transferring cultures to a higher temperature

Time after inoculation in days	T r e a t m e n t				Short conidio- phores in mm.
	Set 4 cultured at 32°C	Set 5 cultured at 23°C for 2 days, then transferred to 32°C	Set 6 cultured at 23°C for 3 days, then transferred to 32°C	Short conidio- phores	
	Height of tall conidiophores in mm.	Height of tall conidiophores in mm.	Height of tall conidiophores in mm.	Height of tall conidio- phores in mm.	
2	Surface mat with heads	No sign of tall conidiophores	No visible sign of conidiophores	No visible signs of conidiophores	
3	"	1.5, 1.5, 1.5, 1.5, 1.5. Mean 1.5	Only a few	Only a few	7, 7, 7, 8, 6. Mean 7.0
4	"	2.0, 2.0, 2.0, 2.0, 2.5 Mean 2.0	"	"	8, 9, 7, 8, 8. Mean 8.0
6	"	5, 4.5, 4.5, 4, 4.5. Mean 4.5	"	"	8, 11, 11, 10, 10. Mean 10.0
9	"	8, 7, 7, 7, 6. Mean 7.0	"	"	11, 11, 12, 9, 12. Mean 11.0

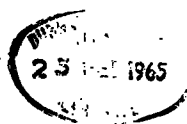
Discussion of the results.

It may be seen from the results of this experiment that high temperatures favour the production of short conidiophores below c. 1 mm tall and inhibit the growth of tall conidiophores. Low temperatures inhibit short conidiophore production and favour tall conidiophore growth. When cultures were transferred from 23°C to 32°C the tall conidiophores produced were very little taller than those cultured continuously at 32°C. It would thus appear that high temperatures have an almost immediate influence on inhibiting conidiophore growth. Similarly when cultures are transferred from 32°C to 23°C the tall conidiophores grow to heights similar to those of cultures grown continuously at 23°C.

Experiment 33 to determine whether light given while the cultures are at a high temperature is effective in inducing conidiophore elongation when they are subsequently returned to a lower temperature.

Experimental Procedure.

The cultures were grown on Zurzyka agar in large oxoid caps placed in 125 ml conical beakers capped with 150 ml squat beakers. They were inoculated with a spore suspension and then placed in an illuminated incubator maintained at a temperature of $21 \pm 0.5^\circ\text{C}$. The light intensity at the level of the cultures was 3.4 foot-candles or 36 metre-candles.



Three days after inoculation the average height of the tall conidiophores produced by the cultures was 3.5 mm. They were divided into 5 sets with 5 replicates in each; all but the first set were placed into biscuit tins. On the following day sets 2 - 4 were illuminated for 30 minutes and this treatment was repeated at 24 hour intervals for the next three days. The temperatures at which the various sets were illuminated are recorded in Table 22. Thirty minutes prior to each period of illumination the tins containing sets 3 and 4 were transferred to an incubator maintained at a temperature of $32 \pm 0.5^{\circ}\text{C}$. It had previously been found that the temperature inside the tins equilibrated with that of the incubator within this 30 minute period. The light intensity was the same as that of the incubator maintained at the lower temperature. At the end of the illumination period the lids of the tins were replaced; set 3 was immediately transferred to the 21°C incubator while set 4 was transferred an hour later. Set 1, 2 and 5 were cultured in the incubator at the lower temperature all the time.

Results.

The cultures were examined 48 hours after the final period of illumination. The mean height, dry weight and carotene content of the tall conidiophores in each set were determined and the results are recorded in Table 22.

TABLE 22 Influence on conidiophore growth of illuminating cultures daily at different temperatures

Set	Treatment	Tall conidiophores						Carotene as % of Set 1
		Height in mm.	Mean dry weight in milligrams	Dry weight as % of Set 1	Mean carotene in μ gm/gm dry weight			
1.	Illuminated continuously at 21°C	20, 24, 22, 22, 22. Mean 22.0	162	100%	150.0			100%
2.	Illuminated for 30 min. per day at 21°C	11, 10, 12, 11, 11. Mean 11.0	95	58.6%	32.0			21%
3.	Illuminated for 30 min. per day at 32°C	12, 12, 10, 13, 11. Mean 12.0	96	59.3%	58.0			39%
4.	Illuminated for 30 min. per day at 32°C. Left at same temp. in dark for 1 hour, then transferred to 21°C.	11, 12, 12, 11, 11.5 Mean 11.5	122	75.3%	60.0			40%
5.	In darkness	6.5	Conidiophores not as abundant as in other treatments					

Discussion of the Results.

The results of experiment 33 indicate that the inhibitory influence of high temperatures on conidiophore growth is not effective during the photoinductive process. Light given at high temperatures is effective in inducing conidiophore elongation if the cultures are subsequently returned to darkness at lower temperatures. This would appear to indicate that it is continued incubation at high temperatures which is effective in inhibiting conidiophore elongation rather than to any inhibition of the actual photoinductive process. The result of this experiment could either be interpreted as supporting the 'staling' hypothesis suggested on page 97 or as indicating that certain stages of the inductive process which can proceed in darkness are temperature sensitive.

It should be noted that cultures which are left in darkness at 32°C for a further hour after illumination had a higher conidiophore dry weight than the other cultures. The mean carotene content of both sets illuminated at 32°C was approximately twice that of the set illuminated at 21°C; there was thus a doubling of the concentration of carotene for an approximately 10°C rise in temperature.

SECTION V

INFLUENCE OF THE MEDIUM ON TALL CONIDIOPHORE GROWTH

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SUMMARY

Neither volume of the culture medium nor transference of mycelial mats to fresh culture media influences the duration of the induction period. Tall conidiophores cultured on media with pH's ranging from 4.6 - 7.9 grow at about the same rate. Conidiophores continue to elongate when they are transferred to 2.0 molar sucrose solutions. The density and carotene content of tall conidiophores - but not their height - is influenced by the concentration of glucose in the medium; the optimum concentration for maximum tall conidiophore density is in the 5 - 10% range. None of a number of substances tested could substitute for light in inducing tall conidiophore elongation.

1. INFLUENCE OF THE VOLUME OF THE CULTURE MEDIUM

If the changes in the medium, which Gardner maintains must take place prior to conidiophore production, involve the accumulation of a certain substance (or substances) produced by the mycelium, it is probable that the volume of the culture medium would have an influence on the length of the induction period. The following experiment was conducted in order to investigate this particular point.

Experiment 34 to determine whether the volume of the culture medium influences the length of the induction period.

Experimental Procedure.

Fifteen 50 ml beakers containing rafts floating on 2% malt solution were prepared. The beakers were divided into three sets with five replicates in each. The volume of the malt solution in each set is recorded in Table 23.

The rafts were inoculated with spores and placed under illumination from a fluorescent tube in a room maintained at a temperature of $20 \pm 1^{\circ}\text{C}$.

Results.

The time when tall conidiophores were first observed in each culture was recorded. The mean values for each set were calculated and the results are recorded in Table 23.

TABLE 23Influence of volume and depth of the medium on conidiophore height

Set	Culture Medium		Time taken for tall conidiophore appearance in hours:		Conidiophore height 4 days after inoculation in mm:	
	Volume in mls	Depth in mm		<u>Mean</u>		<u>Mean</u>
1	20	22	49, 50, 51, 50, 50	50	6, 7, 8, 7, 7	7
2	35	31	48, 51, 51, 50, 50	50	7, 6, 7, 6, 6	6
3	50	41	51, 53, 50, 50, 51	51	7, 7, 8, 6, 7	7

It may be seen from Table 23 that, within the limits of the experiment, volume of the culture medium does not influence the duration of the induction period.

2. INFLUENCE OF TRANSFERRING MYCELIA TO FRESH CULTURE MEDIA

Experiment 35 to determine what influence transference of mycelia to fresh medium has on the duration of the induction period.

Experimental Procedure.

Fifteen 50 ml beakers containing rafts floating on 20 mls of a 2% malt solution were prepared. The rafts were inoculated with spores and then placed beneath a fluorescent tube in a room maintained at a temperature of $20 \pm 1^{\circ}\text{C}$. The light intensity at the level of the culture was 10 foot-candles or 107 metre-candles.

Thirty-six hours after inoculation the cultures were divided into three sets with five replicates in each. The treatment given to each set was as follows.

Set 1. The rafts supporting the mycelia were removed from the beakers with a pair of sterile forceps. Each raft was washed in a sterile 2% malt solution and then transferred to beakers containing 20 mls of fresh 2% malt solution.

Set 2. Each raft was lifted clear of the culture medium, dipped in sterile 2% malt solution, and then replaced in the beaker containing the original culture solution.

Set 3. The cultures were not disturbed.

After a further 26 hours growth, making a total of 62 hours after inoculation, the typical height and the height of the few taller conidiophores in each beaker were measured. The mean values for each set were calculated and are recorded in Table 24.

It is possible to obtain an estimate of the maximum duration of the induction period in the cultures by subtracting from the period since inoculation the time required for the tallest conidiophore to reach the height attained. The mean rate of conidiophore elongation during the period following the induction period would not exceed c. 500 μ /hour. The mean induction period of each set was calculated using this estimate of the rate of conidiophore elongation.

TABLE 24

Influence on the induction period of transferring
mycelial mats to fresh culture media

Set	Treatment	Height of tall conidio- phores in mm.		Estimated age of taller conidiophores in hours	Estimated duration of induction period (hrs)
		Typical	Taller conidio- phores		
1	Transferred	4.0	5.5	11	51
2	Washed and replaced	4.0	6.5	13	49
3	Not disturbed	5.0	7.5	15	47

In the experiment described above the mycelia were cultured continuously in light. In the following experiment the mycelia were cultured in darkness, transferred to fresh media in red light and then subsequently grown in light. According to Gardner, under these culture conditions the conidiophores would start to elongate 48 hours after being transferred to the fresh media.

Experiment 36 to determine the duration of the induction period
when cultures are transferred to fresh media prior to illumination.

Experimental Procedure.

Ten beakers containing rafts floating in 20 mls of 2% malt solution were prepared. The rafts were inoculated with spores and the cultures were placed in darkness in a room maintained at a temperature of $20 \pm 1^{\circ}\text{C}$.

Two days after inoculation the cultures were divided into

two sets with five replicates in each. The sets were then treated in red light in the following manner.

Set 1. The rafts supporting the mycelia were removed from the beakers, washed in sterile 2% malt solution, and then placed in beakers containing 20 mls of fresh 2% malt solution.

Set 2. The cultures were not disturbed.

All the cultures were then placed in light under a fluorescent tube. Twenty-two hours later the height of the taller conidiophores in each culture was measured and the mean value for each set calculated. The results are recorded in Table 25. The estimated duration of the induction periods after transfer are also presented in Table 25. They were calculated in the manner described in the previous experiment.

Results.

TABLE 25

Set	Treatment	Mean height of taller conidiophores 22 hrs after transfer (in mm)	Estimated age of taller conidiophores (in hours)	Estimated duration of induction period after transference and illumination (in hrs)
1	Transferred	4.5	9	13
2	Not transferred	5.5	11	11

It is possible that a two to three-day old mycelium has already accumulated from the medium all that it requires for subsequent conidiophore elongation. The following experiment was conducted in order to determine whether a three-day old

mycelium would produce tall conidiophores when illuminated while floating on sterile distilled water.

Experiment 37 to determine whether tall conidiophores would be produced by mycelia which had been cultured in darkness and then illuminated while floating on water.

Experimental Procedure.

Ten 50 ml beakers containing rafts floating on 20 ml of 2% malt solution were prepared. The rafts were inoculated with spores and the cultures divided into two sets with five replicates in each. The sets received the following treatments.

Set 1. Placed in light under a fluorescent tube in a room maintained at a temperature of $20 \pm 1^{\circ}\text{C}$.

Set 2. Placed in darkness in a cupboard in the same room.

Three days after inoculation the beakers were taken from the dark cupboard in red light. The rafts supporting the mycelial growth were removed from the cultures, washed in sterile distilled water, and placed in 50 ml beakers containing c. 30 mls of sterile distilled water. The cultures of this set were then placed beside those of the other set in light. The height of the tall conidiophores in each culture was measured at intervals and the mean value for each set calculated.

Results.

The results are presented in Table 26.

TABLE 26

Influence of transferring mycelial mats cultured in
darkness to distilled water in light

Set	Treatment	Mean height of tall conidiophores in mm				
		72 hrs	96 hrs	115 hrs	125 hrs	150 hrs
1	Cultured continuously in light	6.5	11.5	17.5	22.0	
2	Transferred to light after three days	Transferred	4.5	8.5	10.5	13.5

Discussion of the Results of Experiments 35, 36 and 37

The results obtained in this series of experiments do not confirm Gardner's hypothesis that certain changes occur in the culture medium during the induction period which are essential for conidiophore elongation. It was found that the duration of the induction period is not appreciably increased by transferring cultures to fresh media either 36 hours after inoculation or just prior to their illumination. In experiments 35 and 36 the duration of the induction periods of transferred cultures were respectively only 4 and 2 hours longer than the controls. If Gardner's hypothesis held true one would expect the induction periods of the transferred cultures in these experiments to have been 48 hours longer than the controls. In experiment 37 it was shown that a three-day old mycelial mat cultured in darkness is capable of supporting tall conidiophore growth even though it was subsequently only supplied with water. The length of

the induction periods of the cultures transferred to water was 14 hours.

The results from these three experiments clearly show that conidiophore elongation is not dependent upon the accumulation in the culture medium of certain chemical substances; a mycelial mat cultured in darkness for 3 days needs only a supply of water for subsequent conidiophore elongation in the light. Thus the essential changes which occur on illumination and prior to the induction of conidiophore elongation take place in the mycelium and not in the culture medium.

3. INFLUENCE OF pH OF THE MEDIUM

The following experiment was conducted in order to determine if pH of the medium had an influence on tall conidiophore production and elongation. It has been shown that pH influences growth and fruiting of a number of fungi. Lilly & Barnett (1947), for example, found that Sordaria fimicola does not produce perithecia unless the pH of the medium is above 6.0.

Experiment 38 to determine what influence pH of the medium has on tall conidiophore elongation.

Experimental Procedure.

A phosphate rather than a citric acid buffer was used as it provided a medium which differed only in phosphate

concentration from the Zurzyka medium employed in many of the other experiments. A series of phosphate buffers was prepared using double the concentration of potassium dihydrogen phosphate and disodium hydrogen phosphate suggested by Machlis & Torrey (1959). Similarly a double strength solution of Zurzyka culture medium was prepared. The culture solution and the phosphate buffers were autoclaved separately and then mixed to provide buffered culture solutions of the required composition. The concentration of phosphate in the buffered solutions was of the order of nine times greater than that normally contained in the culture medium. The pH of the solutions were measured, after sterilization, with an E.I.L. meter and it was found that the pH's tended to be slightly lower than was expected on the basis of the data given by Machlis & Torrey.

The cultures were grown on large rafts floating in 50 mls of buffered solution in 125 ml conical beakers which were capped with 150 ml squat beakers. Each raft was inoculated centrally with a small piece of mycelium cut from the edge of a colony growing on 2% malt agar. There were three replicates in each set. The beakers were placed in an incubator maintained at a temperature of $21 \pm 1^{\circ}\text{C}$ and the light intensity at the level of the cultures was 3.4 foot-candles or 36 metre-candles.

Results

The height of the conidiophores and the pH of the culture medium fourteen days after inoculation are recorded in Table 27. The optical densities of the culture media at 410 m μ at the end of the experiment were also determined. During the course of the experiment no difference was observed between the replicates in production, density or rate of growth of the tall conidiophores. At the end of the experiment there were dense stands of orange coloured conidiophores in all cultures, with only scattered fruiting heads having been produced.

TABLE 27

Influence of pH of the culture medium on conidiophore height

Set Phosphates in the solutions	Original pH of medium	Final pH of medium	Mean final height of conidio-phores (mm)	Optical density of medium at 410 m μ
1 Normal Zurzyka medium	4.6	3.1	35	-
2 KH PO	4.4	3.4	40	0.085
3 KH PO and Na HPO	5.4	4.1	40	0.097
4 " "	5.8	5.3	42	0.154
5 " "	6.9	6.5	40	0.217
6 " "	7.4	6.8	41	0.281
7 " "	7.6	6.9	37	0.345
8 Na HPO and KCl to provide K	7.9	7.0	40	-

Discussion of Results

It thus appears that the duration of the induction period and the rate of conidiophore elongation are not influenced by pH of the medium over the range 4.6 - 7.9. One fact which is clearly shown by this experiment is that during growth the pH of the medium falls, i.e. it becomes more acidic in nature. This fall in pH is probably due to the nature of the nitrogen source in the medium. Ingold (1961) points out that when the nitrogen supply is in the form of an ammonium salt, ammonium nitrate in this case, the cation is commonly absorbed and utilized much more rapidly than the anion and this brings about a fall in pH.

At the end of the experiment there was only one really marked difference between the various sets of cultures and this was in the colour of the media. This ranged from a pale straw colour in set 2 to a deep yellow orange colour in set 7. A yellow, pH sensitive, pigment is passed into the medium during growth. This was clearly demonstrated by observing the change in colour when the pH of these solutions was altered by the addition of acid or alkali. Thus the difference in colour and optical density (see Table 27) between the culture media of the various sets was probably due in the main to the differences in pH rather than to differences in the amounts of pigment present in the medium.

4. INFLUENCE OF GLUCOSE CONCENTRATION

Experiment 30 to determine the influence of glucose concentration on tall conidiophore production and elongation.

Experimental Procedure.

Zurzyka agar culture media containing the concentrations of glucose listed in Table 28 were prepared. Large oxoid caps containing the various culture media were placed in 125 ml conical beakers capped with squat beakers. There were five replicates in each set of cultures which were placed in a light box. The experiment was carried out in a room maintained at $22 \pm 1^{\circ}\text{C}$ and the light intensity at the level of the cultures was 8.5 foot-candles or 91 metre-candles.

Results.

The cultures were examined 18 days after inoculation. The mean height, dry weight and carotene content in each set of cultures were determined and the results are presented in Table 28.

TABLE 28

Influence of concentration of glucose in the medium on carotenogenesis and tall conidiophore production under conditions of continuous illumination

Set	Glucose conc. in the medium	Tall conidiophores		
		Mean ht. in mm above medium	Mean dry wt. in milligrams	Mean carotene content in $\mu\text{ gm/gm}$ dry wt.
1	1%	55	49	412
2	5%	55	160	272
3	10%	55	144	291
4	20%	50	130	199

Experiment 40 to determine the influence of glucose concentration on the photoinduction of conidiophore elongation

Experimental Procedure.

Zurzyka agar culture media containing the concentrations of glucose listed in Table 29 were prepared. Large oxoid caps containing the various culture media were placed in 125 ml conical beakers capped with squat beakers. Two sets of each concentration of glucose were prepared and there were five replicates in each set. One set of each pair was placed in a light box and illuminated for one minute every hour; the light intensity at the level of the cultures was 8.5 foot-candles or 91 metre-candles. The second set of each pair was placed in darkness in a similar box. The experiment was carried out at a temperature of $22 \pm 1^{\circ}\text{C}$.

Results.

The cultures were examined 18 days after inoculation. The mean height, dry weight and carotene content of the tall conidiophores in each set were determined and the results are presented in Table 29. There was only sparse production of short conidiophores in all the cultures grown in darkness.

