ECOLOGICAL STUDIES ON KERATINOPHILIC FUNGI

by

R. IBBOTSON, B.Sc. (Nottingham)

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CONTENTS

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SUMMARY

The seasonal distribution of keratinophilic fungi has been studied in the soils surrounding an outdoor swimming pool and in soils of summer pens of hedgehogs. The species isolated were conidial and cleistocarpic Arthroderma uncinatum, Trichophyton terrestre, Chrysosporium keratinophilum, Microsporum cookei and Microsporum gypseum. A correlation between some species and the numbers of hedgehogs in the pens was seen, due to the influence of keratin provided by the quills and scales of the hedgehogs. No such relationship was seen at the swimming pool, although conidial A. uncinatum was seen to decrease when the swimming pool was open to the public in the summer. However, this appears to be the normal seasonal occurrence since a similar pattern of distribution in soils collected from outside the swimming pool was seen.

The effects of the addition of fungicides to soil upon the isolation of keratinophilic fungi have been studied, using the hair-baiting technique. The general pattern showed an initial fall in percentage colonisation of baits followed by a fairly rapid recovery to give greater colonisation of greasy baits compared with controls, and a return to the same or less than the control on degreased wool. This contrasts with the normal observation that the majority of keratinophilic fungi grow better on degreased than greasy wool.

A study of the keratinophilic flora of soils of different pH values and from coastal regions has been made as ground work for the experimental section.

The new intake of students living in halls of residence at Nottingham University was studied in relation to tinea pedis over an academic year. The initial level in October 1972 was 9%; by May 1973 it had increased to 24% but returned to 11% in October 1973. In addition to any seasonal influence, there seemed to be a [illegible] correlation between the sports played by the students and the incidence of tinea pedis. The showers in halls of residence and the University sports centre were thought to be the main points of cross transfer of the infection.

In the experimental section, the effects of pH, temperature, fungicides and sea water upon certain keratinophilic fungi have been examined *in vitro*.

Aspects of the nutrition of *C. keratinophilum*, *A. uncinatum* and *T. terrestre* were studied and the most suitable combinations of carbon and nitrogen sources for optimal growth were determined.

A study of the competitive saprophytic ability of several dermatophytes was made using various techniques. By use of the fluorescent antibody technique, it was found that *A. uncinatum* was a good competitive saprophyte in the presence of keratin and can thus be regarded as a true soil inhabitant.

Finally, the mating structures of *Arthroderma benhamiae*, *A. tuberculatum*, *A. uncinatum*, *Nannizzia cajetani*, *N. gypsea* and *N. incurvata* were studied using the scanning electron microscope.

SECTION I

OBSERVATIONAL

INTRODUCTION

A dermatophyte is considered to be a fungus parasitising keratinised tissue of man and animals and causing dermatophytosis (Ainsworth, 1971). The philosophical question of whether keratin is dead or living, and hence whether keratinophilic fungi are saprophytes or parasites, is open to discussion. However, not all fungi capable of utilising keratin are known to be pathogenic to man. e.g. Ctenomyces serratus and Chrysosporium keratinophilum, but all keratinophilic fungi should be treated as potential pathogens. Kaplan, Georg and Ajello (1958) suggested that keratinophilic fungi could be separated, on the basis of their normal occurrence into geophilic, zoophilic and anthropophilic. Some geophilic dermatophytes have been isolated from man or animals, e.g. Trichophyton ajelloi (Georg, Kaplan, Ajello, Williamson and Tilden, 1959; Lindqvist, 1960; Pier and Hughes, 1961; Otčenášek and Dvořák, 1963; Rakhmanov, Fedorova and Yashkul', 1969; Refai and Ali, 1970 and Hoffmann, Kolipp and Koch, 1970), Microsporum cookei (Lindqvist, 1960; Marples, 1961; Ridley, 1961; Mariat and Tapia, 1966; Schick, 1966, 1968; English, 1967; Lundell, 1969; Hoffmann et al. 1970 and Frey, 1971) and Trichophyton terrestre (Marples, 1961; Marples and Smith, 1962; Otčenášek and Dvořák, 1963; Gip and Martin, 1964; Gentles, Dawson and Connole, 1965; Connole, 1965 and Hoffmann et al. 1970). These three species were also isolated by Smith, Rush-Munro and McCarthy (1969) but were considered to be contaminants and although some of the literature cited concerns isolations from healthy

animals, there is evidence for the pathogenic nature of each of these species.

However, the numbers of infections caused by these three geophilics are small compared with those caused by Microsporum gypseum, which has been found by many people to infect man and animals (Ajello, 1953; Menges, Love, Smith and Georg, 1957; Lindqvist, 1960; Alsop and Prior, 1961; Kaplan and Ivens, 1961; Ridley, 1961; Otčenášek and Dvořák, 1963; Mohapatra and Gugnani, 1964; Smith and Marples, 1964; Keep and Pile, 1965; Koch, 1965; Bensch and Gemeinhardt, 1966; Chmel, 1966; Donald and Brown, 1966; Fischman, Londero and Santiago, 1966; Gip and Hersle, 1966; Herpay and Rieth, 1966; Mata and Mayorga, 1966; Alteras and Evolceanu, 1967; Carter, 1967; Connole, 1967; Immel, 1967; Al-Doory, Vice and Olin, 1968; Kaben and Ritscher, 1968; Alteras, Nesterov and Ciolofan, 1968; Magalhães and Boesch, 1968; Blank, Taplin and Zaias, 1969; Steinerová and Dubrovova, 1969; Alteras and Cojocaru, 1970, 1971; Chmel and Buchvald, 1970; Hoffman et al. 1970; Kadlec and Podivinska, 1970; Pöhler and Schönborn, 1970; Baran, 1971; Findlay, Roux and Simson, 1971; Weisbroth and Scher, 1971; Alteras, 1971, 1972; Belukha and Luk'yanova, 1972; Koehne, 1972 and Schönborn and Pöhler, 1972).

Zoophilic dermatophytes are known to be capable of saprophytic survival in soil e.g. Trichophyton mentagrophytes (Schönborn, 1966
Baxter, 1969). Emmons, (1950) suggested that there was a sound basis for the hypothesis that pathogenic fungi causing human and animal disease were more commonly present in soil and vegetation than mycologists generally realised. In 1951, he stressed that greater attention should be given to the occurrence of these fungi in soil. Ajello (1956) and Grin and Ožegović (1963) have also discussed the significance of

a study of soil to dermatomycoses. Muende and Webb (1937) found a ringworm fungus growing saprophytically on dung in an animal shed and Watling (1963) reported various keratinophilic Aleurisma spp. on hawk pellets. The hypothesis of a saprophytic life of dermatophytes, proposed by Vanbreuseghem (1952), has been upheld by the fact that dermatophytes have been isolated from soil in all five continents, Otčenášek, Dvořák and Kunert (1967) have reviewed the literature up to 1964 about the distribution of geophilic dermatophytes in the soil. More isolations throughout the world have been made since that time. These include reports from Australia (Frey, 1965; Donald and Brown, 1966), Belgium (Ajello, Varsavsky and Delvingt, 1965), Colombia (Rogers, 1971), Czechoslovakia (Steinerová and Buchvald, 1967), South Dakota (Knudtson and Robertstad, 1970), France (Coudert, Michel-Brun and Battesti, 1967; Percebois, Burdin and Helluy, 1965), Germany (Staib and Evangelinos, 1968; Köhler and Hoffman, 1965), Great Britain (Baxter, 1965, 1969), Hong Kong (Turner, 1965), India (Padhye, Misra and Thirumalacher, 1966; Randhawa and Sandhu, 1965; Garg, 1965; Gugnani, Shrivastav and Gupta, 1967; Roy, Ghosh and Dutta, 1972), Italy (Ajello, Varsavsky, Sotgiu, Mazzoni and Mantovani, 1964), Mozambique (Magalhães and Boesch, 1968), New Zealand (Marples, 1965; Durie, 1961), Ohio (Kurup and Schmitt, 1970), Poland (Cheçinski, 1970; Prochacki and Bieżunska, 1968), Polynesia (Marples, 1965), Portugal (Cabrita and Figueiredo, 1973), Roumania (Alteras and Evolceanu, 1967, 1969; Evolceanu and Alteras, 1967; Căpuşan, Bociat and Popesco, 1970), Sweden (Gip and Hersle, 1966; Pålsson, 1968), Texas (Al-Doory, 1967), Thailand (Taylor, 1966), USSR (Karatygiria, 1971) and Yugoslavia (Janković-Brmbolić, 1967).

Trichophyton mentagrophytes, T. ajelloi and Chrysosporium spp. have been found on dry leaf litter (Szathmáry, 1970) and T. terrestre, T. ajelloi, Microsporum cookei and M. gypseum were found by Balabanoff and Usunov (1967) on organic waste of plant origin. However, it can be seen from the plethora of literature that soil serves as a natural reservoir for fungi which can cause ringworm and are pathogenic or potentially pathogenic to man. Because of this it is important to determine more about the ecology of keratinophilic species in general and the autecology of individual species. This would help in the study of the epidemiology of dermatophyte infections.

Edaphic factors affecting the distribution and growth of fungi which can attack keratin (both dermatophytes and related saprophytic forms) have been given relatively little attention in the past. Pugh (1971) commented on the fact that so few workers record the reaction of soils from which they isolated keratinophilic fungi that it was difficult to assess the importance of this factor. He also stressed the lack of data which would help to explain the observed distribution patterns of keratinophilic fungi. Böhme, Rawald and Stohr (1969) studied the effect of pH on keratinophilic fungi, Buchvald, Steinerová and Hraško (1967) also studied pH in addition to calcium carbonate and humus content of the soil. Chmel, Hasilíková and Hraško (1971) studied humus content and humidity of the soil in relation to the isolation of keratinophilic fungi. These workers appear to be among the few who have studied edaphic factors influencing the incidence of these fungi.

The present research was undertaken to investigate the distribution in nature of certain keratinophilic fungi with special reference to areas where biotic factors, which provide a keratin source for these fungi, are present. It is generally

known that keratinophilic fungi can frequently be isolated from the environment of animals (Ajello, 1953; Hejtmánek, 1957; Menges, Love, Smith and Georg, 1957; Carmichael, 1961; Pier and Hughes, 1961; Durie and Frey, 1962; Ajello et al. 1964; Pugh, 1964; Frey, 1965; Petzoldt and Böhm, 1966; Staib and Evangelinos, 1968 and Pugh and Evans, 1970a) and that specific keratinophilic fungi can be regularly found on hedgehogs (Marples and Smith, 1960, 1962; English, Evans, Hewitt and Warin, 1962; La Touche and Forster, 1963; English, 1964; English, Smith and Rush-Munro, 1964; Quaife, 1966; Fox, 1968 and Morris and English, 1969). Hedgehog nests were examined by English and Morris,(1969), but the soil on which hedgehogs live has not been studied.

People who use swimming baths have been sampled for tinea pedis (Rosenthal, Baer, Litt, Rogachevsky and Furnari, 1956; English and Gibson, 1959; Fichtenbaum, 1966; Alteras, Cojocaru and Hontaru, 1967; Cordonnier, Lundy-Mahieu, Parent and de Beer, 1971 and Gentles and Evans, 1973). Cubicles and floors of swimming pools were studied in the past (Ajello and Getz, 1954; Gentles, 1956, 1957; English and Gibson, 1959; Gip, 1961, 1967; Gip and Aschan-Åberg, 1968 and Cordonnier et al. 1971), but little work has been reported on isolations from soil at these sites. Only Fichtenbaum (1966) isolated Trichophyton ajelloi and T. terrestre from earth and dust from an outdoor pool and Evolceanu and Alteras (1967) isolated several keratinophilic fungi from a grassy bathing place at a lakeside in Roumania.

Since hedgehogs were kept at the university and were being placed in summer pens for only part of the year, and an outdoor swimming pool was in close proximity, where visitors only came for part of the year, a study was made to discover and compare any seasonal changes in the occurrence of keratinophilic fungi taking place at these two sites.

Work in the past on coastal soils (Pugh and Mathison, 1962; Dabrowa, Landau, Newcomer and Plunkett, 1964; Gip and Paldrok, 1966; Marcelou-Kinti, 1968; Orrù, Pinetti and Aste, 1968; Kishimoto and Baker, 1969) has shown that while a number of keratinophilic fungi (including some dermatophytes) had been isolated from these environments, certain species such as Trichophyton ajelloi were not normally found in these situations. It was decided to determine if this still held true for soil samples at the coastal ecosystem at Gibraltar Point, 10 years after Pugh and Mathison carried out their work and then to continue these studies to find out why there was this distinct distribution.

The use of horn and hoof meal as a long-lasting fertiliser in horticultural practice has been known to increase the frequency of Microsporum gypseum (Alsop and Prior, 1961) and Somerville and Marples (1967) showed that cowhorn shavings also increased isolations of this species. Since soils from a farm which had been treated with refuse, from a broiler factory, were readily available it was thought to be of interest to study the effect of this treatment upon the presence of the dermatophytic flora.

Fungicides are also frequently used both in horticulture and agriculture and while the work already mentioned was in progress, other research in the Mycology laboratory at Nottingham University was concerned with the effects of application of fungicides on soil biology. This provided an opportunity to study the effects of the fungicides on keratinophilic fungi.

It is well known that pH has a distinct effect upon the growth of fungi. Böhme and Ziegler (1969) have documented the literature relating to this subject. Their findings agreed in general with those of Marples (1965) and Pugh (1966) but

disagreed with those of Rodziewicz (1963) for Trichophyton ajelloi. Marples isolated this species mostly from highly acidic soils while Böhme and Ziegler and Pugh found that T. ajelloi was found mostly at pH 6 or less. Rodziewicz however, isolated most of his T. ajelloi from soils at pH 8. Soils at Sutton Bonington Agricultural School which had been treated to give values of pH 3.5 to 8.5, increasing by units of 0.5, were readily available and studies were carried out to determine the effect of pH on the isolation of keratinophilic fungi.

During the course of this research at Nottingham, a good opportunity arose to study the same group of people over a 12 month period or more, and a tinea pedis survey was carried out to investigate the distribution of the infection over an academic year. Although Legge, Bonar and Templeton, (1929 a,b), Gould, (1931), Muskatblit, (1933), Marples and Bailey, (1957), Marples, (1959), and Doby-Dubois, Berthault and Doby, (1961) have studied students in the past, only Legge et al. repeated the tinea pedis survey of the students after a few months. Apart from English and Gibson, (1959), who studied boys at a boarding school twice in one year, no other such studies on students have been reported in the literature.

SAMPLING SITES

Soil samples were collected from:-

a) Highfield's swimming pool, University Park, Nottingham. N.G. Ref. SK545385

b) hedgehogs' pens at Nottingham University Zoology Department. N.G. Ref. SK545385

c) Rothampsted Experimental Station, Harpenden, Herts. N.G. Ref. TL123135

d) Sutton Bonington Agricultural School, Nottingham University. N.G. Ref. SK505262

e) Gibraltar Point, Lincolnshire. N.G. Ref. TF555576

f) a Pembrokeshire farm. N.G. Ref. SM838090

g) University Park soils from the Nottingham University Botany Department, which were used for experimental and some observational work.

Nest material was collected from nesting boxes within the hedgehog pens. Spines and scales were collected from the hedgehogs.

During the tinea pedis survey skin samples were taken from first year students at Nottingham University.

Highfield's swimming pool is an open-air pool with 3 grassy areas. Between the grass and the pool is an expanse of concrete including a sunbathing area and some of the changing cubicles, (Figure 1). Visitors use the pool from the end of May to the end of September.

The hedgehog pens are enclosures 4m square with nesting boxes containing hay, inside on the mixed vegetation (Figure 2). The hedgehogs live in the pens from March to the end of September. Four was the maximum number of hedgehogs in one pen at any time; 1 male and 3 females. Other animals such as shrews, mice and birds were known to enter the pens.