TABLE 29

Influence of concentration of glucose in the medium on
carotenogenesis and tall conidiophore production under
conditions of intermittent illumination

Set	Glucose conc. in the medium	Tall conidiophores		
		Mean ht. in mm above medium	Mean dry wt. in milligrams	Mean carotene content in μ gm/gm dry wt.
1	1%	27.5	45	141
2	5%	24.5	152	72
3	10%	25.5	189	80
4	20%	22.5	129	54
<u>Control cultured in light</u>				
5	5%	50.0	174	214

Discussion of the Results of Experiments 39 and 40

It may be seen from Tables 28 and 29 that the height of tall conidiophores does not appear to be significantly influenced by the concentration of glucose in the medium. There are, however, considerable differences in conidiophore dry weight between the various glucose treatments in experiments 28 and 29. As the height of the tall conidiophores is approximately the same in each experiment, it seems clear that the differences in dry weight result from variation in the density of tall conidiophore production per unit surface area of the medium; this in turn presumably reflects differences in the density of foot cell production per unit area of the mycelium. The optimum glucose concentration for maximum tall

conidiophore density is in the region of 10% in experiment 30 and 5% in experiment 29. In both experiments the carotene content of the tall conidiophores is highest on media containing 1% glucose and lowest on media containing 20% glucose.

Thus the concentration of glucose in the medium influences the density of tall conidiophore production and their carotene content, but not their final height. Fewer conidiophores per unit surface area of the medium are produced at low glucose concentrations but they are as tall as others formed at higher concentrations.

5. INFLUENCE OF OSMOTIC PRESSURE OF THE MEDIUM

It was shown in experiment 37 that a three-day old mycelial mat which had been cultured in darkness would produce tall conidiophores when transferred to distilled water and then illuminated. Conidiophore elongation under these circumstances is thus solely dependent upon a supply of water from the medium. The following experiment was conducted to determine whether or not tall conidiophores would be produced and elongate when mycelial mats were illuminated while floating on a range of solutions of varying osmotic pressures. Tall conidiophore elongation on a particular solution would indicate that the suction pressure of the fungal cells was greater than the osmotic pressure of the solution on which they were floated.

Experiment 41 to determine the suction pressure developed by the tall conidiophores.

Experimental Procedure.

The cultures were grown on large rafts floating on 20 mls of Zurzyka solution in 100 ml squat beakers. Each was inoculated with a drop of a spore suspension and then placed in darkness in a room maintained at $20 \pm 1^{\circ}\text{C}$. The following solutions of sucrose were prepared:-

Sucrose 0.5 M, 1.0 M, 2.0 M and 3.0 M.

Six days after inoculation the culture media were transferred to an incubator maintained at a temperature of $21 \pm 1^{\circ}\text{C}$. The light intensity at the level of the flasks was 3.4 foot-candles or 36 metre-candles. Twelve hours later the rafts supporting the mycelial mats were transferred to 100 ml beakers containing 20 mls of the solution described above. There were four replicates of each solution.

Results.

The height of the conidiophore stalks were measured at regular intervals and the mean conidiophore height was calculated for each set. The results are presented in Table 30.

TABLE 30Influence of the molarity of sucrose solutions on conidiophore growth

Set	Solutions to which the mycelial mats were transferred	Osmotic pressure of solns. in atmospheres	Mean height of tall conidio- phores in mm at days after transfer				Density of conidio- phore growth
			One	two	three	eight	
1	Untreated cultures	c.10	5.0	11.0	15.5	19.0	4
2	Distilled water	0	4.5	9.5	12.5	14.0	4
3	0.5 Molar Sucrose	14	4.0	7.5	12.0	14.0	4
4	1.0 Molar Sucrose	35	3.0	6.0	8.5	10.0	3
5	2.0 Molar Sucrose	c.122	2.0	2.5	3.0	3.0	1
6	3.0 Molar Sucrose	c,200	No sign of tall conidiophore production				

In the experiment described above no provision was made to ensure that the humidity in the culture vessels was maintained at a high level. It was shown in experiment 27 that humidity has a marked influence on conidiophore height. The strong sugar solutions would tend to lower the relative humidity in the culture vessels, and because of this it was decided to repeat the experiment in such a way as to ensure that the atmosphere above the cultures was kept as humid as possible.

Experiment 42Experimental Procedure.

Small rafts were floated on Zurzyka culture solution in 400 ml beakers and then inoculated with drops of a spore suspension. These beakers were placed in darkness in a room maintained at a temperature of $20 \pm 1^{\circ}\text{C}$.

Six days after inoculation the rafts were floated on sucrose solutions in large oxoid caps; the caps were then transferred to the culture vessels. The culture vessels employed were the 125 ml conical beakers lined with wet blotting paper described on page 4 . Figure 19 on page is a drawing of one such culture. Due to the presence of the rafts covering most of the surface, very little of the sucrose solutions were exposed to air and the relative humidity in the culture vessels was maintained at a high level. There were four replicates in each set of sucrose solutions.

Results.

The height and density of the conidiophores in each culture were measured at regular intervals. The mean values for each set of cultures was calculated and the results are presented in Table 31 and graphically in Figure 24.

TABLE 31

Influence of the molarity of sucrose solutions on conidiophore growth

Set	Solutions to which the mycelial mats were transferred	Osmotic pressure of solns. in atmospheres	Mean height of tall conidiophores in mm at days after transfer				Density of conidiophore growth
			One	Two	Four	Six	
1	Water	0	3.0	9.0	19.0	25.0	4
2	0.75 Molar Sucrose	23	3.0	6.5	14.0	19.0	2
3	1.0 Molar Sucrose	35	3.0	6.5	15.0	17.0	2
4	1.5 Molar Sucrose	66	2.0	5.0	11.0	14.0	2-
5	2.0 Molar Sucrose	c.122	2.0	3.0	7.0	9.0	1+

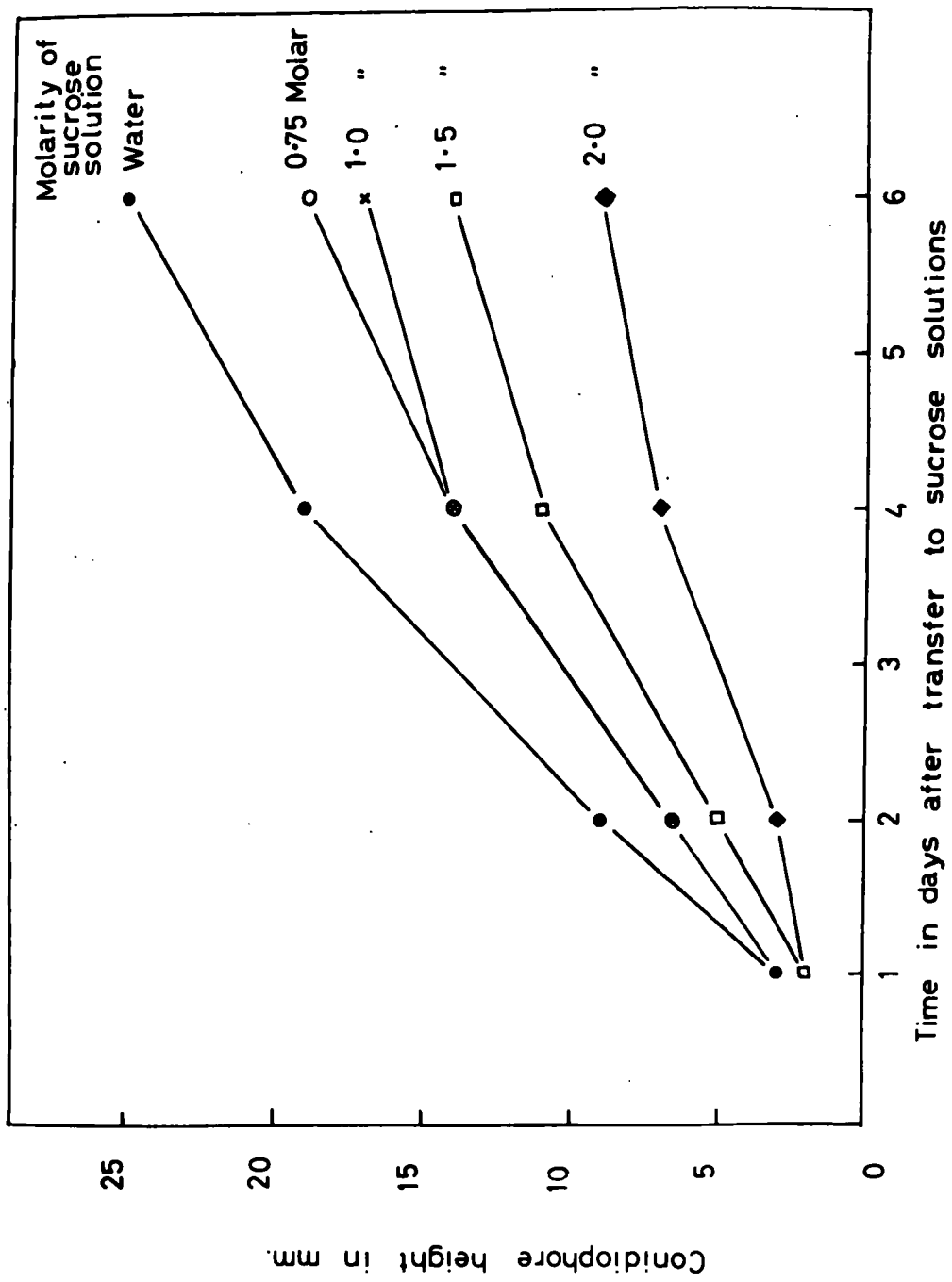


FIGURE 24 INFLUENCE OF DIFFERENT MOLAR SOLUTIONS OF SUCROSE ON CONIDIOPHORE ELONGATION

Discussion of the Results of Experiments 41 and 42.

It is a well established fact that many species of the Aspergilli can grow on solutions of high osmotic pressure. Fraser & Chambers (1907) found that the growth of Aspergillus herbariarum is favoured by sugar concentrations in the culture medium of 40% and Brancato & Golding (1953) reported that Aspergillus niger can grow on culture solutions containing 6 - 7 molal concentrations of glycerol (these solutions would have osmotic pressures of 134.4 and 156.8 atmospheres). In both instances the cultures were grown from germination in in the solutions of high osmotic pressure.

In experiment 41 tall conidiophores were produced on all except the 3.0 molar sucrose solution, but even at this high concentration the mycelial mats became orange as they aged, indicating that they were still metabolically active and that carotenogenesis had continued. The tall conidiophores produced by mycelial mats floating on 2.0 molar solutions in experiment 41 were comparatively short, but in experiment 42 in which provision was made to maintain a high relative humidity around the conidiophores they attained a height of 9.0 mm.

The rate of conidiophore growth at different sucrose concentrations in experiment 42 is shown in Figure 24. It can be seen that the rate of elongation is progressively

depressed by higher sucrose concentrations. The fact that conidiophores continue to grow on sucrose solutions as concentrated as 2.0 molar would seem to indicate that the suction pressure they developed is in excess of c. 122 atmospheres.

The question arises as to when this suction pressure is developed by the conidiophores, before or after transfer of the mycelial mats. There may well be some sucrose uptake during the first day after transfer but Gardner (1950) found that sucrose is not a very good carbohydrate source for growth, which might infer that it does not have a very good sucrose digesting enzyme system. Only radioactive isotope studies could show conclusively whether or not there is much uptake of sucrose during the first day after transfer, but as conidiophore elongation is not appreciably delayed it would seem reasonable to infer that the suction pressure developed is in the main the result of substances already present in the mycelium.

6. INFLUENCE OF INCORPORATING VARIOUS SUBSTANCES IN THE CULTURE MEDIUM

It is possible that tall conidiophore elongation which is normally induced by light could also be induced by incorporating a chemical factor in the medium. Cantino &

Horenstein (1959) found that a certain morphological form of Blastodiplodia emersonii which is produced in light could also be induced in darkness when a mixture of succinic and glyoxylic acid was added to the culture medium.

A series of experiments was conducted in which substances thought likely to induce conidiophore elongation were incorporated in the media of cultures grown in darkness. If a substance could be found which would induce tall conidiophore growth in darkness it would provide valuable information as to the biochemical differences which exist between cultures grown in darkness and in light.

Table 32 is a list of the substances used in these experiments.

Discussion of the Results of the Series of Experiments listed in

Table 32.

The type of investigation described above is very much of the 'hit or miss' variety but it was thought desirable that a number of the more obvious substances should be tested in this manner. It may be seen from the results presented in Table 32 that none of the substances tested induced tall conidiophore growth in darkness. It is clear that there must in fact be important biochemical differences between cultures grown in light and darkness which reflect the morphological variation but it would really require a very intensive investigation to reveal these differences. Such an investigation could well be rewarding but was beyond the scope of the present work.

Influence of the incorporation of various substances

Substance	Reason Used
1. 3-indolacetic acid	I.A.A. is a higher plant growth hormone known to influence the elongation of cells with cellulose walls.
2. Kinetin	A substance known to influence cell growth in higher plants.
3. Gibberellin	A substance which is also known to influence cell elongation in higher plants.
4. Riboflavin	A pigment which might be involved in the photo-inductive process leading to conidiophore elongation.
5. Riboflavin-5-phosphate (F.M.N.)	"
6. Carotene (from extract of carrot)	"
7. Succinic and glyoxylic acid	Cantino & Horenstein (1959) found that the incorporation of these two substances in the medium was effective in inducing the morphologic response normally induced by light.
8. Stale culture medium	Gardner (1950) suggested that certain changes must take place in the medium prior to conidiophore elongation. If this was in fact the case, such changes might involve the accumulation in the medium of a growth substance (s).
9. Macerated tall conidiophores.	If a growth substance is involved in inducing conidiophore elongation, macerated conidiophores might contain it in form which is able to induce conidiophore elongation in cultures grown in dark.
10. Glucose	Taller conidiophores are developed by cultures grown on Zurzyka medium, containing 5% glucose, than on 2% malt.

the medium on growth of cultures in darkness

Conc. employed	Effect
25, 50, 100 & 200 ppm. in 2% malt agar.	No tall conidiophore elongation.
1, 10 & 100 ppm. in Zurzyka agar.	"
"	"
10, 50 & 100 ppm. in 2% maltagar.	"
"	"
10, 100 & 1000 ppm. in Zurzyka agar.	"
"	"
in 2% malt agar.	"
0.01 & 0.001 molar mixtures in Zurzyka agar.	"
"	"
Culture media of various ages used.	"
"	"
Various quantities of tall conidiophores used.	"
"	"
1, 5, 10, 20%	"

SECTION VI

INVESTIGATION INTO THE PIGMENTS PRESENT IN THE FUNGUS

AND THE COURSE OF THEIR PRODUCTION

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2. HISTORY OF PIGMENT PRODUCTION	142
3. INFLUENCE OF PIGMENT INHIBITORS ON THE PHOTOINDUCTIVE PROCESS	151

SUMMARY

The pigments present in cultures grown in darkness include very low concentrations of β -carotene and higher concentrations of a yellow, 70% methanol soluble pigment which is pH sensitive and fluoresces in ultra-violet light. At least two additional carotenoids are present in cultures grown in light. The carotene content of illuminated cultures increases linearly with time and this increase continues after growth has stopped.

The incorporation in the culture medium of the riboflavin inhibitors, 1-lyxoflavin and mepacrine, and the β -carotene inhibitor, diphenylamine, did not influence the photoinduction of conidiophore elongation.

1. PIGMENTS EXTRACTED FROM THE FUNGUS

Extraction of pigments from the tall conidiophores.

It was necessary to find a suitable solvent to extract the pigments from the mycelium and tall conidiophores.

Experimental Procedure.

The following extractions were made from tall conidiophores of three week old cultures. Tall conidiophores were added to 5 ml portions of the solvents listed below. The solvents were examined the following day and the results are presented in Table 33.

TABLE 33

Effect of treating tall conidiophores with various solvents

Solvent	Colour of solvent after 1 day	Colour of conidiophores after 1 day
1. Acetone	Orange	Pale yellow
2. Ethyl alcohol	Yellow	Deep yellow
3. 70% Methanol	Pale yellow	Orange
4. Chloroform	Very pale yellow	Orange
5. Ether	Very pale yellow	Orange
6. Water	Very pale yellow	Orange
7. Benzene	Colourless	Orange
8. Petroleum ether	Colourless	Orange
9. Normal hexane	Colourless	Orange

Acetone proved to be the best solvent tested for the extraction of the orange pigment from the tall conidiophores.

Solvents 2 - 6 extract some of the pigments but the tall conidiophores were still coloured after treatment. The orange pigments which remained in the tall conidiophores after treatment with 70% methanol could subsequently be extracted with acetone.

Experiment 43: determination of the absorption spectra of pigment extracts.

It was clear that a separation of the pigments present in tall conidiophores could be made by first treating them with 70% methanol and then with acetone. The following procedure was employed to obtain the two extracts:-

- (i) Tall conidiophores and mycelium (3 grams fresh weight) were broken up with a glass rod in 10 mls of a solution of 70% methanol 30% water (v/v).
- (ii) The fungal material was then washed twice with 10 ml volumes of the 70% methanol solution.
- (iii) The fungal material was squeezed dry of the methanol.
- (iv) 10 mls of acetone was then added to the fungal material.

The extraction procedure described above was performed on tall conidiophores and mycelium which had been cultured for two weeks on Zurzyka solution in both light and darkness.

The extracts obtained are described below.

I. Extracts from cultures grown in light.

The methanol extract was pale yellow while the acetone

extract was bright orange. The absorption spectra of both extracts are shown in Figure 25a. The absorption spectrum of the methanol extract had no peaks in the visible part of the spectrum while that of the acetone extract had absorption maxima at 455 and 482 m μ .

II. Extracts from cultures grown in darkness.

A pale yellow pigment was extracted from the fungus when it was treated with 70. methanol but the acetone extract appeared to be colourless. The absorption spectra of both extracts are shown in Figure 25b. The absorption spectrum of the methanol extract was almost identical with that obtained from cultures grown in light.

Experiment 44: Determination of the carotene pigments present in cultures grown in light and darkness.

Experiment 44a - Cultures grown in light.

Experimental Procedure.

Hundred ml flasks containing 15 mls of Zurzyka solution were inoculated and then cultured in light in a room maintained at 22 - 23°C.

The mycelium was separated from the culture solution three weeks after inoculation and the carotene pigments present were extracted using Goodwin's method (page 13). The carotenes present were taken up in light petroleum (40 - 60°C fraction) and then chromatogrammed on a 20 x 1 cm

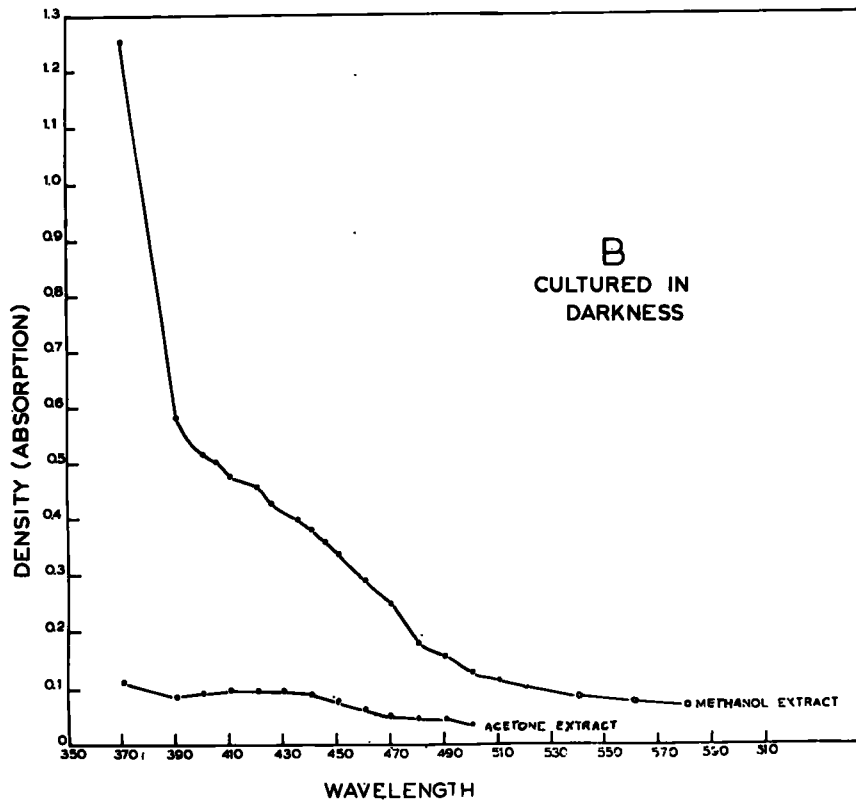
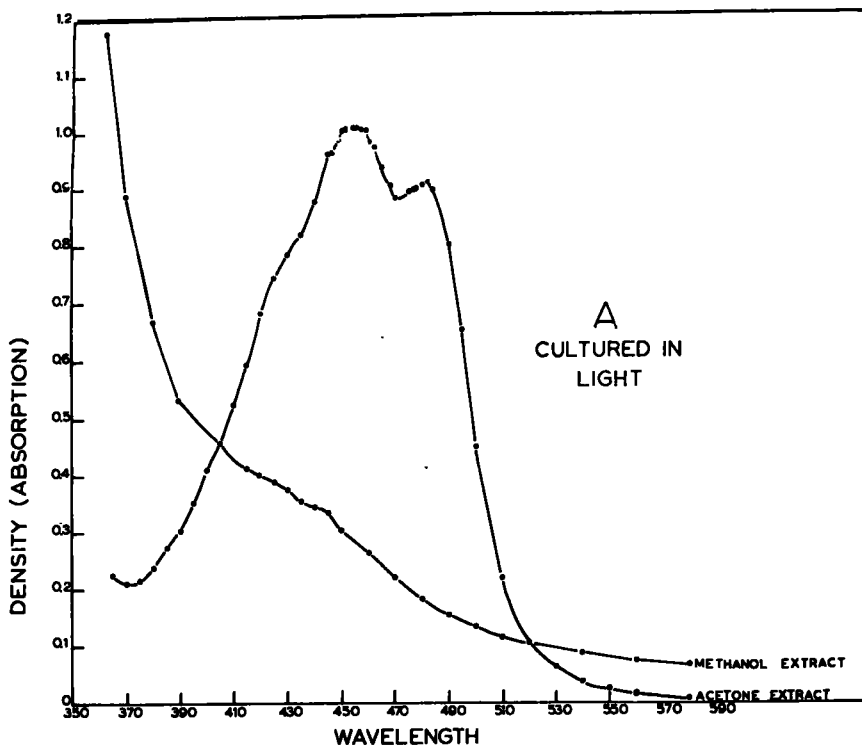


FIGURE 15 ABSORPTION SPECTRA OF EXTRACTS

column of active alumina. The column was then developed with a 90% light petroleum 10% diethyl ether v/v mixture. The bands developed on the column from top to bottom are presented in Table 34.

TABLE 34

Pigments developed on the alumina column from extracts
of the fungus grown in light

Depth of band	Colour	Whether it fluoresced in U.V.
1. 4 mm	Yellow	Yes (yellow)
2. 5 mm	Yellow-orange	Yes
3. 5 mm	Red	No
4. 6 mm	Pale orange	Yes (white)
5. 30 mm	Gap	
6. 25 mm	Orange	No
7. Eluted	Pale yellow	

The column was carefully cut into sections with a glass knife and the various pigments were treated with ether. All except the first two yellowish bands were eluted in this manner, taken up in suitable solvents and analysed with the spectrophotometer.

The absorption maxima of the various pigments are recorded in Table 35.

TABLE 35Absorption maxima of pigments extracted from cultures grown in light

Pigment	Maxima in m μ	Solvent
3. Red	457 485 520	Hexane
	450 482 520	"
	457 482-5 520	"
4. Pale orange	420-5 445 475	Hexane
	420-5 442 472	"
6. Orange	425-30 452 475-80	Hexane
	425-30 452 478-80	"
	425 452 488	Petroleum ether
	435 462 492	Benzene

The orange pigment of band 6 is undoubtedly β -carotene; Shibata (1956) found that the absorption spectrum of β -carotene in benzene has absorption maxima at 463 and 492 m μ with a shoulder at 436 m μ . The absorption spectrum of the orange pigment from band 6 in benzene is shown in Figure 26; it is almost identical with that of pure β -carotene in benzene. The absorption spectra of the red pigment from band 3 and the pale orange pigment from band 4, both in hexane, are shown in Figures 27a and 27b. The absorption spectrum of the former pigment corresponds most closely with the celaxanthin (456, 486.5 and 520 m μ) and Rhodoxanthin (458, 489 and 524 m μ in light petroleum) in regards to the position of its absorption maxima. There are a number of

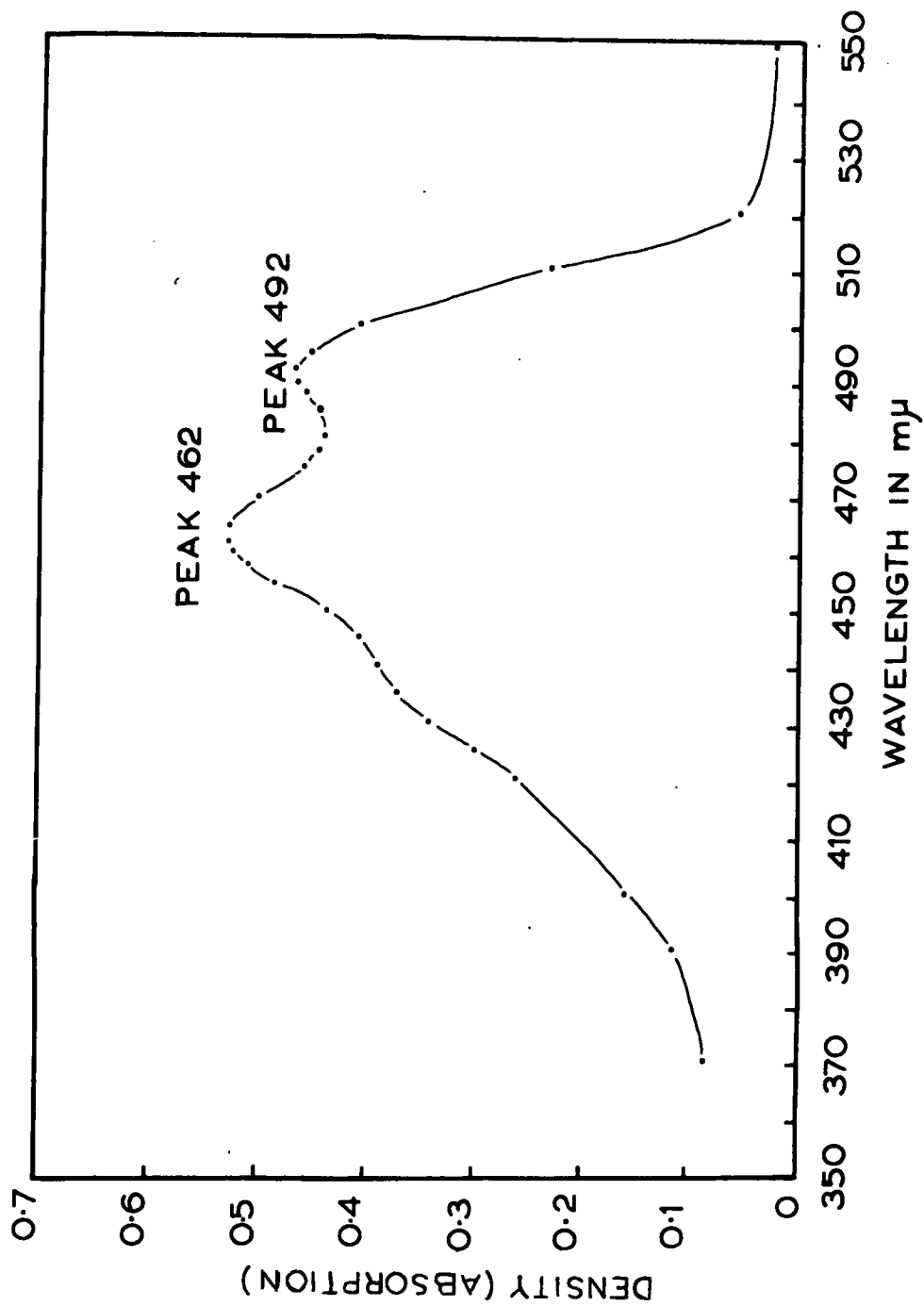
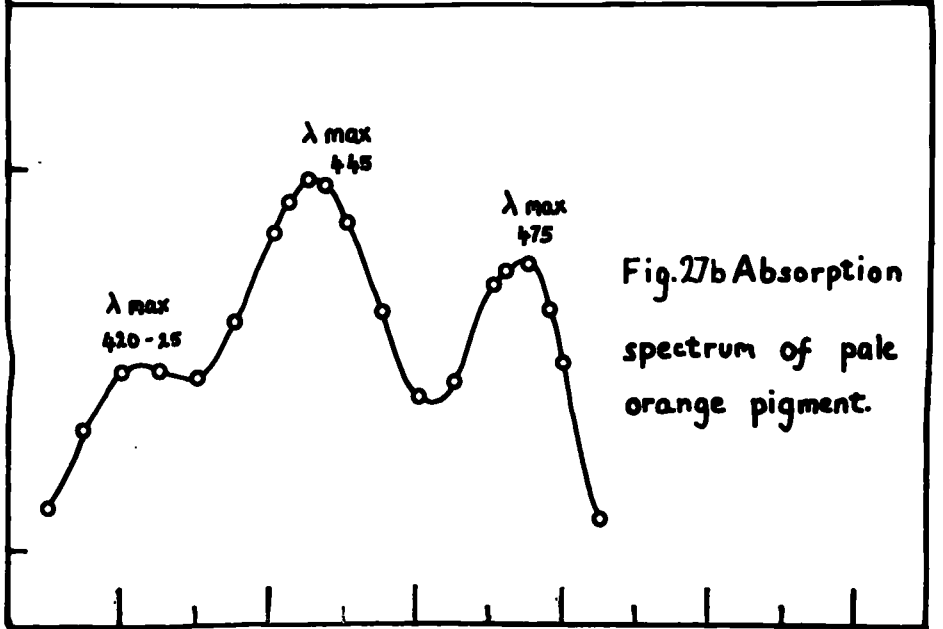
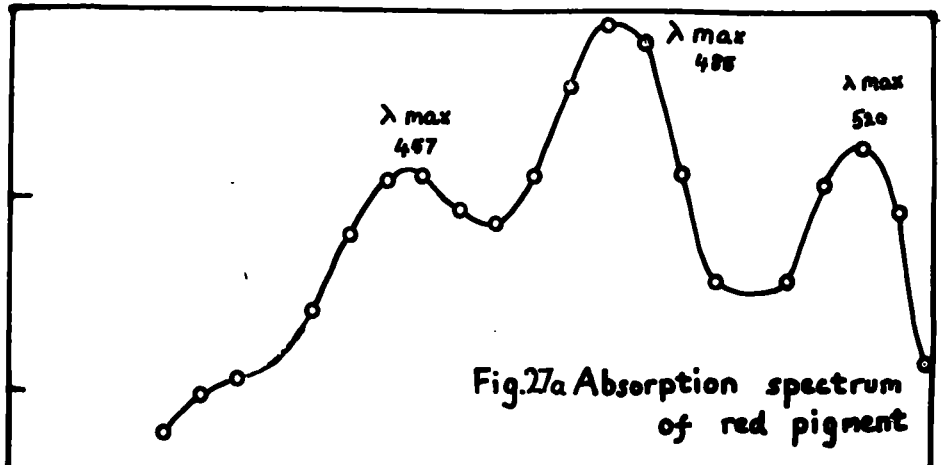


FIGURE 26 ABSORPTION SPECTRUM OF ORANGE PIGMENT
DISSOLVED IN BENZENE

Optical density



Wavelength in nm

carotenoid pigments which have absorption maxima, when dissolved in hexane, which correspond to those of the pale orange pigment extracted from band 4. They include α -carotene (420, 445 and 475 m μ) and lutein (420, 447 and 477 m μ).

Experiment 44b - Cultures grown in darkness.

Experimental Procedure.

Hundred ml flasks containing 15 mls of Zurzyka solution were inoculated and then placed in darkness in a room maintained at 22 - 23°C.

The mycelium (25 gm fresh wt.) was separated from the culture solution 3 weeks after inoculation and the carotene pigments present were extracted using Goodwin's method (page 13). The carotenes extracted were taken up in light petroleum (40 - 60°C fraction) and then chromatogrammed on a 20 x 1 cm column of active alumina. The bands developed on this column from top to bottom are presented in Table 36.

TABLE 36

Pigments developed on the alumina column from extracts of the fungus grown in darkness

No.	Depth of band	Colour	Whether it fluoresced in U.V.
1	4 mm	Yellow	Yes (yellow)
2	4 mm	Yellow-orange	Yes
3	3 mm	Colourless	Yes (white)
4	15 mm	Orange-pink	No
5	5 mm	gap	
6	2 mm	Faint yellow	Yes
7	4 mm	Colourless	Yes

The column was carefully cut into sections with a glass knife and the orange pink pigment of band 4 was extracted from the alumina with ether. The pigment was then transferred to hexane and its absorption spectrum was determined with the Unicam spectrophotometer. The absorption spectrum of this pigment is shown in Figure 28. Absorption peaks are present at 452 m μ and 478 = 480 m μ and there is a shoulder at 425 - 435 m μ . The absorption spectrum of pure β -carotene in hexane shows peaks at 451 m μ and 482 m μ with a shoulder at 425 m μ ; thus it seems clear that this pigment is in fact β -carotene.

The yellow pigments of the first bands developed on the alumina column could not be eluted with organic solvents or hydrochloric acid, but were eluted with sodium hydroxide solutions. The pigment extract was pH sensitive turning colourless at low pH's and deep yellow at high pH's. It had an absorption spectrum similar to that of the methanol extracts shown in Figures 25a and b, with no peaks of absorption in the visible range of the spectrum.

Attempts were made to extract the 70% methanol soluble pigment from cultures grown in darkness without exposing the extracts to light prior to spectroscopic examination. In other instances the pigment was extracted at low temperatures in a refrigerator. In no instance was an

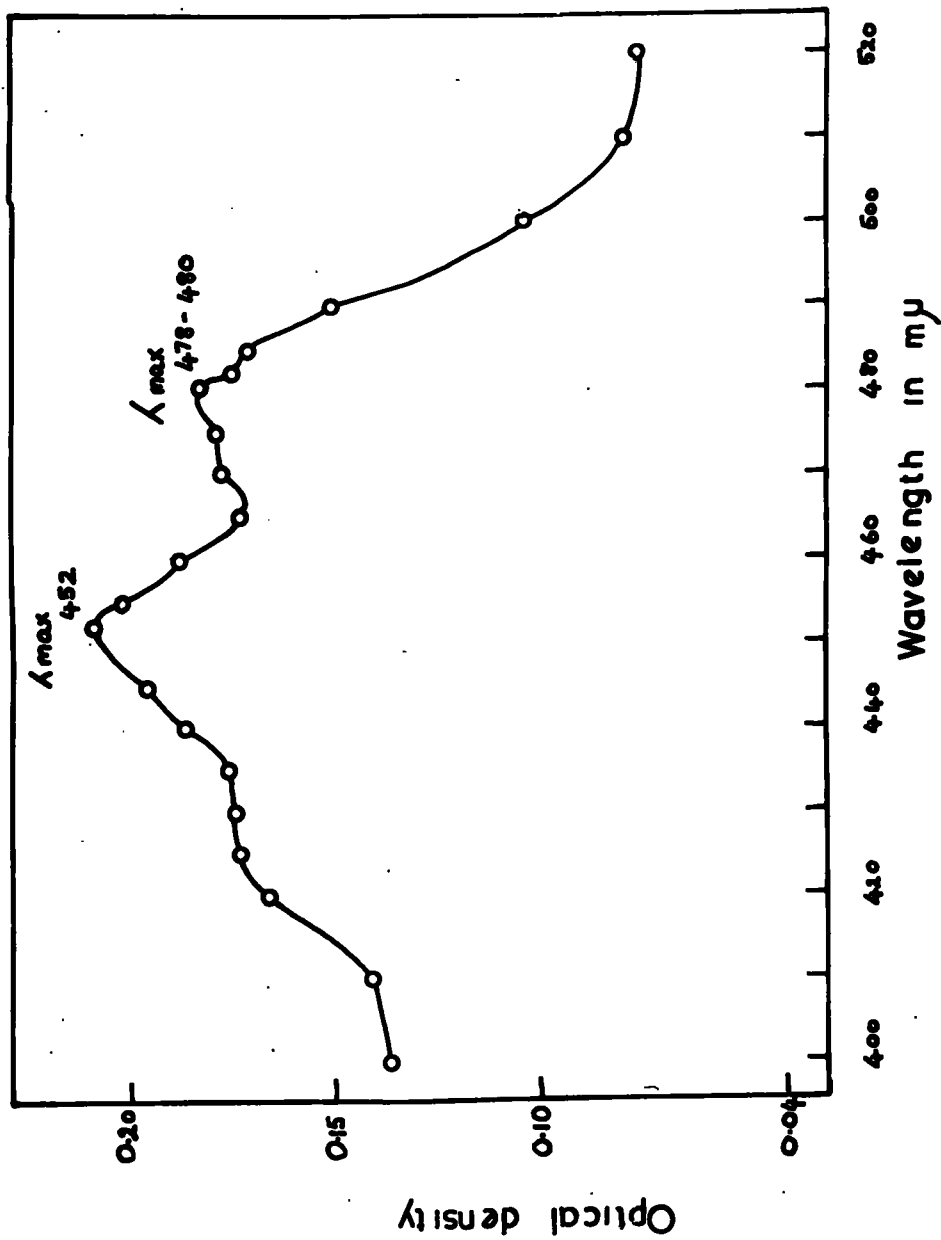


Figure 28 Absorption spectrum of orange pigment extracted from cultures grown in darkness.

extract obtained which had an absorption maximum in the visible part of the spectrum; the absorption spectra obtained were all similar to those shown in Figures 25a and b.

Experiment 45: Extracts using C.T.A.B. solutions.