FIGURE 1

Highfield's swimming pool

FIGURE 2

Hedgehog pens

Field trials of fungicides were taking place at Sutton Bonington and Rothampsted. The fungicides being tested at Rothampsted were formalin, applied at 500 l/ha., captan at 9 kg/ha., dicloran at 4 kg/ha., thiram at 13.4 kg/ha and quintozene at 11.2 kg/ha. The last three had been applied before these doses with half dosages of the same fungicides, i.e. dicloran 2 kg/ha, thiram 6.7 kg/ha and quintozene at 5.6 kg/ha. Initial sampling after these applications was not very informative and since the results from the trials, which were being run by entomologists were also not informative, second treatments were given and resampling took place more frequently to determine immediate effects. At Sutton Bonington, Triarimol at 2 kg/ha., Milcol at 5 l/ha., captan at 5.6 kg/ha. and dicloran at 1.65 kg/ha were being tested. All fungicides were applied in suspension with the exception of formalin which was in solution.

A further 3 soils were collected from Sutton Bonington. The soil was officially classed as Astley Hall complex and the texture is sandy loam of variable depth (1-2m) over Keuper Marl, with imperfect drainage.

TABLE 1

Treatment of Sutton Bonington soils to give different pH values.

pH of soil	annual dressing
4.2	0.27×10^3 kg S ha^{-1}
6.7	0.65×10^3 kg $Ca(OH)_2$ ha^{-1} (c. 5 cwt/acre)
8.2	6.5×10^3 kg $Ca(OH)_2$ ha^{-1} (c. 50 cwt/acre)

Gibraltar Point is a coastal nature reserve in Lincolnshire. Several samples of soil were taken from the marine ecosystem:-

a) mud uncolonised by higher plants
b) below spring high water mark, colonised by Sueda and Salicornia
c) below high water mark
d) above high water mark
e) new foredunes (Figure 3)
f) mature eastern main dunes (Figure 4)
g) trapping chambers of the Heligoland bird traps. (Figure 5)

Samples of soil were collected from two areas at a Pembrokeshire farm. One sample was from a field with early potatoes growing in it. There had been a great deal of turkey broiler factory debris (feathers, feet, heads and insides) used as manure on soil. The second sample was dug out from a hedge, i.e. not receiving the same treatment as the potato field.

A survey was made for the incidence of tinea pedis among the new intake of students at Nottingham University in October 1972 and October 1973. The students in the 1972 intake were sampled 3 times altogether, the second survey taking place in May 1973 and the third in October 1973 when the new intake was also sampled. The October surveys took place in the first two weeks of the students' attendance at the university in order to determine the initial incidence of tinea pedis.

GIBRALTAR POINT

FIGURE 3
New foredunes

FIGURE 4
Mature eastern main dunes

FIGURE 5
Heligoland bird traps

MATERIALS AND METHODS

Sampling procedure

Soil samples from the swimming pool and hedgehog pens were collected once a month for a year. AtRothampsted and Sutton Bonington soils were collected at various times after treatment with fungicides.

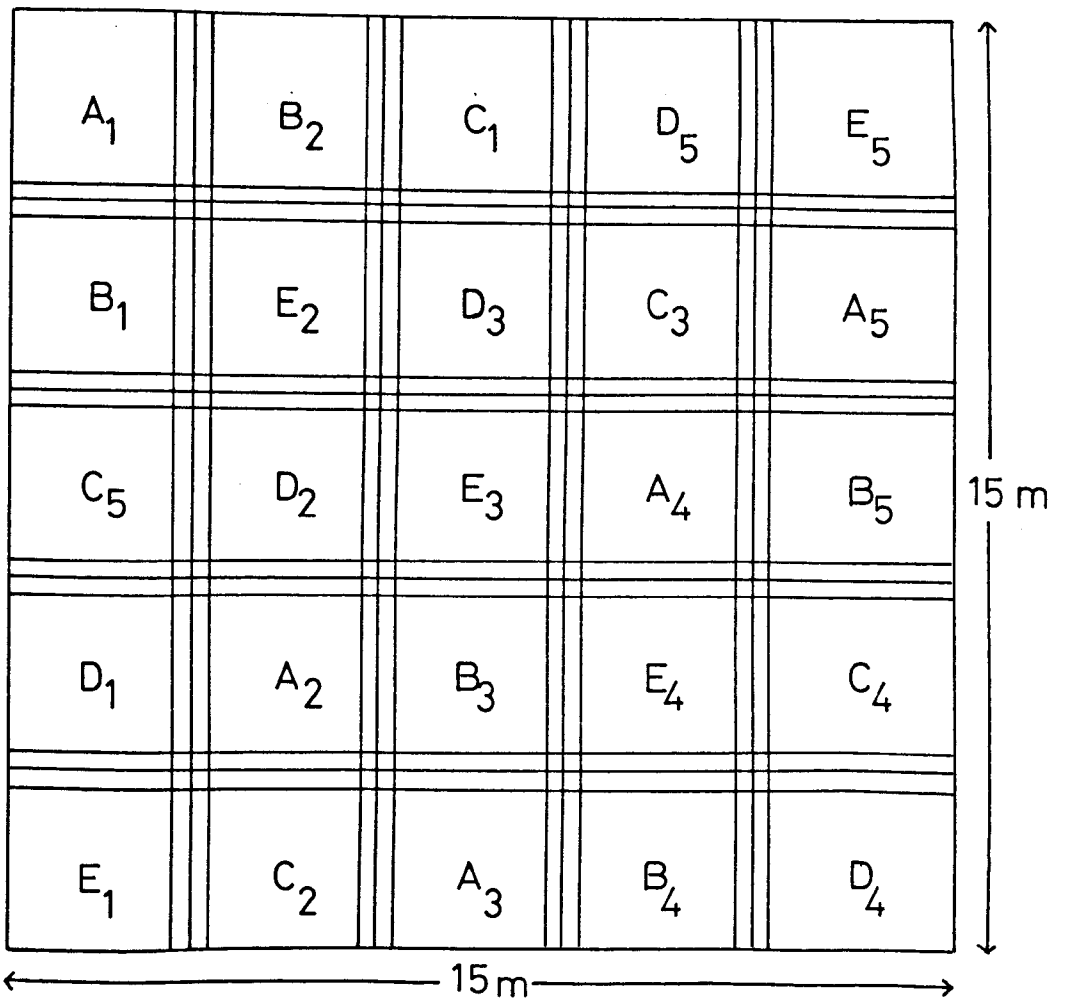
All soil was collected in sterile polythene bags, sealed, labelled and returned to the laboratory. Samples not used immediately were stored at 2°C. In the case of the swimming pool and hedgehog pens, soil was taken from the top 10 cm of the profile. The soils that had been treated with fungicides were collected in cores 20 cm long using an auger to obtain them.

At the swimming pool the samples were collected from 7 sections across the main grassy patch at random; one further sample was taken from behind the diving board. Samples were taken in the 8 hedgehog pens along the running tracks of the hedgehogs. Two "controls", which were not under the influence of the biotic factors, were taken for each of these 2 sampling sites from outside the "experimental" area.

Numbers of people attending the swimming pool were recorded and the numbers of hedgehogs in the pens each month were noted. Soil temperature was measured every month when samples were collected from these two sites. Rainfall data were obtained from the Department of Geography, Nottingham University.