The extracting solution used was a 4% solution of C.T.A.B. (cetyltrimethylammonium bromide). The experimental procedure was as follows:-

- (i) The fungus was cultured in flasks painted black which were placed in darkness.
- (ii) Two weeks after inoculation the culture solutions were decanted in red light and the mycelium was washed twice with distilled water.
- (iii) The fungus material was then washed three times with a 0.1 M solution of KH_2PO_4 (pH 4).
- (iv) Extracted for several hours with a 4% C.T.A.B. solution.
- (v) The absorption spectrum of the extract was determined with the solution being exposed to light as little as possible.

The extracts obtained in the above manner were yellow and all had absorption spectra similar to those of the methanol extracts shown in Figures 25a and b.

Cultures of Schizophyllum commune, Collybia velutipes and Phycomyces blakesleanus grown in darkness were also subjected to the same extraction procedure. The extracts of both the first two species had a distinct yellow colour

and similar absorption spectra to those described above for Aspergillus giganteus. The extract of Phycomyces had only a faint yellow tint.

Discussion of the nature of the pigments extracted from cultures grown in darkness.

I. The yellow pigments of bands 1 and 2.

The distinction between these two yellow pigments on the column was not always clear and for the purpose of this discussion they will be considered as a single pigment. They could be extracted from the fungus either with a 70% methanol or a 4% C.T.A.B. solution. It may be seen from the absorption spectrum of the pigment shown in Figure 25b that there is no peak of absorption in the blue end of the spectrum. One would expect that the pigment concerned in the light sensitive system involved in conidiophore growth would have such a peak. The fact that the pigment can be extracted from the mycelium with 70% methanol and is absorbed near the top of the column would rather seem to indicate that it might be a xanthophyll. When, however, an extract in petroleum ether was passed through a column of calcium carbonate, no pigment was adsorbed, although if a xanthophyll had been present one would expect it to have done so. The pigment is slightly water soluble and a yellow pigment with a similar absorption spectrum is found in the culture medium. the culture media

of cultures grown both in light and in darkness become progressively deeper yellow with age.

This pigment can also be extracted from the mycelium with a 4% C.T.A.B. solution; C.T.A.B. is a substance with amphipathic properties which is used to extract the visual pigments from the retinas of animals.

Extracts of the yellow pigment were also made in darkness at low temperatures but their absorption spectra were the same as that shown in Figure 25b.

Wolf (1959) reported that the yellow pigment photoreceptor present in cultures of Physarum polycephalum is a pteridine. This yellow pteridine could be extracted from cultures with 70% methanol and adsorbed in the first few cms. of alumina columns. The pigment could not be eluted from the column with organic solvents but was eluted by 0.1 N HCl. The pigment is pH sensitive and in acid conditions has an absorption spectrum with a peak at 420 m μ . Many of the characteristics of this pigment resemble those of the yellow pigment extracted from Aspergillus giganteus, viz. (a) they can both be extracted with methanol; (b) they are both adsorbed near the top of the alumina columns; (c) they are both pH sensitive and (d) cannot be eluted from the column with organic solvents. There are, however, a number of differences between the pigments which make it unlikely that they are identical,

viz. that the pigment extracted from Physarum polycephalum had an absorption maximum at 420 m μ , became deeper in colour at low pH and could be eluted from alumina columns by 0.1 N HCl.

Wolf found that a second yellow pigment is extracted from cultures of Physarum polycephalum with methanol and that this pigment is only weakly adsorbed on alumina columns. It has an absorption spectrum very similar to that of the yellow pigment extracted from Aspergillus giganteus (Figure 25b). The difference in the behaviour at various pH's and the fact that the Physarum polycephalum pigment is only weakly adsorbed by alumina seems to indicate, however, that there is no close relationship between the two pigments.

Cantino (1959) found that light induces morphological changes in Blastocladiella emersonii. The only pigment that he could find in cultures grown in darkness was one which is soluble in 80% alcohol. This particular pigment has an absorption spectrum with a peak at 255 m μ but with no absorption maxima in the 400 - 500 m μ region. There may possibly be some relationship between this pigment and that extracted from Aspergillus giganteus. It would be interesting to compare these pigments further, particularly as Cantino found no evidence to suggest the presence of a carotenoid in cultures of B. emersonii grown in darkness.

A third pigment, indicator yellow, a degradation product of the visual pigment of animals, rhodopsin, also has an

absorption spectrum similar to that shown by the methanol soluble pigment of Aspergillus giganteus. It was because of this similarity that the 4% C.T.A.B. solution, which is used to extract rhodopsin from the retinas of animals, was tried as an extracting solution. None of the extracts obtained showed an absorption maximum in the blue end of the spectrum.

Yellow extracts were also obtained when cultures of Schizophyllum commune and Collybia velutipes, which had been grown in darkness, were treated with C.T.A.B. solutions. This particular aspect of the work needs further investigation. The amphipathic properties of substances such as C.T.A.B. might facilitate the extraction of photoreceptor substances which are composed of protein and chromophore (pigment) moieties. One suspects that the majority of photoreceptors are of this nature, but in the past most of the techniques used would have extracted only the chromophore portion of the photoreceptor.

A further possibility, which is discussed more fully on page 170, is that the pigment may be a reduced flavin nucleotide.

The fact that the methanol soluble pigment does not have an absorption peak in the blue end of the spectrum does not rule out the possibility that it may be a degradation product of the photoreceptor. There is some evidence which suggests that similar pigments are present in other fungi, and there is the distinct possibility that improved extraction procedures

may eventually yield a fraction which does in fact show an absorption maximum in the blue end of the spectrum.

II. β -carotene of band 4.

The presence of β -carotene in three week old cultures grown in darkness was definitely confirmed, but attempts to establish its presence by chromatographic procedures in one week cultures were not successful. If it is present in young cultures it can only be present in very low concentrations, the upper limit being c. 18 μ grams/gram dry weight (experiment 48). However, one cannot eliminate β -carotene as the photoreceptor simply on the grounds that it is present in such low concentrations in young cultures grown in darkness. A concentration of 18 μ grams/gram dry weight represents something of the order of 4×10^{16} molecules of carotene per gram dry weight, and as the photochemical reaction is of the 'low energy' variety one would imagine that such a concentration would be sufficient for the reaction to proceed efficiently.

III. Faint yellow pigment of band 6.

Quantities of this pigment sufficient for spectral analysis were not obtained. The pigment is not present in one week old cultures which rather suggests that it is unlikely that it is the photoreceptor.

Pigments present in cultures grown in light.

All the pigments discussed above are found in cultures grown in light. In addition, two other carotenoids, the pigments of bands 3 and 4, are also present.

2. HISTORY OF PIGMENT PRODUCTION

Experiment 46 to determine the history of carotene production under various culture conditions.

Experimental Procedure.

The fungus was grown on Zurzyka's culture solution in 100 ml flasks. Each flask was inoculated with three drops of a spore suspension. The flasks were placed 80 cm below a fluorescent light in a room maintained at a temperature of $22 \pm 1^{\circ}\text{C}$. The light intensity at the level of the cultures was 10 foot-candles or 107 metre candles. The sets were treated as follows.

Set 1. The flasks contained 25 mls of culture solution and they were shaken in one plane on a Gallenkamp shaker.

Set 2. The flasks contained 25 mls of culture solution and were not shaken.

Set 3. The flasks contained 10 mls of culture solution and were not shaken.

At intervals four flasks were removed from each treatment and the carotene content in each was determined. The mean carotene content per gram dry weight and the mean dry weight of each set was calculated.

Results.

The results are presented in Tables 36a and b and in the form of graphs in Figures 29a and b. The curves of the graph were fitted by eye.

TABLE 36a

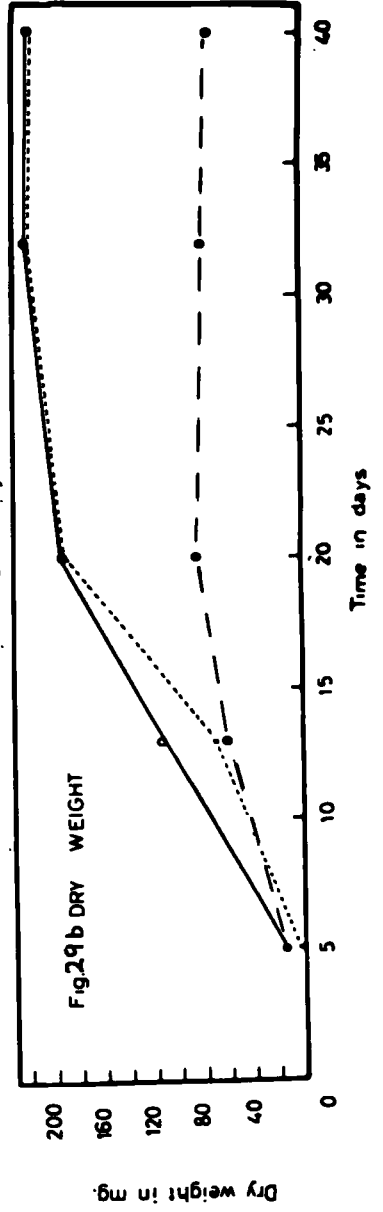
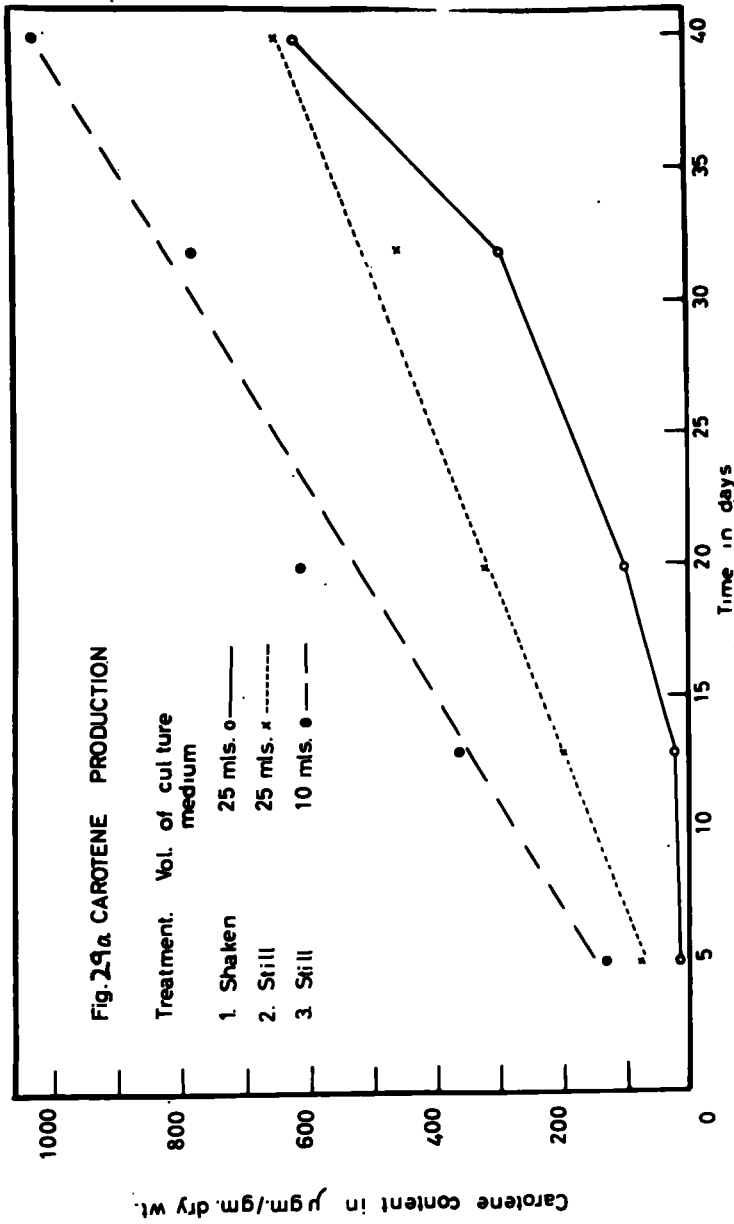
History of carotene^{2,25} under various culture conditions

Days after inoculation	Carotene content in μ gm/gm dry weight					
	Set 1 cultures shaken, 25 mls		Set 2 cultures still, 25 mls		Set 3 cultures still, 10 mls	
		Mean		Mean		Mean
6	6, 10, 9, 7	8	60, 80, 82, 74	74	90, 120, 130, 92	108
13	15, 16, 28, 17	19	195, 218, 171, 195	195	399, 267, 429, 365	365
20	106, 97, 96, 101	100	283, 319, 278, 295	319	612, 602, 732, 501	612
32	284, 270, 285, 291	283	458, 477, 438, 467	458	637, 779, 813, 888	779
40	610, 625, 630, 630	625	666, 680, 620, 650	654	1130, 990, 872, 1100	1023

TABLE 36b

Growth under the same culture conditions

Days after inoculation	Dry weight in milligrams					
	Set 1 cultures shaken, 25 mls		Set 2 cultures still, 25 mls		Set 3 cultures still, 10 mls	
		Mean		Mean		Mean
6	10, 8, 14, 7	13	4, 2, 2, 3	3	10, 18, 18, 12	16
13	144, 116, 88, 116	116	60, 76, 81, 72	72	64, 69, 63, 71	64
20	196, 176, 223, 190	196	208, 193, 187, 181	193	93, 75, 95, 88	88
32	201, 224, 266, 204	224	227, 216, 220, 217	220	81, 80, 89, 74	81
40	222, 185, 256, 220	221	222, 219, 218, 214	218	72, 76, 73, 82	76



Discussion of Results

It may be seen from an examination of Figure 29a that the carotene content of the cultures in sets 2 and 3 increased almost linearly with time. The carotene content in all sets continued to increase after growth had stopped. Goodwin & Willmer (1952) found that in Phycomyces blakesleeanus the bulk of the carotene is formed after growth has been completed. Growth was poorest in the cultures of set 3 but the carotene content per gram dry weight was higher in this treatment than in any other, reaching a level where one part per thousand of the dry weight was made up of carotene.

At first, growth in set 1 was better than the other sets but its carotene content was by far the lowest. The carotene content in this set thirteen days after inoculation was only about a tenth of that of the unshaken cultures in set 2. During the last three weeks of the experiment the carotene content of set 1 increased considerably and at the end of the experiment there was practically no difference between the shaken and unshaken cultures of sets 1 and 2.

The initially low carotene level in set 1 may be due to a number of factors. Carotenogenesis may have been inhibited by a poor supply of oxygen in submerged cultures. This is unlikely to have been the case, however, as growth was better in this set than in any other. A more probable

explanation lies in the nature of the mycelial growth in the submerged cultures. The shaking caused the mycelium in each flask to form a few large conglomerations, and the carotene content of these seemed to reside mainly in the outer surfaces. The mycelial masses were totally submerged during the first two weeks of the experiment but as they increased in size parts were exposed to the air. Conidiophore production was suppressed while the mycelium was submerged but when it subsequently became exposed to air conidiophores were produced. The increase in the carotene content in set 1 coincided with the appearance of these conidiophores. The low initial carotene content was probably due mainly to the nature of the large mycelial conglomerations in which carotene production is restricted to the outer surfaces rather than to the fact that the conidiophores have a much higher carotene content than the mycelium.

Experiment 47 to investigate the history of carotenogenesis.

A second experiment was undertaken in order to determine if the initially low carotene content of the shaken cultures was due to the inhibition of conidiophore production or the nature of the mycelial masses produced.

Experimental Procedure.

In order to delay conidiophore production in submerged cultures the volume of culture medium in each flask was increased to 50 mls. The experiment was carried out under

the same conditions as described above for set 1 in experiment 46 except that shaking was not started until three days after inoculation.

Results.

The results are presented in Table 37.

TABLE 37

Days after inoculation	Carotene content in μ gm/gm dry weight		Dry weight in milligrams	
		Mean		Mean
8	60,83,54,55	63	260,255,280, 285	270
15	160,190,186, 216	188	478,453,467, 478	469
30	459,463,490, 512	481	445,462,473, 470	455
41	590,610,589, 617	602	460,455,482, 483	457

Discussion of the Results of Experiments 46 and 47.

In experiment 46 the mycelium was made up of fewer and larger conglomerations per flask than in experiment 47. This might have been due to differences in size of the inocula or because of the delay before shaking was begun in experiment 47. It may be seen from the results presented in Table 37 that in experiment 47 there was no marked lag in carotene production, although no aerial conidiophores were formed

until nearly five weeks after inoculation. Thus it seems clear that the lag in carotene production in the shaken cultures of experiment 46 was probably primarily due to the fact that the fungal material in each flask was made up of only a few conglomerations which in effect reduced the carotene producing area.

Determination of the amount of β -carotene present in cultures grown in darkness.

Ethyl ether extracts of cultures of the fungus grown in darkness contain several pigments. One was shown to be β -carotene while another is an unknown yellow pigment which is adsorbed near the top of the alumina column. This yellow pigment, but not the β -carotene, may also be extracted from the fungus by a 70% v/v methanol/water solution. This fact suggested a method of obtaining a more accurate determination of the amounts of β -carotene present in cultures grown in darkness. If the fungal material is first treated with 70% methanol to remove the unknown yellow pigment, the β -carotene could subsequently be extracted with acetone and a quantitative determination could then be made using this extract. The method used to determine the amount of β -carotene present in a culture was as follows.

- (a) The fungal material was treated with successive portions of 70% methanol solution until the extract was clear. These extracts were discarded.

(b) The method described on page 14 was then followed to obtain a quantitative determination of the amount of β -carotene present.

(c) Due to the method employed of treating the material with 70% methanol before making the carotene determination, the figure obtained for the dry weight of the cultures would tend to be lower than the actual value. Accordingly the dry weight of similar cultures which had not been treated with the solvent were obtained, and these figures were substituted in the formula used to obtain the value for carotene content per unit dry weight.

The following experiment was conducted in order to determine the amount of β -carotene present in cultures grown in darkness.

Experiment 48 to determine the amount of β -carotene present in cultures grown in darkness.

Experimental Procedure.

The cultures were grown in 100 ml conical flasks containing 10 mls of Zurzyka solution. The flasks were inoculated with drops of a spore suspension and then divided into two sets.

The treatments were as follows.

Set 1. The flasks were placed in darkness.

Set 2. The flasks were placed in light in a light box.

The light intensity at the level of the cultures was 8.5 foot-candles or 91 metre candles.

Both sets of cultures were kept in a room maintained at a temperature of $19 \pm 1^{\circ}\text{C}$.

At intervals after inoculation three flasks were removed from each set and the β -carotene content of the cultures was determined using the method described above.

Results.

The results are presented in Table 38 and in graph form in Figure 30.

TABLE 38

β -carotene production in cultures grown in light and darkness

Days after inoculation	β -carotene content in $\mu\text{ gm/gm dry wt.}$	
	Set 1 cultured in darkness	Set 2 cultured in light
6	18	69
12	14	119
32	35	301

Discussion of the Results of Experiment 48.

It may be seen from the results presented in Figure 30 that the concentration of β -carotene in cultures grown in darkness is very low; at the end of the experiment the concentration was only c. 1/10th of that present in cultures grown in light. The carotene content of cultures grown in light increased linearly with time. Small quantities of other carotenes were in fact present in the extract but the main pigment present is undoubtedly β -carotene.