A latin square was laid out at Sutton Bonington. The plot consisted of a square 15 x 15m divided into 25 3 x 3m subsquares. Fungicides were applied in suspension and each treatment occurred once in each row (Figure 6). Plots were sampled by placing a grid on each 3m square. Numbers taken

FIGURE 6



A = Milcol

B = Dicloran

C = Captan

D = Triarimol

E = Control

KEY

D = Dicloran

Q = Quintozene

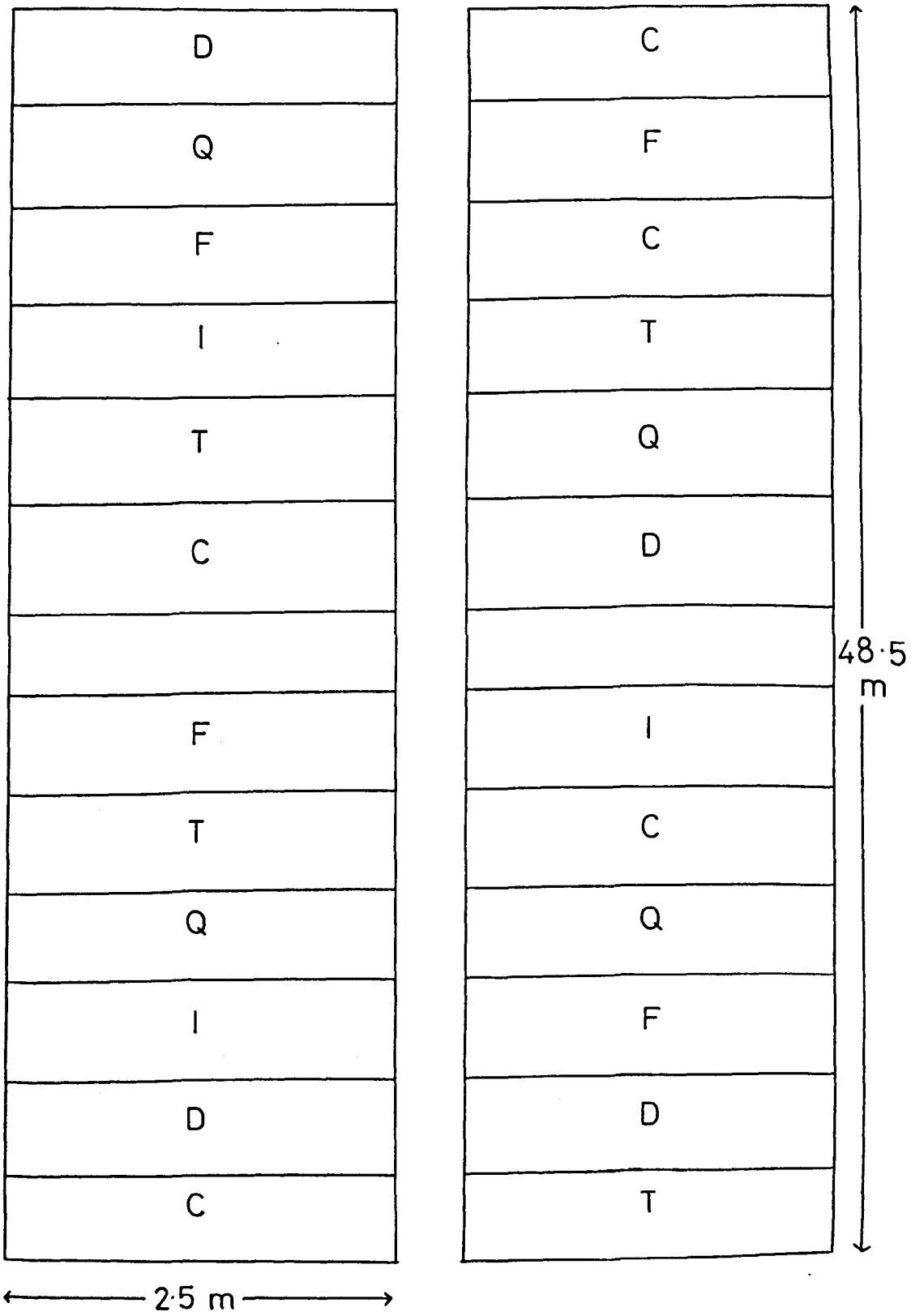
F = Formaldehyde

I = Control

T = Thiram

C = Captan

FIGURE 7



from tables of random numbers (Fisher and Yates, 1963) were then used to locate a point in the 3m square. Three random cores per 3m square were taken, making a total of 15 samples per treatment.

A split plot design was used at Rothampsted. Four replicates of each treatment were used (Figure 7). Polythene bags were thrown randomly and soil samples were collected from each point in a sectorwhere a bag landed, i.e. 16 samples per treatment in all.

The collection of soils of different pH value was made from samples of surface soil from the length of each plot. The samples were collected separately, at random and placed in a bucket, mixed and then subsamples were taken and placed in polythene bags, labelled and taken to the laboratory.

To collect nest material from the hedgehogs' nesting boxes sterile polythene bags were turned inside out (English and Morris, 1969), a handful of hay gathered and the bag was then turned the correct way over the material so that it was untouched by hand.

Hedgehogs were picked up and dropped from a height of one foot onto a large sheet of paper. They were also rubbed with thick gloves and scratched with unbent paper clips, to obtain quills and scales.

Students were chosen randomly from the first year intake and they were asked to give a sample scraping of skin from between the 4th and 5th toes on both feet. A fresh wooden spatula was used each time to take the sample. Although some workers use a scalpel to take the sample and frequently draw blood in doing so, this method was not used because of lack of

medical supervision. The skin particles were collected on black paper which was folded and placed in an envelope, upon which details of collection were noted. The envelopes were taken to the laboratory and if not used immediately were kept at 2°C. All students who gave samples of skin, filled in questionnaires designed to establish hygiene standards and social behaviour.

Laboratory procedure

All soil samples were passed through a 2 mm sieve and a layer of this soil was poured into 6 9 cm petri dishes for each sample, with 10 for the soils of different pH values. The soils were kept moist with sterile distilled water. Half the plates were baited with 1 cm portions of degreased Animal Wool, B.P. and half with greasy wool. All plates were incubated at 25°C and examined every week for 4 weeks. Presence of fungi on the wool baits was recorded for the swimming pool and hedgehog pen soils. When the biotic factors were present at these sites, percentage colonisation of the wool by each fungus was determined by counting the fungi on ten hairs picked randomly from the soil surface from each petri dish. This method was used for the soil samples from all the other collecting sites. Hairs were examined in lactophenol mounts. Isolates were cultured on Sabouraud Dextrose agar to confirm identification. Soil crumb plates (Warcup, 1950) were set up for the swimming pool and hedgehog pen soils and soils treated with fungicides. A 0.1 g crumb of soil was placed in 6 9 cm petri dishes and covered with 15 ml S.D.A. supplemented with 40 mg/l chloramphenicol and 500 mg/l cycloheximide. These plates were agitated to disperse the soil particles and when the agar had solidified the plates were placed at 25°C and 35°C (half the plates at each temperature) and studied for two weeks.

Hedgehog nest material was cut into 3 cm pieces and 5

pieces, picked randomly, were placed on each of four plates of supplemented S.D.A. Two plates were incubated at 25°C and 2 at 35°C. The quills and scales were plated out and incubated in the same manner.

The skin samples from students were plated out onto malt extract agar (Gentles and Dawson, 1956), supplemented with chloramphenicol at 40 mg/l and cycloheximide at 500 mg/l plus ink blue at 50 mg/l (Baxter and Cooper, 1964; Quaife, 1968). The plates were incubated at 25°C for 5 weeks to allow development of dermatophytes even if they were initially inhibited by yeasts which appear early after inoculation. Plates were examined daily for evidence of decoloration of the blue medium. Cultures were taken from these decoloured areas and identified. (Figure 8). A microscopical study was not made of the skin samples because there was insufficient material.

Cycloheximide and chloramphenicol were used to eliminate bacteria and unwanted soil saprophytes. Chloramphenicol is preferable to streptomycin and penicillin because it can be autoclaved.