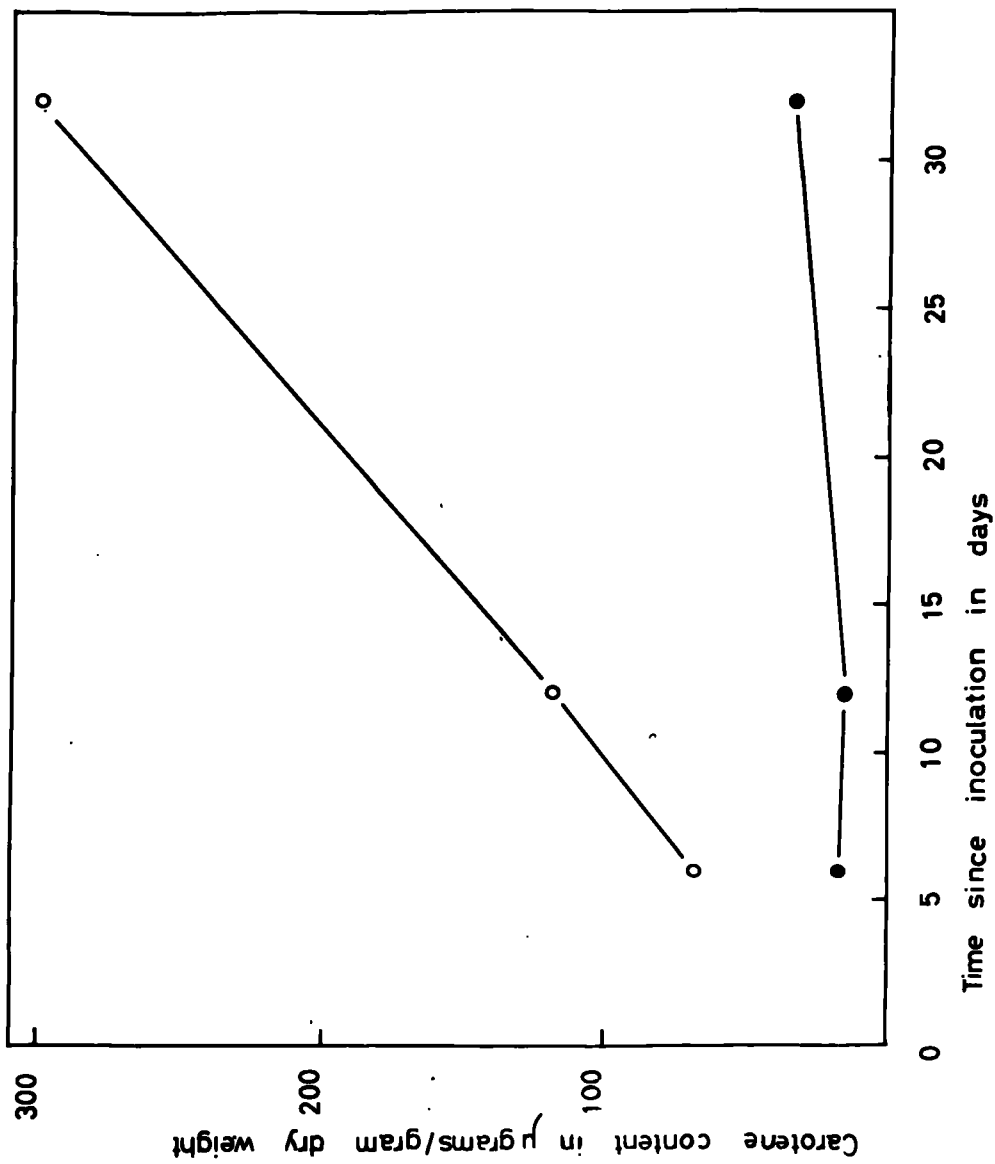


FIGURE 30 CAROTENE PRODUCTION IN CULTURES GROWN IN LIGHT (o) & DARKNESS (●)

Experiment 49 to determine the course of the production of the methanol soluble yellow pigment in cultures grown in light and darkness.

Experimental Procedure.

Forty 100 ml beakers containing 15 mls of Zurzyka solution were inoculated with a spore suspension. Half the cultures were placed in darkness in a light-tight cupboard in a room maintained at a temperature of $21 \pm 1^{\circ}\text{C}$. The other half were placed in light in a light box in the same room. The light intensity at the level of the cultures was 4.0 foot-candles.

At intervals five flasks from each set were removed and the pooled mycelium was treated in the following manner.

- (a) The mycelium was separated from the medium and washed twice with distilled water.
- (b) The mycelium was placed in a tared glass stoppered bottle which was then weighed.
- (c) Forty mls of 70% methanol was poured into each bottle.
- (d) The optical density of an aliquot of the solution was determined 24 hours later.
- (e) A value in arbitrary units for the concentration of the pigment in the mycelium was obtained using the formula
$$\frac{\text{O.D.} \times \text{Volume of the solution}}{\text{Dry weight}} .$$

Results.

The values obtained using the method described above are recorded in Table 39.

TABLE 39

History of the production of the methanol soluble pigment in arbitrary units

Days after inoculation	Cultures grown in light	Cultures grown in darkness
7	9.5	8.4
14	20.6	32.6
22	32.6	31.5
27	39.0	33.0

The results are presented graphically in Figure 31.

Discussion of the Results of Experiment 49.

It may be seen from Figure 31 that the concentration of the methanol soluble pigment of cultures grown in light increased almost linearly with time, while that in cultures grown in darkness increased more rapidly at first but then appeared to stabilize.

3. INFLUENCE OF PIGMENT INHIBITORS ON THE PHOTOINDUCTIVE PROCESS

One method commonly used to obtain information on the nature of the pigment photoreceptor involved in a system influenced by light is to include in the medium a chemical

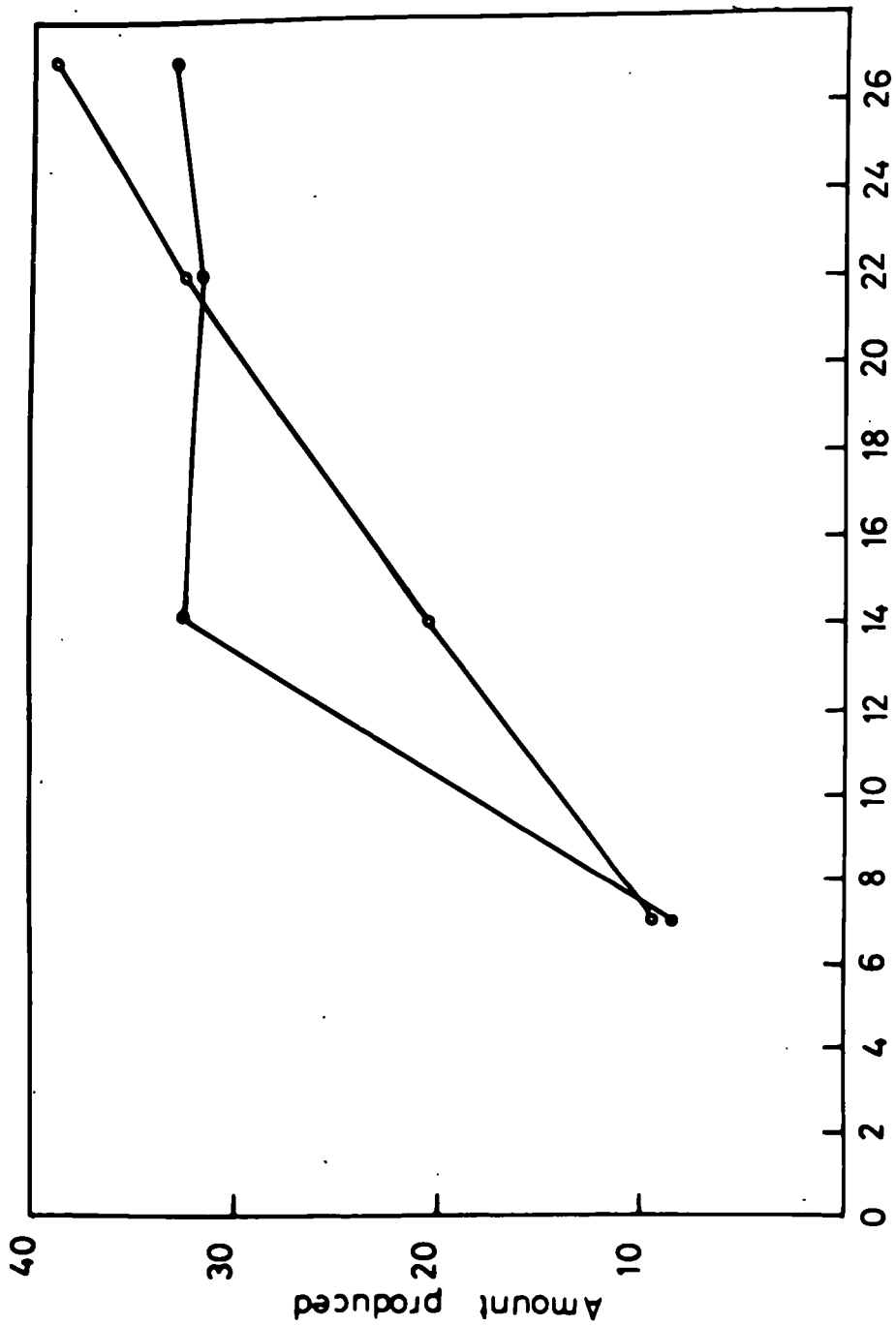


FIGURE 31 PRODUCTION OF THE YELLOW METHANOL SOLUBLE PIGMENT IN CULTURES GROWN IN LIGHT (○) & DARKNESS (●)

substance which inhibits the action of a particular pigment, and then to find out whether its presence has any inhibitory influence on the system. The following series of pigment inhibitors were used in the experiments reported below.

I. L-lyxoflavin. This is a substance which has been shown to inhibit the action of riboflavin. Page (1956) reported that the incorporation of this substance in the culture medium inhibits mycelial growth of Pilobolus kleinii in light and that this inhibition can be relieved by adding riboflavin to the medium. Page found that the induction of trophocyst formation by light was inhibited by the presence of 100 μ grams per ml of l-lyxoflavin in the medium. This inhibition was completely relieved by the addition of 10 μ grams per ml of riboflavin and partially by increasing the duration of the illumination period. Page concluded from the results he obtained that a flavin rather than a carotenoid is the photoreceptor in the induction of trophocyst formation.

The way in which l-lyxoflavin inhibits riboflavin activity is suggested by work conducted by Huennekens (1956). He found that l-lyxoflavin is incorporated in flavin mono- and dinucleotides (LMN and LAD) and that these nucleotides function as flavin co-enzymes in the cell. LMN and LAD were tested for co-enzyme activity with D-amino acid oxidase and TPNH-cytochrome apo-reductase respectively, and it was found that neither was utilized as well as the riboflavin analogues FMN and FAD.

II. Mepacrine. Many workers regard mepacrine as a flavoprotein inhibitor; Haas (1944) found that it was effective against a number of well-authenticated flavoprotein electron transfer enzymes. Carlile (1962) found that the presence of mepacrine in the medium inhibits the growth of Phycomyces blakesleeanus more in cultures grown in light than in cultures grown in darkness. This inhibition was relieved by adding riboflavin to the medium. Carlile explained his results by postulating the presence of an active flavoprotein system in cultures grown in light which is inhibited by mepacrine. This flavoprotein system is supposed to be non-functional in cultures grown in darkness.

III. Diphenylamine. Goodwin (1952) found that when Phycomyces blakesleeanus was cultured on a medium containing 25 - 30 ppm of diphenylamine, 1% glucose, 0.1% asparagine and the normal concentrations of mineral salts and vitamins, the concentration of β -carotene in the sporangiophores and mycelium was reduced to less than 3% of that normally found. The presence of diphenylamine also reduced the growth rate, but the final amount of growth achieved is reduced by only 20%.

Experiment 50 to determine whether the presence of 1-lyxoflavin in the medium inhibits the photoinduction of conidiophore elongation.

Experimental Procedure.

Culture media containing the concentrations of 1-lyxoflavin listed in Table 40 were prepared. The cultures were grown in 100 ml flasks containing 10 mls of the medium. They were cultured in darkness in a room maintained at 20 - 21°C.

Eight days after inoculation the flasks were divided in red light into four sets with five replicates in each. All the sets except one were illuminated for two minutes under a fluorescent tube. After illumination the cultures were returned to darkness. Two days later the cultures were again illuminated for the same period.

Results.

The mean height of the tall conidiophores three days after the second period of illumination was determined and the results are presented in Table 40.

TABLE 40

Influence of incorporating 1-lyxoflavin in the medium on the photoinduction of conidiophore growth by light

Set	Concentration of 1-lyxoflavin	Weight of the tall conidiophores in mm.	Mean
1	0	14, 15, 14, 15	14.5
2	100	14, 14, 13.5, 12.5	13.5
3	200	12.5, 13, 14, 14.5	13.5
		Cultured in darkness	
4	0		6.0

Experiment 51 to determine the influence of incorporating
1-lyxoflavin in the medium on mycelial extension.

Experimental Procedure.

Culture media containing the concentrations of 1-lyxoflavin listed in Table 41 were prepared. Petri dishes containing 20 mls of the culture media were inoculated centrally and then cultured in light at a temperature of 20 - 21°C. There were replicates in each set of cultures. The diameter of each colony was measured at intervals.

Results.

The mean diameter of each set eleven days after inoculation was determined and the results are presented in Table 41.

TABLE 41

Influence of 1-lyxoflavin in the medium on mycelial growth

Set	Concentration of 1-lyxoflavin	Diameter of the colonies in mm.	<u>Mean</u>
1	0	43, 43.5, 43, 42.5	43
2	100	44, 43, 44, 43	43.5
3	200	43, 42, 43, 42	42.5

Discussion of the Results of Experiments 50 and 51.

It may be seen from the results presented in Tables 40 and 41 that the presence of 1-lyxoflavin in the medium does not appear to inhibit either the rate of mycelial growth in light or the photoinduction of conidiophore elongation.

Experiment 52 to determine whether the presence of mepacrine in the medium inhibits the photoinduction of conidiophore elongation.

Experimental Procedure.

The thermolabile compound mepacrine dihydrochloride dihydrate was sterilized by passage through a 'Mallipore' filter paper and sintered glass filter. The basic culture medium employed was Zurzyka agar and the mepacrine was added aseptically when the medium had cooled after sterilization but before the agar had set. The concentrations of mepacrine were 25, 50 and 75 ppm. Large oxoid caps were used as culture vessels and these were placed in 125 ml conical beakers capped with 150 ml beakers. Each culture was inoculated with three drops of a spore suspension.

The cultures were placed in two different light regimes, one treatment received continuous illumination in a light box while the other treatment was given a period of 20 minutes light per day in a second light box. The light intensity at the level of the cultures in each light box was 8.5 footcandles or 91 metre candles. There were four sets of cultures in each treatment with five replicates in each set.

Results.

Twelve days after inoculation the cultures were removed from the light boxes and the mean height, carotene content

and dry weight of the conidiophores in each set was determined and the results are presented in Table 42.

TABLE 42

Influence of incorporating mepacrine in the medium on the photoinduction of conidiophore growth and carotenogenesis

Light regime	Concn. of mepacrine	Tall conidiophores			
		Mean ht. in mm above medium	Mean carotene content in µg/gm dry wt.	Mean dry wt. in milligrams	No. of heads produced
Cultures illuminated continuously	0	52	299	139	Large number
	25 ppm	52	286	151	"
	50 ppm	50	272	155	"
	75 ppm	50	274	167	"
Cultures illuminated for 20 mins. per day	0	14	108	112	No heads formed
	25 ppm	12	96	115	"
	50 ppm	11	83	127	"
	75 ppm	12	98	118	"

Experiment 53 to determine the influence of incorporating mepacrine in the medium on mycelial extension in cultures grown in light and darkness.

Experimental Procedure.

The media containing mepacrine were prepared in the manner described on page 156 . Petri dishes containing 20 ml

portions of the culture media were prepared. Each of the sets listed in Table 43 consisted of 12 petri dishes which were divided into three groups cultured in different light regimes. The cultures were inoculated centrally with drops of a spore suspension dispersed from a hypodermic syringe. The groups of cultures were placed into separate light boxes and grown in the light regimes listed in Table 43. The light intensity at the level of the cultures in the two light boxes which were illuminated was 8.5 foot-candles or 91 metre-candles.

Results.

Eleven days after inoculation the mean colony diameter for each group of cultures was calculated and the results are presented below in Table 43.

TABLE 43
Influence of incorporating mepacrine in the medium
on mycelial growth

Set	Concn. of mepacrine in the medium	Colony diameter in mm. in different light regimes					
		Cultured in darkness		Illuminated for 20 mins. at 24 hour intervals		Illuminated continuously	
			<u>Mean</u>		<u>Mean</u>	<u>Mean</u>	
1	0	60, 62, 62, 67, 64	63	64, 69, 62, 62, 63	64	63, 61, 67, 65, 64	64
2	25 ppm	59, 61, 58, 58, 59	59	56, 58, 51, 58, 61	58	58, 58, 58, 57, 59	58
3	50 ppm	55, 58, 57, 57, 56	56	52, 56, 52, 52, 59	54	52, 54, 56, 56, 56	55
4	75 ppm	58, 54, 55, 57, 56	56	53, 54, 48, 49, 47	50	49, 50, 53, 51, 51	51

This experiment was repeated using higher concentrations in order to determine if the rate of mycelial growth on medium containing mepacrine is really less in light than in darkness as would seem to be suggested by set 4 in Table 43.

Experiment 54

Experimental Procedure.

Zurzyka agar containing mepacrine was prepared in the manner described on page 156. Each group of cultures consisted of four petri dishes each containing 20 mls of the culture medium. The cultures were inoculated centrally with drops of a spore suspension dispensed from a hypodermic syringe. Half the petri dishes of each set were wrapped up individually in aluminium foil while the other half were placed into plastic bags to ensure that the conditions of aeration of the cultures were the same. The cultures were placed in an illuminated incubator maintained at $22 \pm 0.5^{\circ}\text{C}$. The light intensity at the level of the cultures was 8.5 foot-candles or 91 metre-candles.

Results.

Eight days after inoculation the mean diameter of each group of cultures was determined and the results are presented in Table 44. Plate 17a is a photograph of cultures from each set.

TABLE 44Influence of incorporating mepacrine in the medium on mycelial growth

Set	Conc. of mepacrine in the medium	Mean colony diameter in mm in the different treatments			
		Cultured in light		Cultured in darkness	
			<u>Mean</u>		<u>Mean</u>
1	0	54, 50, 50, 55, 51	52	52, 56, 53, 54, 52.5	53.5
2	100 ppm	46, 46, 45, 44, 44	45	44, 46, 51, 47, 47	47
3	200 ppm	46, 46, 45, 48, 45	46.5	45, 46, 48, 45, 46	46

Discussion of the Results of Experiments 52, 53 and 54.

It may be seen from the results of experiments 53 and 54 that the presence of mepacrine in the medium slightly inhibits the rate of mycelial growth and that the extent of this inhibition is the same whether the cultures are grown in light or darkness. This contrasts with the results obtained by Carlile (1962) with Phycomyces blakesleeanus (page 153). The photoinduction of conidiophore elongation and carotene production also does not appear to be inhibited to any great extent by the presence of mepacrine. In fact, in both the continuously and the intermittently illuminated cultures the conidiophore dry weight was rather higher on media containing mepacrine than in the controls. This at first sight appears to be rather a strange result. The explanation may lie in the inhibitory influence mepacrine has on mycelial growth. It has previously been reported (see page 96) that although low temperatures

Conc. of mepacrine

in the medium. 200 ppm 100 ppm 0

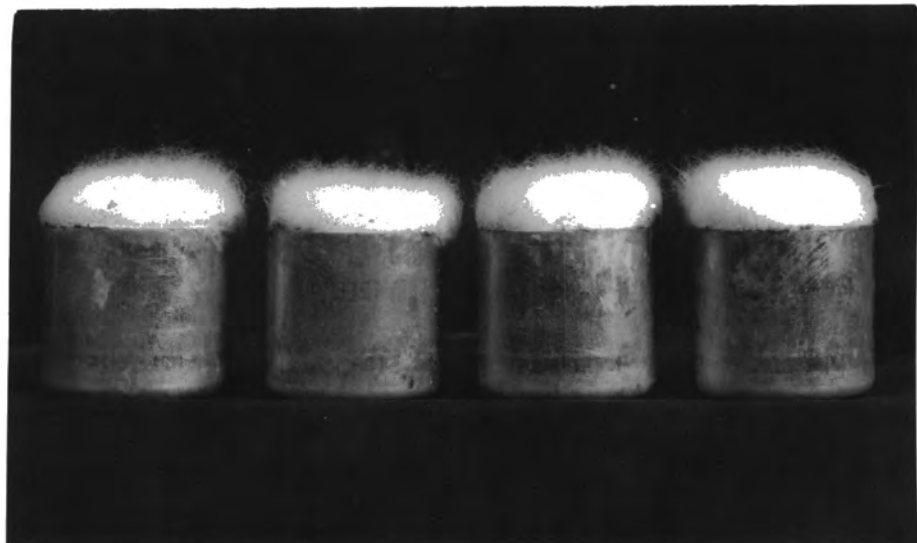
PLATE 17a Influence of the presence of mepacrine in the medium
on photoinduction of conidiophore growth.

3 1/2 cm

Conc. of FMN

in the medium 1,000 ppm 100 ppm 10 ppm 0

Plate 17b Influence of the presence of FMN in the medium on
photoinduction of conidiophore growth.



reduce the rate of mycelial growth they appear to increase total conidiophore growth. It might well be that the promotion of conidiophore growth on media containing mepacrine is correlated with the fact that there is an inhibition of mycelial growth on the same medium.

The presence of mepacrine in the medium appears to have only a slight inhibitory influence on carotene production by the conidiophores.

Experiment 55 to determine whether the presence of diphenylamine in the medium inhibits the photoinduction of conidiophore elongation.

Experimental Procedure.

A modified Zurzyka agar medium containing diphenylamine was used in this experiment. The glucose concentration of the medium was reduced to 1%; Goodwin (1952) found that when Phycomyces was cultured on a medium containing a low concentration of glucose and diphenylamine the production of β -carotene was drastically reduced. The concentrations of diphenylamine employed were 20 and 40 ppm. Large oxoid caps were used as culture vessels and these were placed in 125 ml conical beakers capped with 150 ml beakers. Each culture was inoculated with three drops of a spore suspension. There were five replicates in each set. The cultures were grown in the same light regimes as those employed in experiment 53 .

Results.

Twelve days after inoculation the cultures were removed

and the mean height, carotene content and dry weight of the conidiophores in each set were determined. The results are presented in Table 45.

TABLE 45

Influence of incorporating diphenylamine in the medium
on the photoinduction of conidiophore growth and carotenogenesis

Light regime	Concn. of diphenylamine	Tall conidiophores			
		Mean ht. above medium in mm.	Mean dry wt. in milligrams	Mean carotene content in μ gm/gm dry wt.	Number of heads produced
Cultures illuminated continuously	0	50	43	454	Large number
	20 ppm	50	43	427	"
	40 ppm	43	35	281	"
Cultures illuminated for 20 mins./day	0	19	48	132	None formed
	20 ppm	17	46	110	"
	40 ppm	12	32	86	"

Experiment 56 to determine the influence of incorporating diphenylamine in the medium on mycelial extension in cultures grown in light and darkness.

Experimental Procedure.

Petri dishes containing 20 mls portions of the culture media were prepared. Each group of cultures grown in the

different light regimes consisted of four petri dishes. The cultures were inoculated centrally with drops of a spore suspension dispensed from a hypodermic syringe. The groups of cultures were placed into separate light boxes and grown in the light regimes listed in Table 46. The light intensity at the level of the cultures in the two light boxes which were illuminated was 8.5 foot-candles or 91 metre-candles.

Results.

Eleven days after inoculation the mean colony diameter of each group of cultures was determined and the results are presented in Table 46.

TABLE 46

Influence of incorporating diphenylamine in the medium
on mycelial growth

Set	Conc. of diphenylamine in the culture medium	Colony diameter in mm in different light regimes					
		Cultured in darkness	Mean	Illuminated for 20 mins. at 24 hr. intervals	Mean	Illuminated continuously	Mean
1	0	49,47,48, 51,50	49	47,49,46,46, 47	47	46,47,44, 44,44	45
2	25 ppm	44,43,45, 47,41	44	40,41,42,41, 41	41	35,40,33, 33,34	35
3	40 ppm	27,26,25, 28,29	27	26,25,27,24, 28	26	25,25,24, 27,24	25

Discussion of the Results of Experiments 55 and 56.

It may be seen from the results presented in Table 46 that the presence of diphenylamine in the medium inhibits mycelial growth and that this inhibition is possibly slightly greater in cultures grown in light than in cultures grown in darkness.

Diphenylamine inhibits tall conidiophore growth and carotene production in cultures grown both in continuous and intermittent light. The mean dry weight of conidiophores produced by cultures grown in light, even on the control medium, was low. This was undoubtedly due to the low concentration of glucose in the medium (see page 117). On the whole, conidiophore growth was less inhibited by diphenylamine than carotene production. The presence of diphenylamine in the medium appears to inhibit mycelial rather than conidiophore growth; the rate of mycelial growth of cultures in intermittent illumination containing 20 ppm diphenylamine was only 55% of the control, while the mean conidiophore dry weight was over 80% of the control.

Considering the fact that mycelial growth is inhibited by diphenylamine it seems clear that the photoinduction of conidiophore growth is by comparison not greatly influenced by the presence of the pigment inhibitor. The presence of 20 ppm diphenylamine reduced the concentration of carotene in the conidiophores to just over half that of the controls.

Influence of incorporating FMN in the medium on conidiophore elongation.

Lukens (1963) found that light induced conidiophore elongation in Alternaria solani and inhibited sporulation. Periods of illumination of short duration given at 6 hour intervals were effective in inhibiting sporulation. When riboflavin-5-phosphate mononucleotide (FMN) was incorporated in the medium the short periods of illumination were far less effective in inhibiting fruiting. Lukens concludes that flavins appear to be essential for conidial formation and are photo-inactivated.

Light influences conidiophore growth and sporulation of Aspergillus giganteus in a manner very similar to that described for Alternaria solani. The following experiment was undertaken to determine if the presence of FMN in the medium reduces the effectiveness of the action of light in inhibiting sporulation in Aspergillus giganteus.

Experiment 57 to determine the influence of the presence of FMN in the medium on the photoinduction of conidiophore elongation.

Experimental Procedure.

Zurzyka media containing 10, 100 and 1,000 ppm of the sodium salt of riboflavin-5-phosphate (FMN) were prepared. Five large oxoid caps of each medium were inoculated with a spore suspension and placed into 125 ml conical beakers capped with 150 ml squat

beakers. The cultures were placed in darkness in a light box which was in a room maintained at a temperature of $21 \pm 1^{\circ}\text{C}$.

Four days later the cultures were illuminated for five minutes and this treatment was repeated at intervals of 24 hours for the next five days. The light intensity at the level of the cultures was 8.5 foot-candles or 91 metre-candles.

Results.

The cultures were examined 24 hours after the final period of illumination. The mean height of the conidiophores in each set is recorded in Table 47.

TABLE 47

Set	Conc. of FMN in the medium	Height of conidiophores in mm	Colour of conidiophores	
			<u>Mean</u>	
1	0	11,12,14,14,14	13	Orange
2	10	10,11,9,10,10	10	Orange
3	100	11,12,10,10,12	11	Orange
4	1,000	11,10,10,12,12	11	Orange

Plate 17 is a photograph of cultures from each set. It is clear that the presence of even high concentrations of FMN in the medium does not inhibit the induction of conidiophore growth by light or induce sporulation.

General Discussion of the Results of Experiments involving the use of pigment inhibitors.

Results obtained using pigment inhibitors are difficult to interpret. Unfortunately one cannot necessarily deduce

from a negative result that the pigment under investigation is not involved in the photoinductive process. Negative results have always to be qualified; one does not really know if the inhibitor has been taken up from the medium, or, even if it has, that it has inhibited the action of the pigment. The fact that neither 1-lyxoflavin nor mepacrine, both established riboflavin inhibitors, actually inhibited the photoinductive process might lead one to doubt that riboflavin is the chromophore part of the photoreceptor but it does not necessarily prove that it is not. Similarly one cannot deduce from experiment 55 that β -carotene is not the photoreceptive pigment.

Discussion of the Nature of the Pigment Photoreceptor involved in the light sensitive processes in *Aspergillus giganteus* and other Fungi.

There is some considerable controversy among present day workers as to the nature of the photoreceptors involved in many of the photo-sensitive systems which are present in plants. An extreme view would be that chlorophyll, with its associated pigments, is the only photoreceptor universally accepted to be associated with a particular, known, photochemical reaction. Although light influences the metabolism, morphology and growth of many fungi, the nature of the photoreceptor (s) involved in these processes is unknown. On page 19 is a list of a

number of the processes in fungi which are influenced by light.

The probable reasons why the pigments involved in these reactions have not been identified are:

(a) All of the systems mentioned above most certainly have 'low energy' reactions, and hence only a very small concentration of the photoreceptive pigment need be present for the process to proceed efficiently. This contrasts with the 'high energy' reaction, photosynthesis, where high concentrations of the photoreceptor, chlorophyll, are present.

(b) The number of workers interested in these fields has in the past been comparatively small and they included only a small proportion of biochemists. Recently, however, more interest has been shown in these problems, particularly in some of the morphogenetical aspects. On the whole I feel that the only way in which the photoinduced systems which operate in fungi will be fully explained is through intimate co-operation between physiologists and biochemists. Unfortunately there are still only a few biochemists interested in these problems.

Three main lines of approach are commonly used to determine what pigment photoreceptor is involved in a particular process?

(i) Identification of the pigments present in the fungus.

According to the Grotthus-Draper law, only radiation absorbed by a reacting system is effective in producing chemical changes. If visible radiation is absorbed by a substance, then that substance must be coloured and its colour will depend upon the wavelength of the light absorbed. Only pigments which have been shown to be present in a fungus can be considered as possible photoreceptors, but it must always be remembered that probably only very low concentrations of the pigments are necessary for the process to proceed efficiently.

(ii) Determination of the action spectrum of the process.

Action spectra provide valuable information as to the nature of a photoreceptor, e.g. as light in the blue end of the visible spectrum is the most effective in inducing conidiophore growth one would expect the photoreceptor to be a yellow pigment. Of course the more detailed the action spectra the more useful it is, but one must always bear in mind that absorption spectra of pigments varies with the nature of the solvents in which they are dissolved.

(iii) The use of pigment inhibitors.

Two yellow pigments with absorption maxima in the blue end of the spectrum have been suggested as the photoreceptors involved in the light influenced systems of fungi, viz. riboflavin and β -carotene. It may be that a third class, the pteridines, should also be seriously considered.

The presence of β -carotene in cultures of Aspergillus giganteus grown in darkness has been confirmed. One objection to its consideration as the photoreceptor is that it absorbs very little light in the ultra-violet region of the spectrum although radiation in this region is very effective in inducing conidiophore elongation. One could overcome this objection by postulating that a second substance, possibly a colourless carotenoid, is linked with β -carotene to a protein moiety to form the photoreceptor, and that it is this part of the molecule which absorbs ultra-violet radiation.

The presence of riboflavin in Aspergillus giganteus has not been shown but it has been assumed to be present, as so far all tests on fungi for this substance have been positive. It is possible, on the grounds of a similarity in their absorption spectra, that the methanol soluble yellow pigment might be reduced flavin adenine nucleotide (FAD). Velick (1961) presents absorption spectra for both reduced and oxidized FAD dissolved in water. The reduced form has a sloped absorption spectrum similar to that shown by the methanol soluble pigment, while the oxidised form has absorption maxima at 460 and 380 m μ . Work will be conducted to investigate this possibility further. The main evidence put forward in support of riboflavin as the photoreceptor has been from experiments involving pigment

inhibitors. Both Page (1956) and Carlile (1962) presented convincing evidence using pigment inhibitors which suggested that riboflavin was the photoreceptor involved in the induction of trophocyst formation in Pilobolus kleinii and mycelial growth of Phycomyces blakesleeianus in light. However, in the experiments reported above, using the same riboflavin inhibitors, it was found that there was no inhibition in the photoinduction of conidiophore elongation.

This work, unfortunately, adds nothing to the present discussion of the nature of the pigment photoreceptor in fungi. The only, rather negative, point which does emerge is that perhaps some caution should be attached to the interpretation of work involving pigment inhibitors.

SECTION VII

INVESTIGATION INTO THE NATURE OF THE PHOTOINDUCTIVE PROCESS

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1. EFFECTIVENESS OF ILLUMINATION GIVEN IN THE ABSENCE OF GASEOUS CARBON DIOXIDE	173
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4. TREATMENT OF THE MYCELIUM WITH HYDROGEN PEROXIDE	183

SUMMARY

The presence of gaseous carbon dioxide is not essential for the photoinduction or continued growth of the conidiophores. Free oxygen is not required during the actual period of photoinduction, but is required during the subsequent growth of the conidiophores. Photoinduction in the absence of free oxygen is less effective in inducing carotenogenesis than photoinduction in the presence of oxygen. The rate of respiration does not increase during photoinduction. Treating cultures with hydrogen peroxide cannot replace light in inducing conidiophore growth.

1. EFFECTIVENESS OF ILLUMINATION GIVEN IN THE
ABSENCE OF GASEOUS CARBON DIOXIDE

Yanagita (1963) showed that the presence of atmospheric carbon dioxide is essential for spore germination in Aspergillus niger; he found that there was active carbon dioxide fixation by the conidia. This is by no means the only instance of carbon dioxide fixation in fungal tissues. Penicillium crysogenum (Gitterman & Knight, 1952), Neurospora crassa (Heplar & Tatum, 1954), Rhizopus nigricans (Foster & Davies, 1948) and Streptomyces coelicolor (Cochrane, 1952) have all been shown to fix atmospheric carbon dioxide. In particular ^aCatino & Horenstein (1956 and 1959) have made an intensive study of carbon dioxide fixation by Blastocladiella emersonii. Light stimulates the growth of this fungus but only when it is given in the presence of atmospheric carbon dioxide or when the culture solution contains bicarbonate. One wonders if the light induced growth of Aspergillus giganteus conidiophores might also be linked to a process involving carbon dioxide fixation. In order to investigate this possibility it was decided to determine whether illumination given in the absence of carbon dioxide was as effective in stimulating conidiophore growth.

Experiment 58 to determine whether the presence of atmospheric carbon dioxide was essential for either spore germination or tall conidiophore growth.

Experimental Procedure.

Sixteen large oxoid caps containing Zurzyka agar were prepared. These were inoculated with spores and half were placed in the chamber shown in Figure 32 and half in a similar control chamber. The cultures were illuminated by a 25 watt tungsten filament bulb and air was drawn through the apparatus for the whole period of the experiment. The cultures thus grew in an atmosphere which was kept as free from carbon dioxide as possible. The control consisted of a similar chamber but air was not drawn through it. The experiment was carried out at room temperature and this varied between 16° and 19°C.

Results.

There appeared to be no difference in the cultures of the two treatments either in the length of the induction period or the height attained by the tall conidiophores. It would thus appear that gaseous carbon dioxide is not essential either for spore germination or conidiophore growth in Aspergillus giganteus.

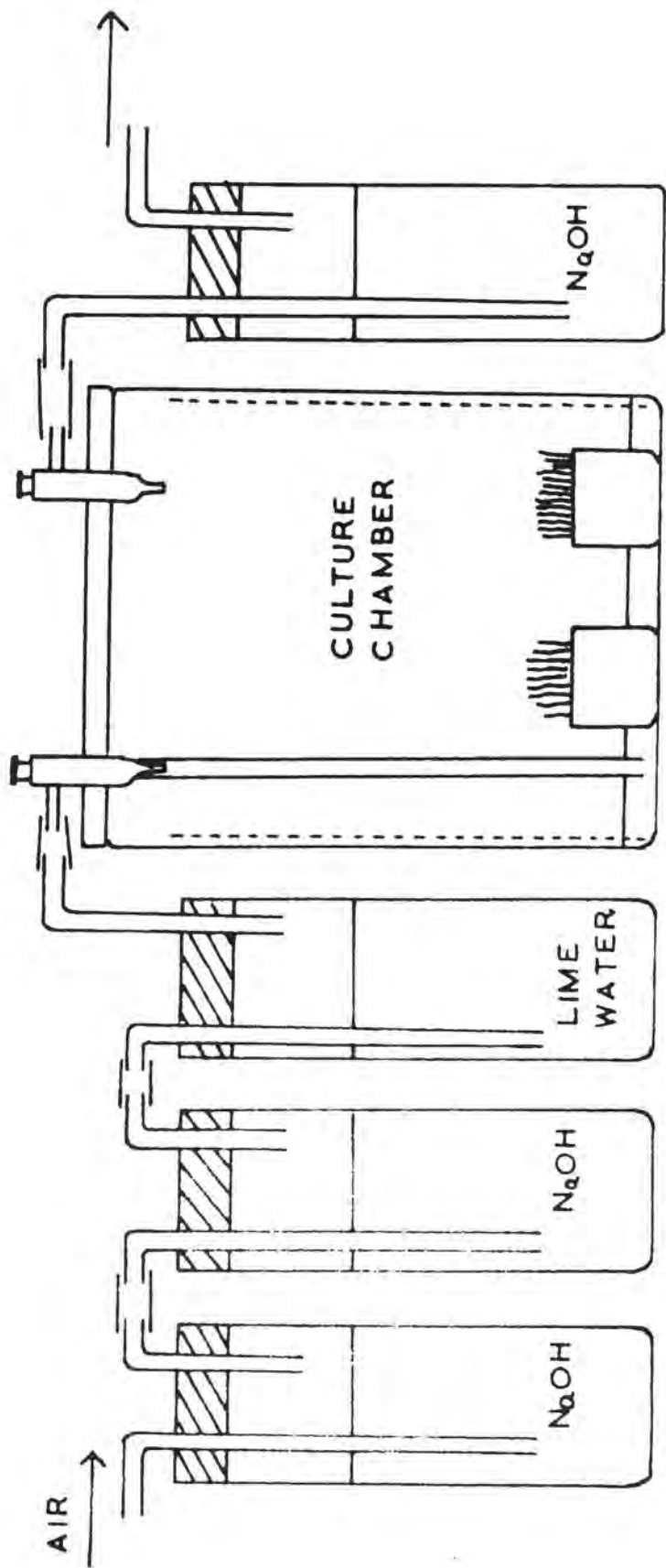


FIGURE 32 METHOD OF GROWING CULTURES IN A CO₂ FREE ATMOSPHERE

2. EFFECTIVENESS OF ILLUMINATION GIVEN IN THE
ABSENCE OF GASEOUS OXYGEN

Photochemical reactions often involve oxidative processes. Zalokar (1954, 1955 and 1957) studied carotene production in Neurospora crassa. He found that mycelial carotenoids are not formed when the fungus is cultured in darkness; even short periods of illumination, however, induce carotenogenesis. Zalkar (1954) reported that illuminating cultures in the absence of oxygen did not induce significant carotene production.