Soil measurements

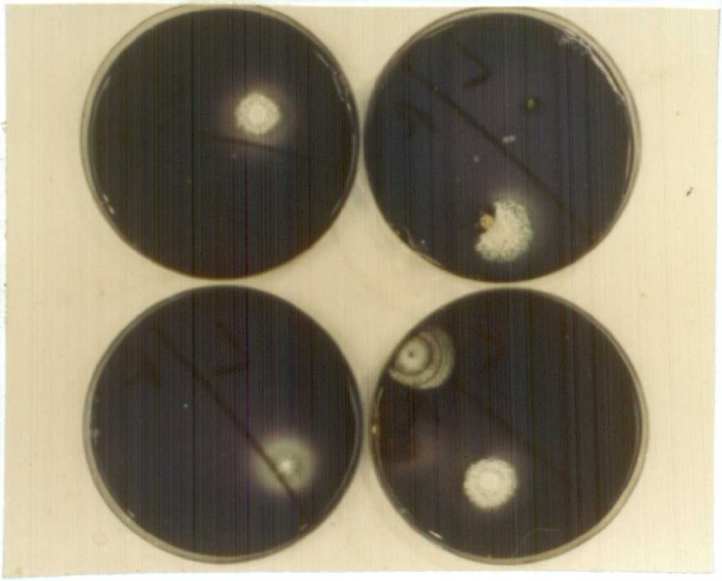
a) pH

The pH of all soils was measured. One part of sterile distilled water (pH 7) was added to one part of soil in a McCartney bottle, shaken for one hour and then left to stand. The pH of the liquid was measured using a Pye Unicam pH meter. pH was recorded for all soil samples from the swimming pool and hedgehog pens every month, whilst that of the soils treated with fungicides was recorded once, at the beginning of the study period.

b) water content

Water content of the swimming pool soils and

FIGURE 8



Decolorisation of ink blue medium

hedgehog pen soils was measured. A known weight of soil was placed in a weighing bottle, placed in an oven at 100°C, allowing water vapour to escape. The bottle was reweighed after 12 h, after it had been cooled in a desiccator. The water content was calculated as percentage dry weight.

c) loss on ignition

Loss on ignition of swimming pool and hedgehog pen soils was measured. The oven dry soil from the water content determination was placed in a crucible after being weighed. It was then placed in a muffle furnace and ignited at 350-400°C for 7-8 h. The crucibles were allowed to cool in the oven and then in a desiccator before being reweighed. Loss on ignition was calculated as percentage oven dry soil. This result represents a combination of organic matter and $CaCO_3$.

Another measurement was made for organic carbon alone.

d) organic carbon content (Walkley and Black, 1934)

Estimation of organic carbon was made using a method which utilises a rapid titration procedure following wet digestion. An 0.25 g portion of finely ground, air-dry soil was placed in a 500 ml, wide-mouthed conical flask. Ten ml of 1N potassium dichromate was added. Then 20 ml of concentrated sulphuric acid was carefully run in. This mixture was shaken for 1 min. and then left for ½ h. Then 200 ml of distilled water was added, plus 10 ml 85% phosphoric acid and 1 ml of diphenylamine indicator. The chromic acid unreduced at the end of this process was titrated with standard ferrous sulphate from a burette until the purple colour changed to green. A calculation of the amount of carbon which has been oxidised is made using the formula

$$\frac{V_1 - V_2}{W} \times 0.003 \times 100$$

where V_1 = volume of 1N potassium dichromate

V_2 = volume of 1N ferrous sulphate

W = weight of soil taken

This measurement was expressed as a percentage of the soil weight.

RESULTS

All fungi isolated by the hair baiting technique were recorded, but since the work concentrated on keratinophilic species only those results have been presented in this section. The other species of fungi isolated can be found in the Appendix I.

Isolates were named according to the form in which they were recorded unless mating experiments of imperfect forms with known strains demonstrated that they were typified by a perfect state. In which case the perfect state name was used, e.g. conidial Arthroderma uncinatum was the name used for the imperfect strain of this fungus and cleistocarpic A. uncinatum for the perfect state. If however, it was not known whether the imperfect state was typified by a perfect state, e.g. in the literature, then Trichophyton ajelloi was the name used. Only imperfect strains of the other keratinophilic fungi were isolated and they were named as such, i.e. Trichophyton terrestre, Chrysosporium keratinophilum, Microsporum cookei and M. gypseum, since it was not known if they were typified by a perfect state.

Ajello (1968) discussed the taxonomic position of Keratinomyces ajelloi Vanbreuseghem and transferred it to Trichophyton because of the affinities with the perfect state Arthroderma. Trichophyton has been used in this study, although it is recognised that there are many published papers using the name K. ajelloi. Further doubt on the true position of this species centres around serological and immunological affinity between Microsporum species and K. ajelloi discovered by Shatik (1965) and Rogers (1968). Malkina (1968) and Arievich and Stephanischeva (1963) also considered that T. ajelloi should be transferred to Microsporum on the grounds of similar biological

and morphological features.

Growth of hyphae on wool baits could be seen within a few days of the soil being sprinkled with the keratin. However, sporulation was not normally seen until after 7 days and these early observations have been recorded only as sterile hyphae in the first week. These results have not been plotted on the histograms on occurrence of keratinophilic fungi.

The seasonal distribution of the keratinophilic fungi isolated from the swimming pool and the hedgehog pens is shown in the histograms (Figures 9 - 20). Figures 21 and 22 show the mean pH of the soil at the time the soil samples were collected and Figures 23 and 24 show the numbers of people and hedgehogs present at the sites throughout the year. The seasonal rainfall and soil temperatures at University Park, Nottingham i.e. the place encompassing the two sampling sites, can be seen in Figures 25 and 26.

Highfield's swimming pool

Experimental soils refer to those from inside the sampling area and control soils refer to those obtained from outside the area.

The conidial state of Arthroderma uncinatum was the most abundant keratinophile isolated at the swimming pool. It was present in at least 50% of the soil samples throughout the year. This species was present in 85% of the total soil samples baited with degreased wool and in 75% of those baited with greasy wool. Conidial A. uncinatum was present in all soil samples in April and then its occurrence fell off gradually until September when another peak was seen. This slight decrease in the isolation rate corresponded with the time that the pool was open to the public.

There was little correlation between presence of conidial A.uncinatum and pH, rainfall or soil temperature, although at

degreased wool

greasy wool

Figure 9 A. uncinatum (conidial)

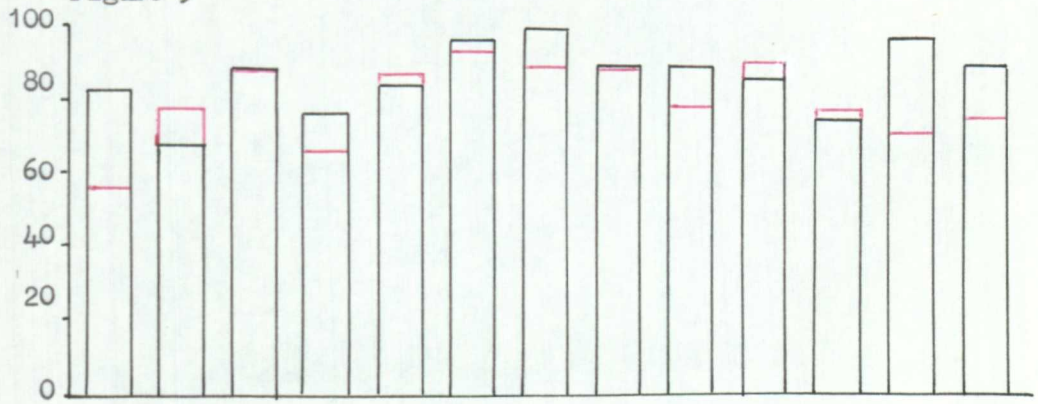


Figure 10 C. keratinophilum

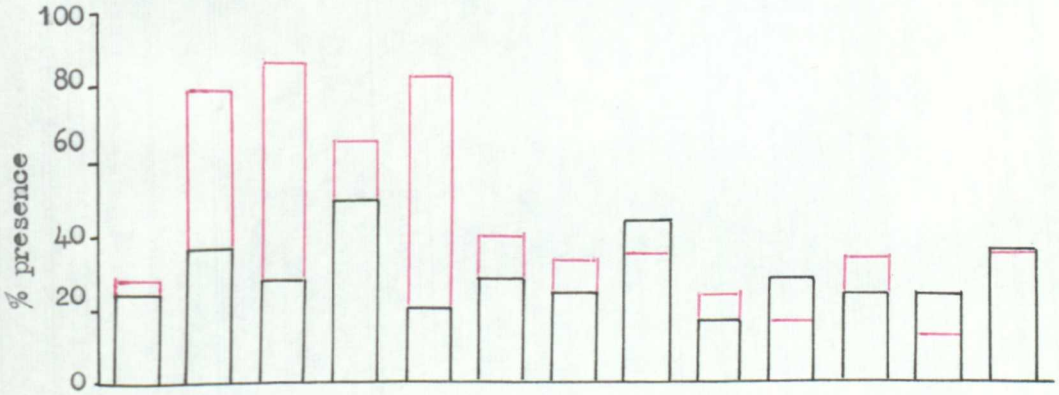
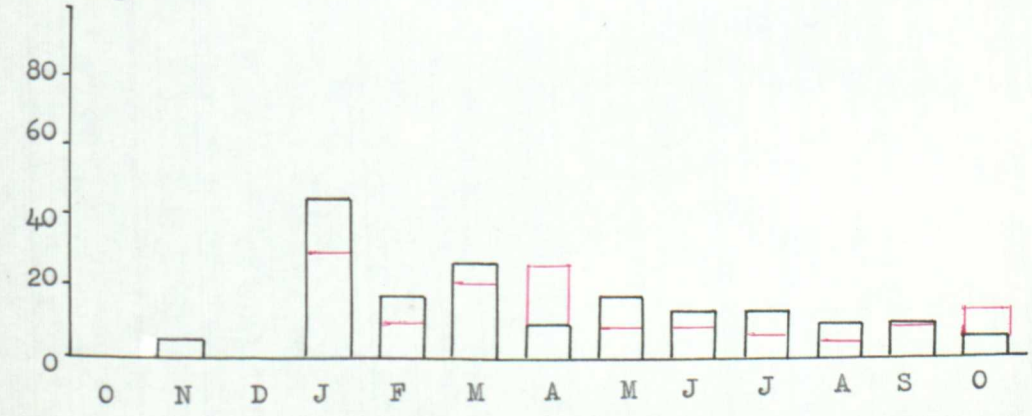