The following experiments were conducted to determine if illuminating cultures of Aspergillus giganteus in an atmosphere of nitrogen is effective in inducing conidiophore growth and carotenogenesis.

Experiment 59 to determine whether conidiophore elongation is initiated when cultures are illuminated while in an atmosphere of nitrogen.

Experimental Procedure.

Fifteen large oxoid caps containing Zurzyka agar were prepared. Each was inoculated with three drops of a spore suspension and then placed into one of two 4 litre beakers lined with damp blotting paper. The beakers were kept in darkness in a cupboard maintained at a temperature of $21 \pm 1^{\circ}\text{C}$.

Four days after inoculation the cultures were divided

in dim red light into three sets with five replicates in each. The sets received the following treatments.

Set 1. Cultures not illuminated. The cultures were kept in darkness in a 4 litre beaker lined with damp blotting paper.

Set 2. Cultures illuminated in the presence of oxygen. The cultures were placed in a glass jar which was in a light box. They were illuminated for half an hour per day during the following four days.

Set 3. Cultures illuminated in an atmosphere of nitrogen. The cultures were placed into the culture jar described on page 11 . This vessel was in the same light box as Set 2 and the cultures were subjected to the same light regime as those of Set 2, but three hours prior to the start of each illumination period the culture jar was flushed out with "white spot" nitrogen for an hour and then sealed. The cultures were thus held for a total of three hours in an atmosphere of nitrogen before each illumination period. At the end of each light period, when the cultures were once again in darkness, the valves were unscrewed to allow an inflow of air into the culture vessel.

Results.

The cultures were examined a day after the final illumination period. The mean height, dry weight and carotene content of the conidiophores in each set were

determined and the results are presented in Table 48. Plate 18 is a photograph of one culture from each set.

TABLE 48

Influence on the photoinduction of conidiophore growth
illuminating cultures which have been kept under
anaerobic conditions for three hours

Set	Treatment	Tall conidiophores		
		Mean ht. in mm.	Mean dry wt. in milligrams	Mean carotene content in μ gm/gm dry wt.
1	Cultured in darkness	A few Below 6	-	-
2	Illuminated in oxygen	14.0	63	124
3	Illuminated in nitrogen	10.5	61	81

Experiment 60

Experimental Procedure.

The cultures were grown on Zurzyka agar in small oxid caps. They were inoculated with drops of a spore suspension and then placed in darkness in two four litre beakers lined with blotting paper. These were then placed in a room maintained at a temperature of $21 \pm 1^{\circ}\text{C}$.

Three days later the cultures were removed from the beakers and divided in dim red light into three sets with five

Illuminated Illuminated Not
 in oxygen in nitrogen illuminated

PLATE 18. Influence of illuminating cultures which have been kept
in nitrogen for three hours.

3 cm
↑
↓

 Illuminated Illuminated Not
 in oxygen in nitrogen illuminated

Plate 19. Influence of illuminating cultures which have been kept
in nitrogen for twenty hours.



replicates in each. The sets were treated as described below:

Set 1. The cultures were placed in a beaker lined with damp blotting paper and kept in darkness.

Set 2. The cultures were placed in an anaerobic culture jar in a light box. Twenty-four hours later they were illuminated for half an hour and this treatment was repeated at 48 hour intervals until the cultures had been illuminated four times.

Set 3. The cultures were placed in a second culture jar in the same light box. This jar was then flushed out with white spot nitrogen for an hour and then sealed. The cultures were left in nitrogen for 19 hours and then illuminated for half an hour. At the end of this light period, while the cultures were once again in darkness, the valves of the culture jar were unscrewed to allow an inflow of air. This set of cultures was subjected to the same light regime as that of set 2 but the cultures were held in an atmosphere of nitrogen for the 20 hour period immediately prior to each illumination period. The experiment was carried out in a room maintained at a temperature of $21 \pm 1^{\circ}\text{C}$.

Results.

The cultures were examined 36 hours after the last illumination period. The mean height and colouration on the conidiophores in each set are recorded in Table 49. Plate 19 is a photograph of one culture from each set.

TABLE 49

Influence on the photoinduction of conidiophore growth of illuminating cultures which have been kept under anaerobic conditions for 20 hours

Set	Treatment	Mean height of the conidiophores in mm	Colour of the aerial growth
1	Kept in darkness	3.5	White
2	Illuminated in oxygen	10.0	Orange
3	Illuminated in nitrogen	3.5	A very faint yellow colour

Discussion of the results of Experiments 59 and 60.

It may be seen from the results of experiment 59, presented in Table 48, that illumination given in the absence of free oxygen is effective in inducing conidiophore growth when the cultures are subsequently returned to darkness in the presence of oxygen. There was little difference between the dry weights of the conidiophores induced in cultures which had been illuminated in the presence (set 2) and in the absence (set 3) of oxygen. This would seem to indicate that the

presence of free oxygen is not essential during the illumination period required to induce conidiophore growth. This would imply that the photoinductive reaction is not an oxidative process.

The carotene content of the conidiophores produced by cultures which had been illuminated in nitrogen was c. two-thirds of that of the conidiophores produced by cultures which had been illuminated in oxygen. This would appear to conflict with the results obtained by Zalokar (1954) from which he concluded that in Neurospora crassa photoactivation of carotenogenesis is an oxidative process, and that illumination in the absence of oxygen fails to promote pigment production. When one examines Zalokar's results, however, one finds the carotene content of cultures illuminated for an hour in the absence of oxygen and then cultured for a further two hours in darkness in the presence of oxygen, was c. one-third of that of cultures in which the illumination was given in the presence of oxygen, and was over twice that of cultures grown continuously in darkness.

It would thus appear that even in Neurospora crassa illumination given in the absence of oxygen is, to a limited extent, effective in inducing carotenogenesis. The effectiveness of illumination given under these conditions may in fact be slightly greater than the results appear to indicate. Carotenogenesis certainly does not proceed in the absence of

free oxygen, thus for one-third of the duration of Zalokar's experiments, which lasted for a total of three hours, carotenogenesis was inhibited. If he had increased the duration of the period in darkness after the illumination period, he may have found still greater induction of carotenogenesis in cultures illuminated in the absence of free oxygen.

The results presented in Table 48 indicate that when illumination is given in the absence of oxygen the photoinduction of carotenogenesis does occur but its effectiveness is reduced.

In experiment 60 the duration of the period during which cultures were held in nitrogen prior to illumination was increased to 20 hours. Under these conditions there appeared to be no photoactivation either of conidiophore growth or carotenogenesis. It is probable that the metabolism of the organism is considerably disturbed by such a long period under anaerobic conditions, and because of this illumination is not effective in inducing conidiophore growth or carotenogenesis.

3. INFLUENCE OF ILLUMINATION ON THE RATE OF RESPIRATION

Hawker & Hepden (1962) found that in Rhizopus sexualis the rate of respiration of cultures increased during zygospore production. Burnett (1952) reports a rise in the rate of respiration when discs of compatible mating-type cultures of Mucor hiemalis and Phycomyces blakesleeianus come in contact.

Carlile (private communication) attaches considerable importance to the possibility that during photoactivation of various fungal processes, stimulated by light, there may be some increase in the rate of respiration.

The following experiment was conducted to determine if the rate of respiration of cultures of Aspergillus giganteus grown in darkness and red light increase when they are illuminated with white light.

Experiment 61 to determine the influence of illumination on the rate of respiration.

Experimental Procedure.

Warburg flasks capped with aluminium foil and containing 5 mls of 2% malt agar were inoculated with drops of a spore suspension. They were attached to manometers, completely covered with aluminium foil, and then placed in a water bath maintained at 22°C. The flasks also contained a 10% solution of sodium hydroxide in the centre well to act as a carbon dioxide absorbent. The experiment was carried out in a darkroom with manipulation facilitated by use of a red safe light.

Manometric readings were taken in red light at intervals after inoculation. The mean rate of respiration in five Warburg flasks is recorded below in Table 50.

Forty-eight hours after inoculation the aluminium foil was removed from the Warburg flasks and manometric readings were taken in red light at 10 minute intervals. After 40

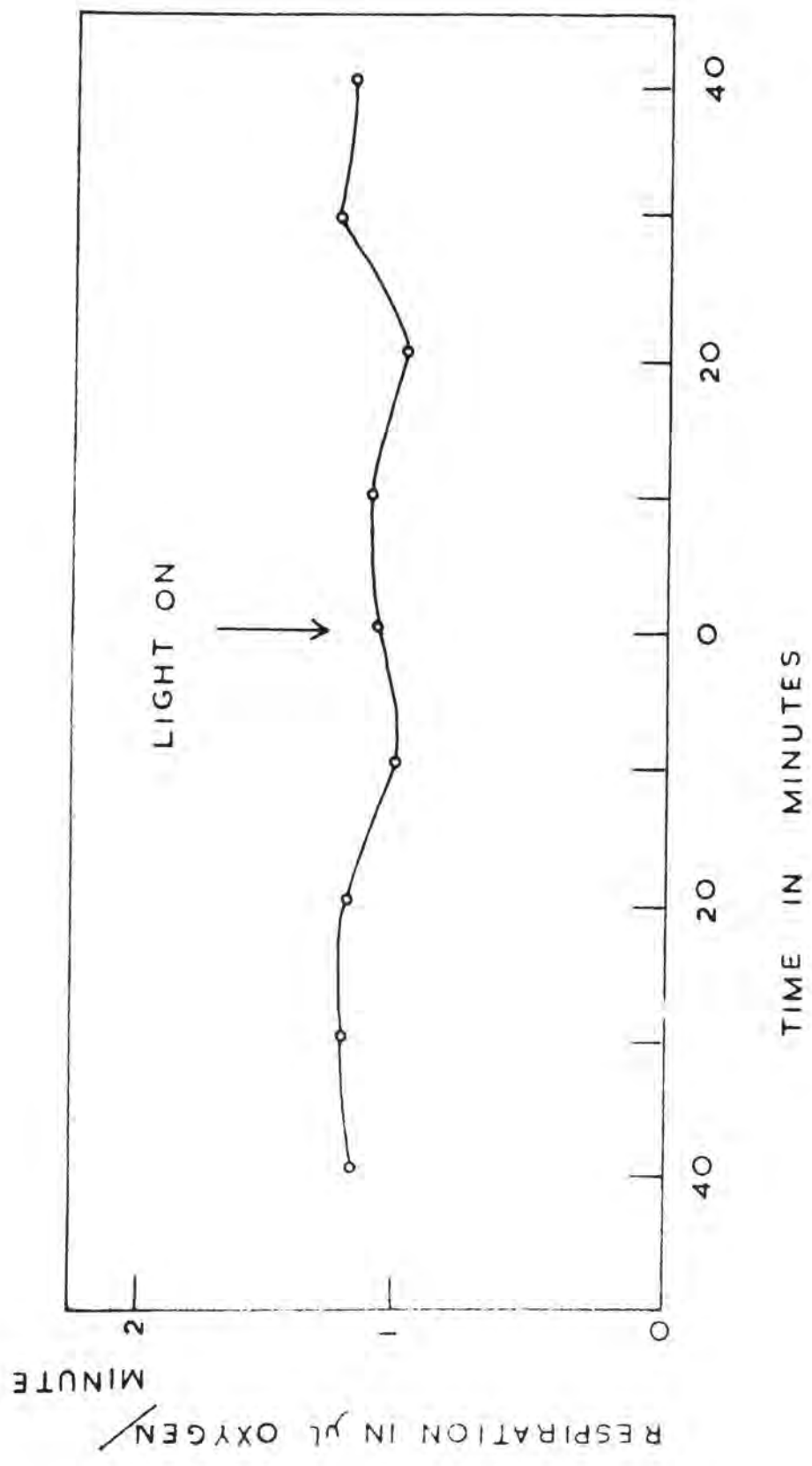


FIGURE 33 INFLUENCE OF LIGHT ON RESPIRATION

minutes the cultures were illuminated with white light; the light intensity at the level of the cultures was 10 foot-candles.

Results.

The mean rate of respiration of five cultures before and after illumination is presented graphically in Figure 33.

TABLE 50

Respiration of cultures in the Warburg flasks

Time (in hours) after inoculation	Rate of respiration: oxygen uptake in μ l/min.
17	1.6
42	3.2
48	2.9

It may be seen from Figure 33 that the rate of respiration was not increased when the white light was turned on.

4. TREATMENT OF THE MYCELIUM WITH HYDROGEN PEROXIDE

Coons (1916) reported that a period of light is necessary for pycnidium formation in Plenodomus foscmaquilans. He found that photoactivation of sporulation could be simulated by the addition of 1/20 cc. of a 3% solution of hydrogen peroxide to the culture medium. Coons concluded that oxidative reactions were involved in the induction of fruiting. Charlton (1953) also found that treating cultures of Alternaria solani with

solutions of hydrogen peroxide could replace a period of light in the induction of sporulation.

The following experiments were conducted to determine if treatment with hydrogen peroxide would induce the growth of conidiophores of Aspergillus giganteus in darkness.

Experiment 62 to determine the influence of exposing cultures to hydrogen peroxide.

Experimental Procedure.

Flasks containing 2% malt agar were prepared and inoculated with a spore suspension. They were placed in darkness in a cupboard maintained at 21 - 22°C. Three days after inoculation the cultures were taken from the cupboard in red light and divided into four sets with five replicates in each. About 5 mls of a solution of hydrogen peroxide were poured into each flask of the cultures of sets 1 - 3. The concentrations of hydrogen peroxide employed were 1, 2.5 and 5 equivalent volumes of oxygen. The fourth set was untreated. After 25 minutes the excess hydrogen peroxide solution was poured out of the flasks, which were then returned to darkness. The cultures were examined four days later.

Results.

No tall conidiophores had been formed by any of the cultures; they were subsequently produced by all the cultures when they were placed in light.

Experiment 63 to determine the effect of adding hydrogen peroxide to the culture medium.

Experimental Procedure.

Fifty ml flasks containing rafts floating on about 20 mls of 2% malt solution were prepared. The rafts were inoculated with a spore solution and placed in darkness in a cupboard maintained at 20 - 21°C.

Three days after inoculation the flasks were divided in red light into four sets with five replicates in each. Drops of a 20 volume hydrogen peroxide solution were added to the culture medium of sets 1 - 3, one drop per culture to set 1, two drops per culture to set 2 and three drops per culture to set 3. The fourth set was left untreated. The cultures were then returned to darkness.

Results.

The cultures were examined three days after adding the hydrogen peroxide. No tall conidiophores had been formed by any of the cultures; they were subsequently produced when the cultures were illuminated.

Discussion of the results of Experiments 61 and 62.

In neither experiment was conidiophore growth induced in darkness by the hydrogen peroxide added to the medium.

SUMMARY

The tall conidiophores are positively phototropic in light of the wavelength range 380 - 530 m μ , but at 280 m μ in the ultra-violet they are negatively phototropic, as are the sporangiophores of Phycomyces. They also show negative phototropic responses when illuminated unilaterally while submerged in liquid paraffin, indicating that the 'lens effect' is in operation in the cylindrical conidiophores.

No evidence was obtained as to the nature of the photoreceptive pigment involved in the phototropic response.

Although the tall conidiophores are strongly phototropic they do not appear to be sensitive to the stimulus of gravity.

1. THE PHOTOTROPIC RESPONSE

(a) INFLUENCE OF LIGHT QUALITY.

The following experiment was conducted in order to determine which light wavelengths are effective in inducing a phototropic response.

Experiment 63 to determine the influence of light quality on the phototropic response of tall conidiophores.

Specimen tubes containing c. 5 mls of 2% malt agar were prepared and five were placed into each growth chamber (see page 10). The tins were arranged on their sides at the distances from a 60 watt bulb calculated to give the same radiant intensity inside each tin. The specimen tubes were placed in an upright position in the growth chambers and were thus illuminated unilaterally by the light passing through the filters. The temperature of the room was maintained at $20 \pm 1^{\circ}\text{C}$.

Results.

The cultures were examined 8 days after inoculation and the results are recorded in Table 51.

Discussion of the Results.

Table 51 shows that light of the wavelength range 380 - 530 m μ which is effective in inducing tall conidiophore elongation is also effective in inducing a positive phototropic response. This probably indicates that conidiophore elongation and phototropism are closely related phenomena,

as in fact one would expect them to be. The same wavelength range also induces phototropic responses in the perithecial necks of Sordaria fimicola (Ingold & Hadland, 1959) and the sporangiophores of Phycomyces blakesleeanus (Curry & Gruen, 1959).

TABLE 51

Influence of light quality on phototropism

Filter	Transmission in m μ	Response of the tall conidiophores
600	380 - 450	Showed a strong positive phototropic curvature
601	380 - 470	"
602	440 - 490	"
603	470 - 520	"
604	500 - 540	Very poor development of tall conidiophores
605	530 - 570	No tall conidiophores produced
608	620 onwards	"
609	650 onwards	"

(b) REVERSAL OF THE PHOTOTROPIC RESPONSE UNDER PARAFFIN

Buder (1918, 1920) reported that when sporangiophores of Phycomyces nitens are submerged in liquid paraffin and then unilaterally illuminated by a horizontal beam of light, they response with a sharp negative phototropic curvature. Gettkant (1954) found that the germ hyphae of a number of fungal spores show reversal in the direction of the phototropic response under paraffin, and more recently Lythgoe (1961) reported that the sporangiophores of Thamnidium elegans also showed this reversal. Buder (1918) suggested that the behaviour of Phycomyces sporangiophores under paraffin can be explained if one postulated that the hyphae behave in a manner similar to cylindrical lenses. Banbury (1959) has recently reviewed this subject.

The following investigation was conducted to determine if the tall conidiophores of Aspergillus giganteus also show this phototropic reversal when submerged in liquid paraffin.

Experiment 64 to determine whether the direction of the phototropic curvature is reversed when conidiophores submerged in liquid paraffin are illuminated unilaterally.

Experimental Procedure.

For this experiment the fungus was cultured in a glass cell constructed from two 8 x 8 x 8 cm lantern slides separated by glass strips one cm wide. A 2% malt agar medium was employed

and after inoculation the cell was placed in an illuminated incubator maintained at a temperature of 21 - 22°C.

Three days later a stand of conidiophores 3 - 5 mm tall had been formed. Liquid paraffin was run into the cell from a pipette until all the conidiophores were submerged. The cell was then placed on the stage of a horizontal microscope beneath a source of white light. After a further 30 minutes growth under these conditions the conidiophores were illuminated unilaterally with white light.

Results.


An hour later all the actively growing conidiophores had turned sharply away from their original direction of growth and were now elongating parallel to the surface of the medium in a direction away from the source of light. During at least the first few hours growth in paraffin the conidiophores continued to elongate at a rate comparable to that of similar conidiophores growing in air and the reaction time for the phototropic curvature was approximately the same. When the cell was examined 20 hours later it was found that in some instances the terminal portion of the conidiophore at right angles to the original direction of growth was as long as 3.5 mm. Conidiophores of a similar height would have elongated in air at a rate of 500 - 600 μ /hour and thus a 3.5 mm length of conidiophore represents a minimum of about 6 hours growth.