Figure 11 M. cookei



Seasonal distribution of keratinophilic fungi at Highfield's swimming pool

degreased wool

greasy wool

Figure 12 T. terrestre

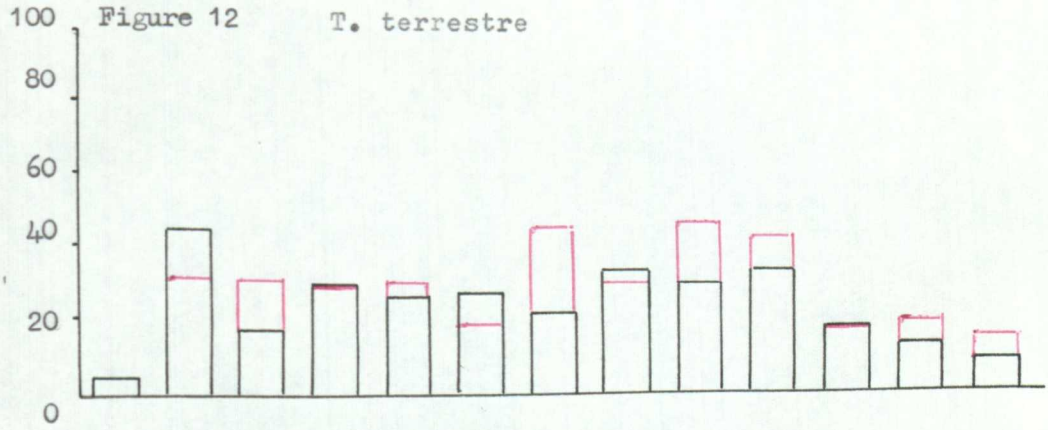


Figure 13 A. uncinatum (cleistocarpic)

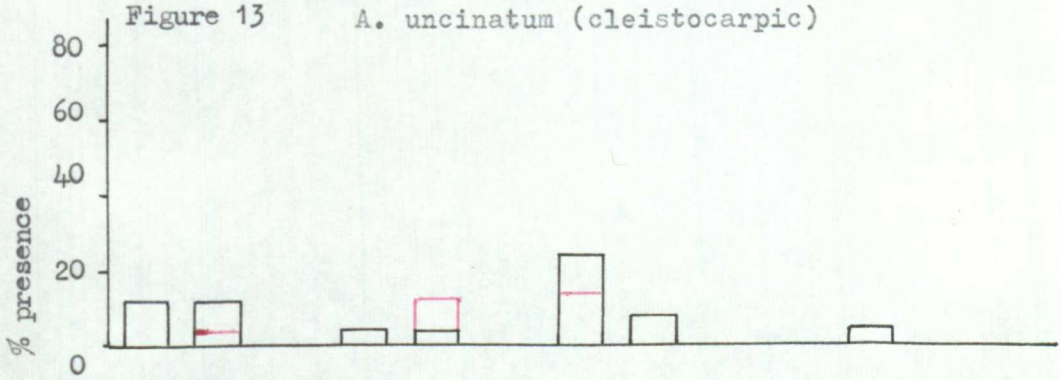
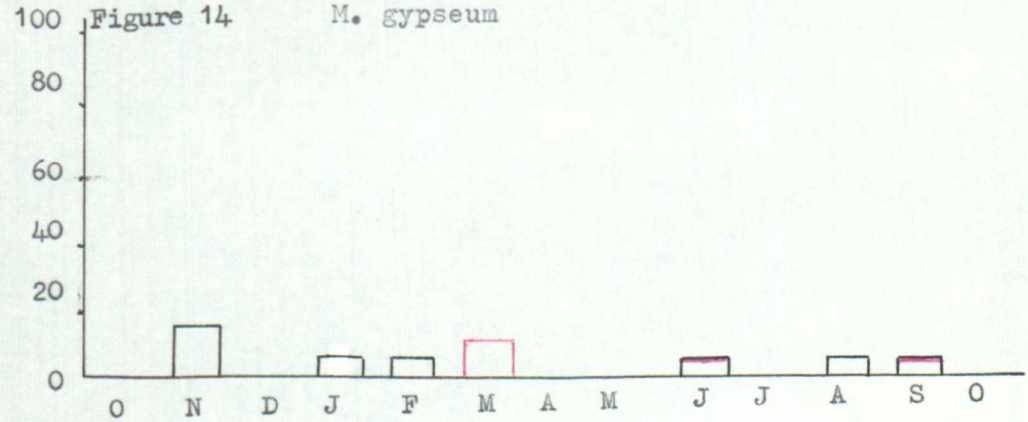


Figure 14 M. gypseum



Seasonal distribution of keratinophilic fungi at Highfield's swimming pool

degreased wool

greasy wool

Figure 15 A. uncinatum (conidial)

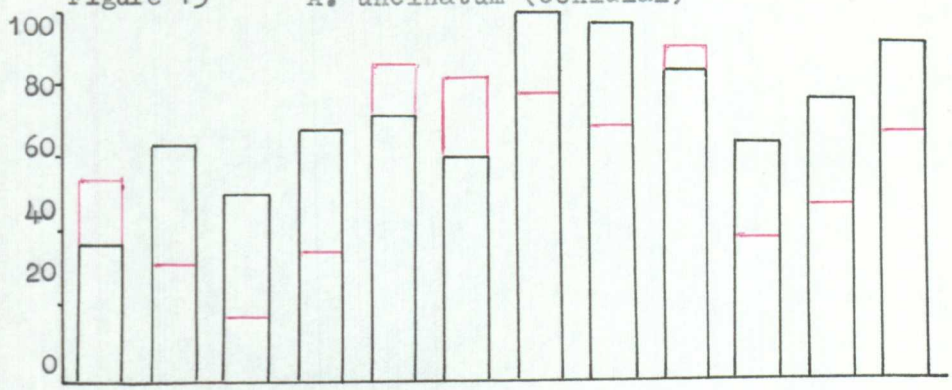


Figure 16 C. keratinophilum

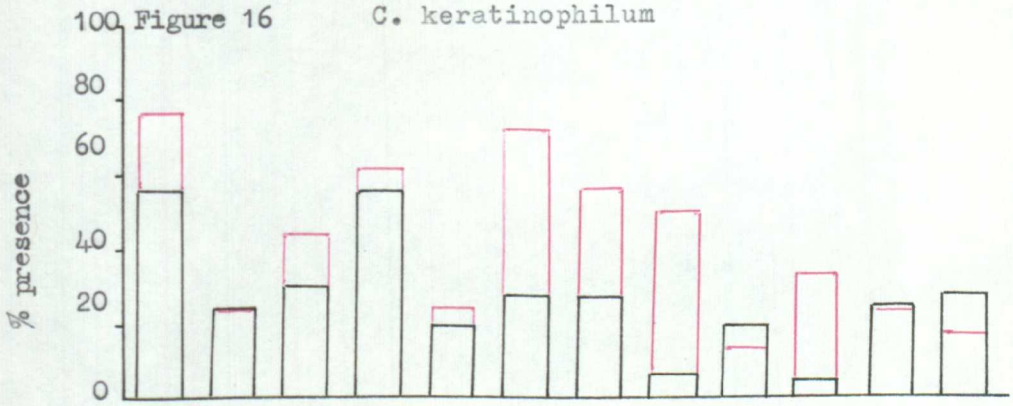
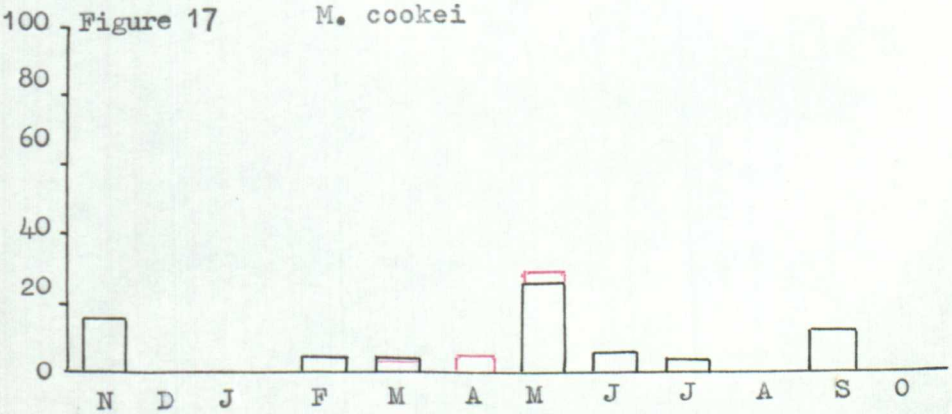
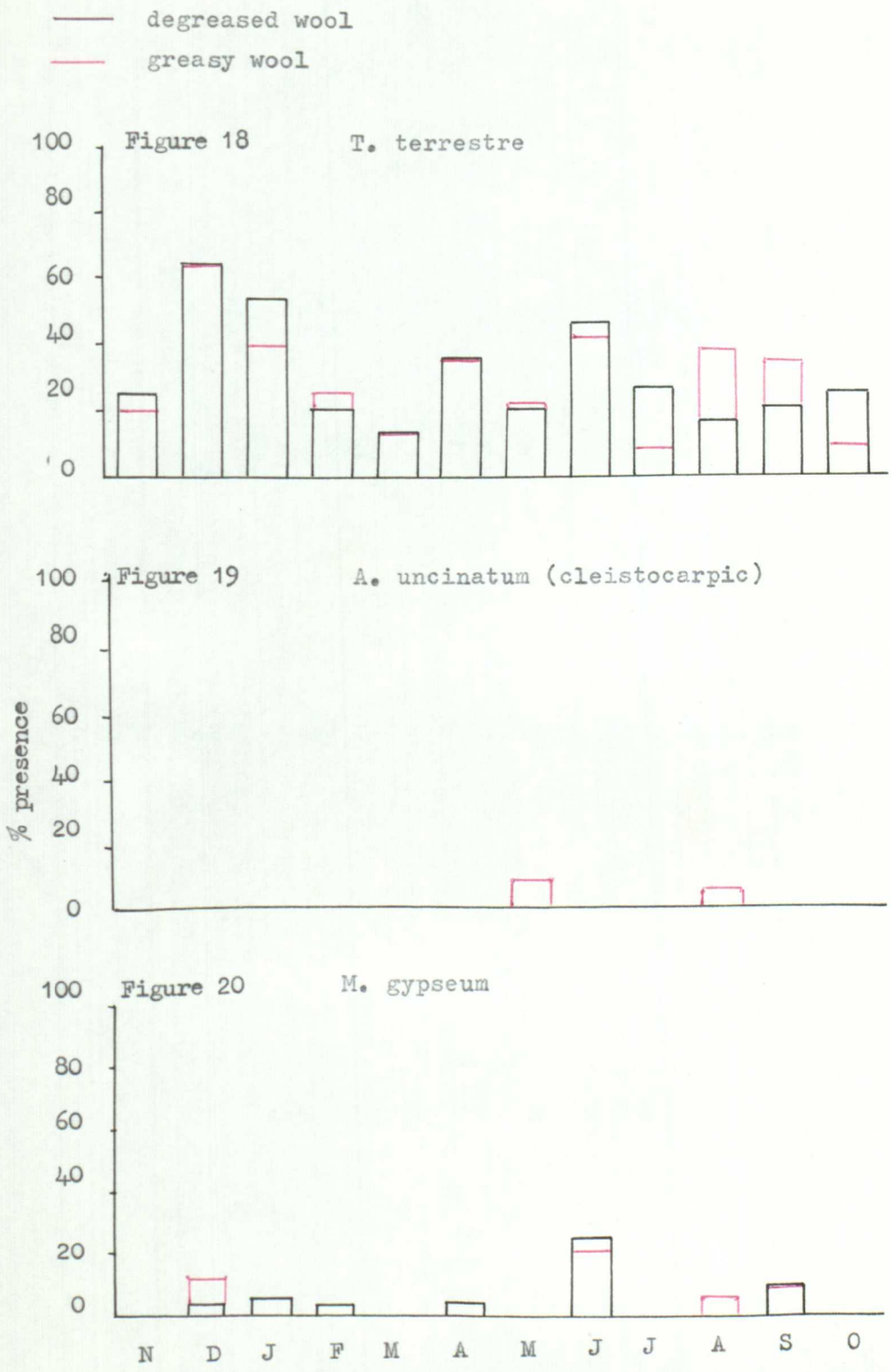