10 mm
←————→

PLATE 20 Conidiophores illuminated unilaterally
while submerged in liquid paraffin.

Direction of incident
light. →

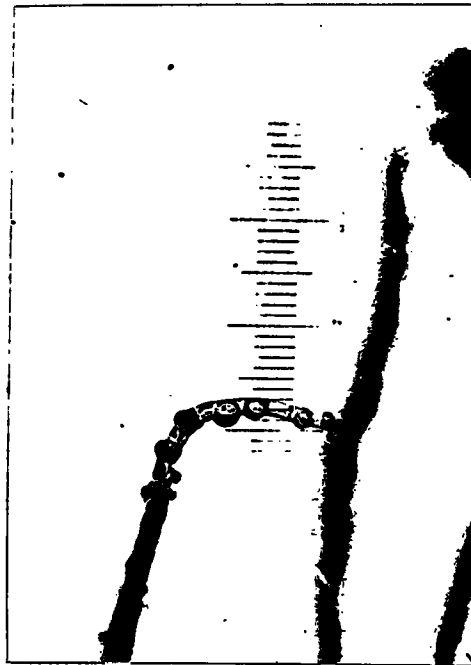
Liquid paraffin



Scale 100 μ



Scale



Direction of
incident light

PLATE 21 Formation of exudation droplets on a conidiophore while submerged in liquid paraffin.

Plate 20 is a photograph of the glass cell containing conidiophores submerged in liquid paraffin which had been illuminated unilaterally for 20 hours.

This experiment also illustrated the nature of the liquid droplets formed on the walls of the conidiophores. While the conidiophores were submerged in paraffin these droplets continued to form and enlarge; they did in fact tend to show up more clearly due to the presence of the paraffin. The fact that these droplets continued to form on the conidiophores under such conditions clearly demonstrates that they are in fact exudation and not condensation droplets. Plate 21 is a photograph of a conidiophore which had formed exudation droplets while submerged in liquid paraffin.

(c) REVERSAL OF THE PHOTOTROPIC RESPONSE IN U.V. RADIATION

Curry & Gruen (1957) found that sporangiophores of Phycomyces respond to unilateral stimulation with ultra-violet radiation below 298 m μ by producing negative phototropic curvatures. The following investigation was undertaken to determine whether or not the conidiophores of Aspergillus giganteus also exhibit a reversal of the phototropic response in the ultra-violet.

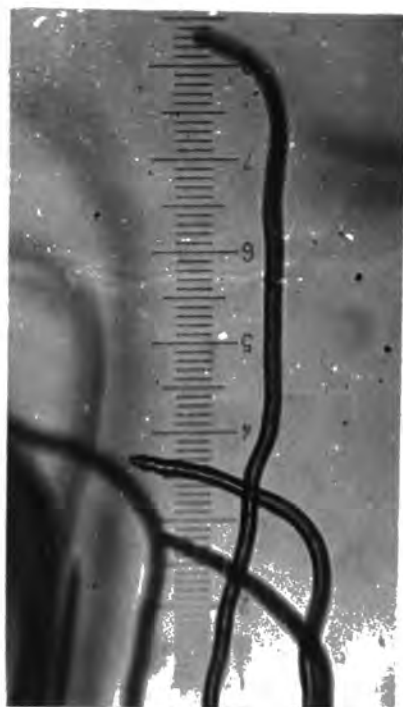
Experiment 65 to determine whether the tall conidiophores show a negative phototropic response when illuminated unilaterally with ultra-violet radiation.

Experimental Procedure.

A Unicam spectrophotometer was used as the source of monochromatic ultra-violet radiation. A special growth chamber was constructed to fit into the cell holder of the spectrophotometer; the chamber consisted of a 2.5 cm x 4.0 cm x 5.0 cm glass box made from glass strips stuck together with Bostik. The two side walls of the chamber were lined with wet blotting paper to maintain a high internal humidity and the end wall was covered with black paper. The open end of the chamber facing the radiation source was covered with cellophane; ultra-violet is transmitted by cellophane but to a much lesser extent by glass. Figure 20 on page 76 is a diagram of one of these cells.

Blocks of inoculated Zurzyka agar were cut from a petri-dish and placed inside the chamber. These were then placed directly beneath the light source in an incubator maintained at a temperature of 21 - 22°C. When the conidiophores were about 4 - 5 mm tall the chamber was transferred to the cell holder of the spectrophotometer and the conidiophores were unilaterally stimulated with ultra-violet radiation of wavelength 280 mμ. The slit width of the spectrophotometer was opened fully to ensure that the culture was illuminated by as broad

Scale 100 μ



Direction of
incident light

PLATE 22 Conidiophores showing negative
phototropic curvatures in response to
unilateral radiation with ultra violet
light.

a radiation beam as possible. The experiment was carried out at room temperature, c. 17°C.

Results.

The cultures were left in the incubator for four to five hours. At the end of this period the chamber was removed and the conidiophores were examined. It was found that the conidiophores which had been illuminated unilaterally in ultra-violet beam had curved away from the source of radiation; the conidiophores were growing at an angle of 90° to their original direction of growth and parallel to the surface of the medium. Plate 22 is a photograph of tall conidiophores showing the negative phototropic response. Thus the tall conidiophores of Aspergillus giganteus, like the sporangiophores of Phycomyces, exhibit a reversal of the phototropic response from positive to negative in ultra-violet radiation of 280 mμ wavelength.

The inversion of the phototropic response of conidiophores in ultra-violet radiation is probably due to the presence in the cytoplasm of a substance which absorbs ultra-violet radiation very strongly. Dennison (1961) demonstrated the presence of the strongly U.V. radiation absorbing substance, gallic acid, in the sporangiophores of Phycomyces. He proposed that it was the presence of this substance which caused the inversion of phototropism in ultra-violet radiation.

(d) CONIDIOPHORE TWISTING DURING THE PHOTOTROPIC RESPONSE

Experiment 66 to determine if tall conidiophores continue to grow spirally while responding phototropically.

Experimental Procedure.

The conidiophores were grown in specimen tubes on 2% malt agar. Conidiophores 3 - 5 mm tall were marked with starch grains; a conidiophore which was marked with starch grains near its tip was then selected for observation and a camera lucida drawing of it was made. The conidiophores were then illuminated unilaterally with a piece of black paper behind the specimen tube to increase the light gradient across it. Camera lucida drawings of the conidiophores were drawn at intervals as they exhibited a phototropic response. Figure 33 is a set of camera lucida drawings of a marked conidiophore responding to unilateral illumination.

Results.

The drawings in Figure 33 show that in this case a starch grain moved in a clockwise direction through about 180° during the period when the conidiophore was curved towards the direction of light.

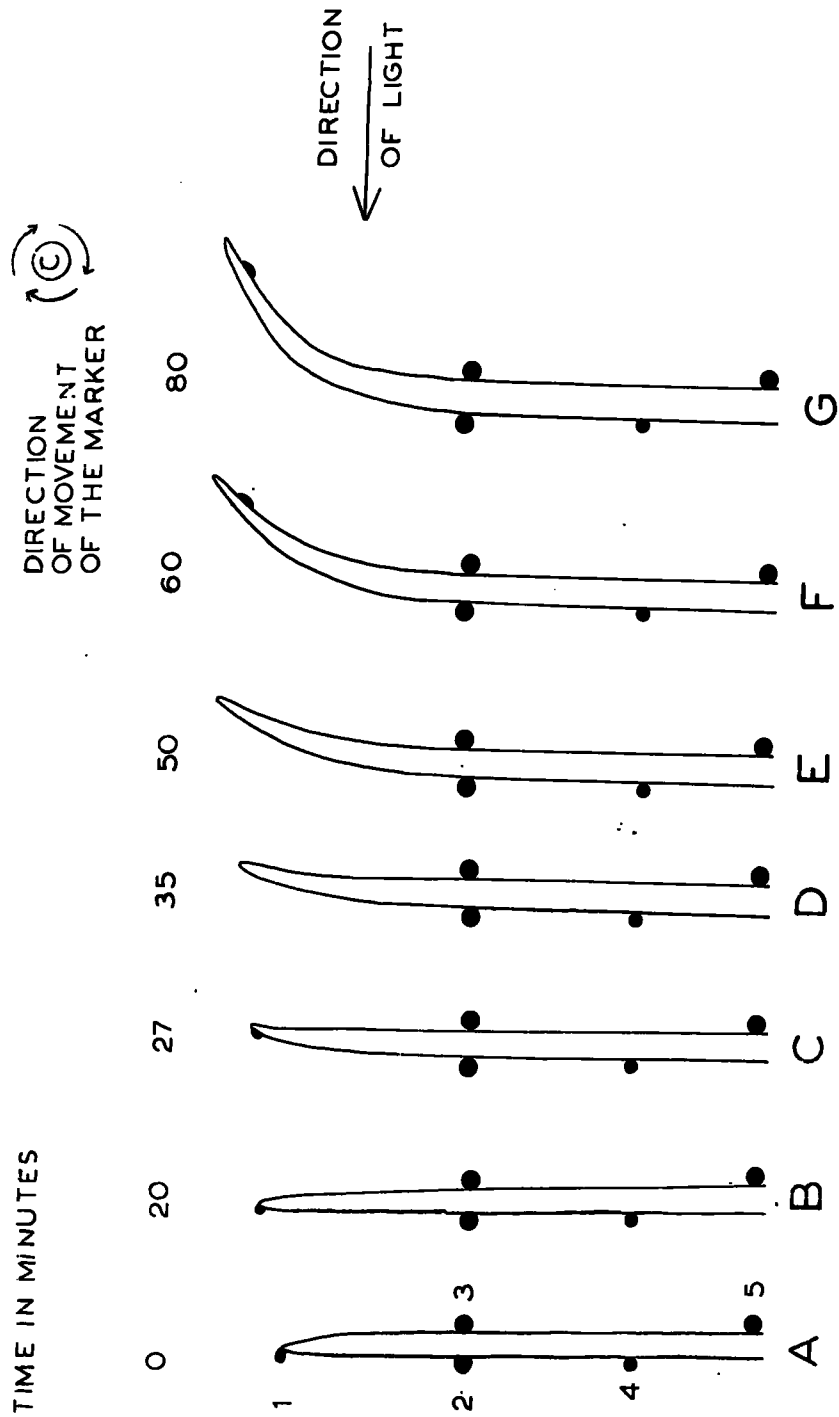


FIGURE 38 CAMERA LUCIDA DRAWINGS OF AN ELONGATING MARKED CONIDIOPHORE WHICH WAS ILLUMINATED UNILATERALLY

(e) INFLUENCE OF PIGMENT INHIBITORS IN THE MEDIUM ON THE
PHOTOTROPIC RESPONSE

The following experiments were conducted in order to determine whether or not the presence of pigment inhibitors in the medium caused an inhibition of the phototropic response of the tall conidiophores.

Experiment 67 to determine the influence of incorporating pigment inhibitors in the medium on the ability of tall conidiophores to response to unilateral illumination.

Experimental Procedure.

Specimen tubes containing culture media which incorporated the pigment inhibitors listed in Table 52 were prepared.

TABLE 52

Influence of incorporating pigment inhibitors in the medium on the phototropic response of the conidiophores

Set	Substance incorporated in the medium	Pigment which it inhibits	Concns. employed in ppm	Culture medium
1	1-lyxoflavin	Riboflavin	10, 100 and 200	2% malt agar
2	Mepacrine	Riboflavin	25 and 75	Zurzyka
3	Diphenylamine	β -carotene	20 and 40	Zurzyka with 1% glucose

The culture media were inoculated with a spore suspension and then placed in light in an incubator maintained at $22 \pm 0.5^{\circ}\text{C}$.

When the conidiophores produced by the cultures were 4 - 5 mm tall they were illuminated unilaterally.

Results.

The conidiophores produced by the culture media containing the various pigment inhibitors all exhibited positive phototropic curvatures in response to the unilateral stimulus.

Experiment 68 to determine the reaction time of the phototropic response of tall conidiophores cultured on media containing pigment inhibitors.

Experimental Procedure.

Specimen tubes containing culture media incorporating the various pigment inhibitors were prepared. They were inoculated with a spore suspension and then placed in light in an incubator maintained at $22 \pm 0.5^{\circ}\text{C}$.

Cultures containing 4 - 5 mm tall conidiophores were placed on the stage of a horizontal microscope and then illuminated from above. One hour later this light was switched off and the conidiophores were illuminated unilaterally by a second light. Photographs of the conidiophores were taken at intervals of five minutes and the time at which the first phototropic response appeared was recorded. The reaction times are recorded in Table 53.

TABLE 53Influence of the presence of pigment inhibitors in the medium on the reaction time for the phototropic response

Inhibitor employed	Reaction time for the response (in minutes)	
	In the presence of the inhibitor	In the absence of the inhibitor
1-lyxoflavin	35, 30, 30	35, 35, 30
Diphenylamine	45, 40, 40	35, 30, 35

Results

The reaction times of conidiophores growing on media containing the various pigment inhibitors were approximately the same as those of conidiophores cultured on media which did not contain the inhibitors. The reaction time was usually in the region of 30 - 35 minutes. Only in the case of conidiophores cultured on media containing 40 ppm diphenylamine were the reaction times rather longer than the controls, but, as was shown in experiment 56, growth is considerably reduced on this medium.

Discussion of the Results of Experiments 67 and 68.

The results of the experiments reported above give no indication of the nature of the photoreceptors involved in the phototropic response of tall conidiophores.

2. THE GEOTROPIC RESPONSE

The positive phototropic response of the tall conidiophores to unilateral illumination has been noted by a number of workers, but no investigation has hitherto been made to determine whether or not they are geo-sensitive. The following experiments were conducted to investigate this point.

Experiment 69 to determine if the tall conidiophores are geo-sensitive.

Experimental Procedure.

Fifteen 100 ml flasks containing 15 mls of Żarzyka agar were prepared. They were inoculated with spores and then placed in darkness in a cupboard maintained at a temperature of $20 \pm 1^{\circ}\text{C}$.

Seven days after inoculation the flasks were divided in red light into three sets with five replicates in each. Each set was illuminated for 15 minutes under a fluorescent tube while the flasks were in the positions described below.

Set 1. The flasks were placed in an upright position during the illumination period, but when they were returned to the cupboard they were placed in a horizontal position with the plane of the agar surface vertical.

Set 2. The flasks were illuminated while in a horizontal position and were kept in this position when they were returned to the cupboard.

Set 3. The flasks were placed in an upright position during the illumination period and were kept in this position when they were returned to the cupboard.

On the following day a further period of illumination was given under the same conditions as described above. The average height of the tall conidiophores in each flask 24 hours after each period of illumination was measured in red light. The mean value for each set was calculated and the results are presented in Table 54.

TABLE 54

Set	Mean height of the tall conidiophores (in mm)	
	24 hrs after first illumination period	24 hours after second illumination period
1	5	14
2	5	12
3	5	12

The angle at which the conidiophores grew from the medium with respect to its surface was noted in each treatment. It was not possible to distinguish between the treatments on the basis of the form or direction of conidiophore growth; the conidiophores grew away from the medium but tended to have a 'splayed' appearance. The conidiophores which had elongated horizontally in darkness did not appear to have reacted in any way to the gravitational stimulus.

Observations of conidiophores elongating in red light

Microscopic observations of conidiophores elongating in red light clearly demonstrated that they were not geo-sensitive. When a source of white light directly above the conidiophores was turned off and subsequent growth observed in red light, it was found that eventually the conidiophores began to deviate from their former direction of growth. While they were being illuminated from above the conidiophores grew vertically upwards from the medium. When the light was switched off the conidiophores continued to grow for some hours in a general upward direction but eventually nearly all of them deviated from this line of growth.

Cultures which had been growing in the cells described on page in white light were left overnight in red light and then examined the following day. No distinct directional orientation of conidiophore growth could be detected; conidiophores were observed growing in all directions, including some which were elongating vertically downwards, and others which were growing in a horizontal plane parallel to the surface of the medium. Plate 23 is a photograph of conidiophores which had grown a number of hours in red light.

The following experiment was conducted to observe the behaviour of conidiophores when they were placed so that they were elongating horizontally in red light.

Scale 100 μ

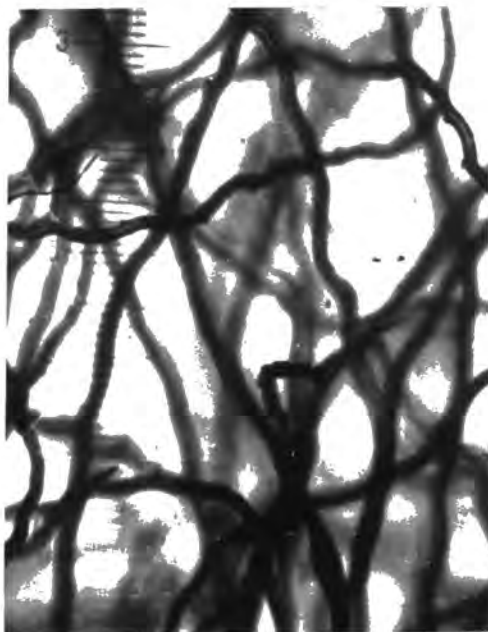


PLATE 23 Conidiophores which have been cultured in red light for several hours.

Experiment 70 to determine whether or not gravity has an influence on the direction of conidiophore growth.

Experimental Procedure.

The cultures were grown in specimen tubes containing c. 5 mls of 2% malt agar. The specimen tubes were placed beneath a fluorescent tube in a room maintained at a temperature of $20 \pm 1^{\circ}\text{C}$. The experiment was conducted on conidiophores which were 7 - 10 mm tall.

Specimen tubes were removed from beneath the light, placed in a horizontal position on the microscope stage, and examined in red light. Conidiophores were kept under observation for periods from three to five hours while they were elongating horizontally and thus receiving a unilateral gravitational stimulus.

Results.

The conidiophores examined continued to grow in a horizontal direction during the periods of observation; no divergence in response to the gravitational stimulus was observed. One conidiophore was kept under observation for about 5 hours and during this period it elongated horizontally for over 2 mm.

Discussion of the Results of Experiments 69 and 70.

The results of this experiment clearly show that the tall conidiophores of Aspergillus giganteus, unlike the sporangiophores of Phycomyces, are not sensitive to gravity.

This fact is not so surprising as it might at first appear if one bears in mind that conidiophore growth of Aspergillus giganteus has to be photoinduced while growth of Phycomyces sporangiophores can proceed equally well in light or darkness. In one sense there is really no need for conidiophores to have a geo-sensitive system as prolonged growth is only possible in light and in these circumstances the direction of elongation will be rigidly determined by the direction of the incident illumination.

This growth system is by no means the only example of fungal structures being photo- but not geo-sensitive. Carlile et al. (1962) found that the conidia of Penicillium isariforme are not sensitive to gravity although they are positively phototropic. Lukens (1963) also found that the conidiophores of Alternaria solani also behaved in the same manner. It thus appears that the growth system shown by Aspergillus giganteus conidiophores is not unique.

It is hoped in the near future to study the light induced growth of both the conidia of various species of the Penicillia and the conidiophores of Alternaria solani, and in particular to compare them to Aspergillus giganteus. It would appear from the studies already reported on these species that they all have the same basic growth system.

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