Figure 17 M. cookei



Seasonal distribution of keratinophilic fungi at the hedgehog pens



Seasonal distribution of keratinophilic fuhgi at the hedgehog pens

Figure 21

Seasonal pH of soils at Highfield's swimming pool

Figure 22

Seasonal pH of soils at hedgehog pens

Figure 23

Numbers of people attending Highfield's swimming pool

Figure 24

Numbers of hedgehogs in pens

Figure 21

Figure 22

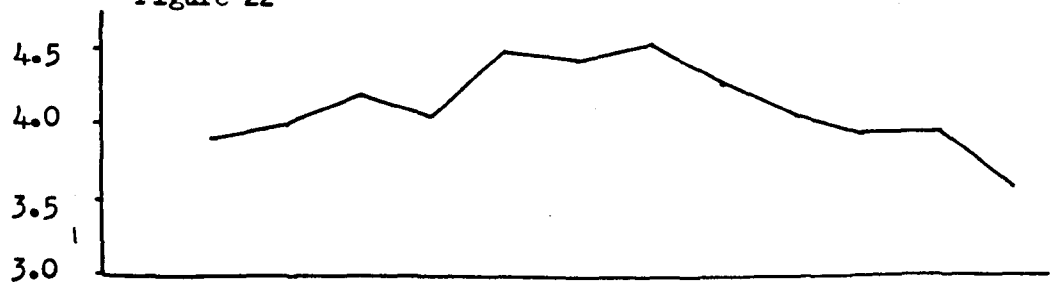


Figure 23

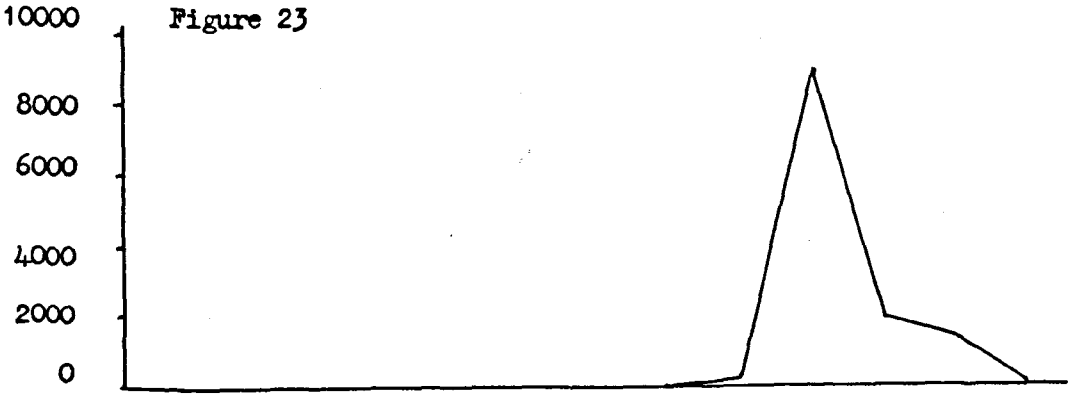


Figure 24

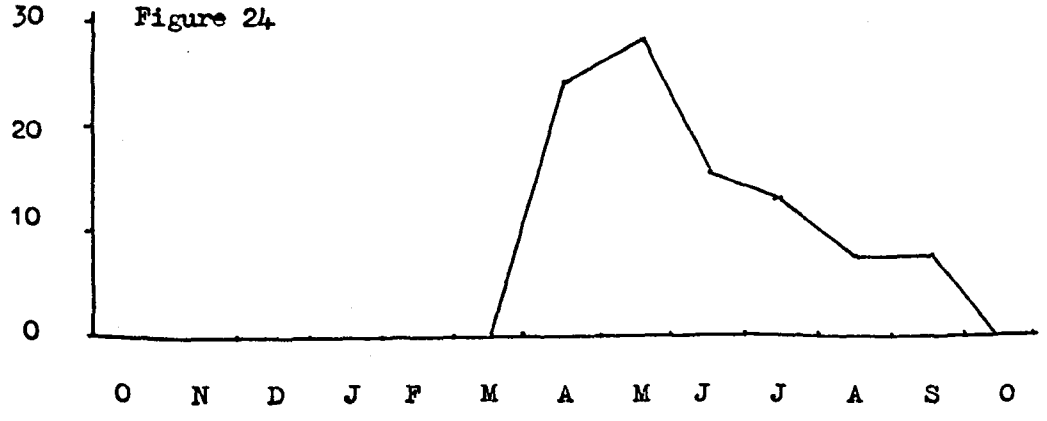
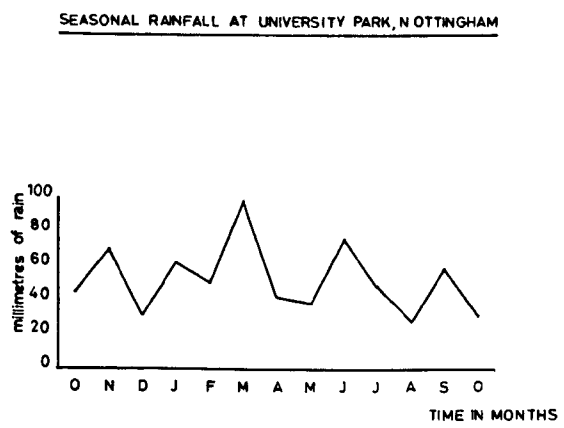


FIGURE 25



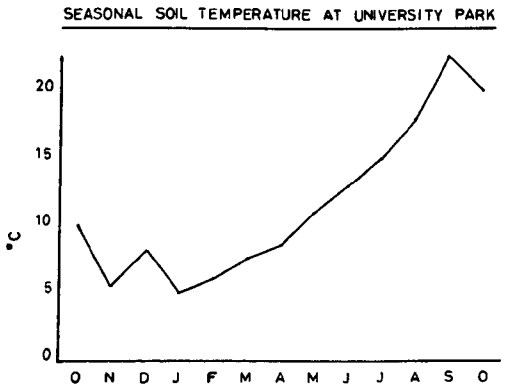


FIGURE 26

soil temperatures around 8°C isolations of this species were at their lowest (50 - 65%).

Arthroderma uncinatum cleistocarpic form was isolated occasionally from the swimming pool soils (2.4% mean presence on degreased wool and 1.2% on greasy wool). There appeared to be no correlations with physical or edaphic factors measured. The maximum percentage presence was 12% in November.

Chrysosporium keratinophilum was isolated from 15% or more of the soils throughout the year. Mean presence on degreased wool was 29% and on greasy wool 43%. There was a peak in winter (85%) but in general the distribution of this species was fairly even. The only general correlation between C. keratinophilum and any physical factors was that the highest percentage presence (>60%) occurred during the winter months and the lowest during the summer.

Trichophyton terrestre was isolated in small numbers from the soils throughout the year. The mean presence on degreased wool was 22% and on greasy wool 25%. There were no obvious maximum or minimum peaks, but the lowest isolations (August to October, 15 - 4%) were at the higher soil temperatures of 18°C to 23°C and there was a generally higher level of isolation from November to June, when the soil temperatures were lower.

Microsporum cookei was, in general, also evenly distributed during the year, but with an almost complete fall off in autumn and early winter; months with low rainfall (October, December, April, August and October), with the exception of November and September, showed lowest percentage presence. The mean presence of M.cookei was 12% on degreased wool and 10% on greasy wool. The maximum and only peak was in January (44%).

M. gypseum was rarely isolated. Its mean presence on degreased wool was 5.3% and on greasy wool 2.1%.

All the keratinophilic fungi recorded, except T.terrestre and C. keratinophilum, were isolated more frequently on degreased than greasy wool. There was no evidence of one species being present when another was absent and vice versa.

Hedgehog pen soils

Conidial A. uncinatum was most frequently isolated, as it was at the swimming pool, but on this occasion the percentage presence was a little lower (mean presence on degreased wool was 72% and 56% on greasy wool) and the maximum peak of 100% occurred a month later, in May. There was a high percentage of conidial A. uncinatum from May to July when the hedgehogs were in the pens.

Cleistocarpic A. uncinatum had a mean presence of 4.1% on degreased wool and 3.7% on greasy wool. The maximum percentage isolation of this species was in June (25%) just after the maximum number of hedgehogs, but no other correlations were obvious. There was also an increase in incidence in the late spring and early summer of C. keratinophilum (24% up to 74%), M. cookei (6% to 26%) and M.gypseum (0% to 8%) which coincided with the maximum number of hedgehogs in the pens. There was a decrease in conidial A. uncinatum, C. keratinophilum, M. cookei and M. gypseum when the hedgehogs were removed from the pens. T. terrestre showed a converse correlation with the presence of hedgehogs, i.e. it had a higher incidence (64%) in the absence of the hedgehogs than in the presence of them (20%). The mean presence of T. terrestre was 30% on degreased wool and 28% on greasy wool.

The mean presence of C. keratinophilum was 27% on degreased wool and 41% on greasy wool.

Conidial A. uncinatum was isolated less when soil temperatures were below 8°C (52%) whereas T.terrestre was generally isolated more between 5°C and 15°C (in December,

January, April and July) than when soil temperatures were above this. Both these observations agreed with those made for the swimming pool soils.

The mean presence of M. cookei was 6.3% on degreased wool and 4% on greasy wool. Results for M. cookei were similar to those made for this species at the swimming pool, i.e. the greatest percentage presence was when there was high rainfall, with the exception of May, but in this month the most hedgehogs (28) were in the pens.

Microsporum gypseum was isolated only very rarely from the hedgehog pens (mean presence was 0% on degreased wool and 1% for greasy wool). The highest incidence of M. gypseum, which was 8% in May, was when the pH of the soils was at its highest, i.e. pH 4.5. At the swimming pool, the pH was in a range 2 units above those of the hedgehog pens and this species was more frequently isolated. No correlation was noted between higher M. gypseum presence and lower soil temperatures as it was at the swimming pool, but low rainfall in May and August coincided with the only occasion M. gypseum was isolated.

Rainfall and soil temperatures

The driest months were December, April, May, August and October, and the wettest were March, July and November.

The soil temperature was low in November and January, with a slight rise in December, and it steadily rose from 5°C to 25°C from January to September, after which a decrease in temperature was noted.

Edaphic factors

Tables 2 and 3 show the mean soil measurements for the swimming pool and hedgehog pen soils and the mean percentage presence of the keratinophilic fungi at the 2 sites.

TABLE 2

Mean soil measurements

Highfield's swimming pool	Water content	Loss on ignition	Organic carbon	pH
Experimental soils	30.41%	8.19%	4.54%	6.32
Control soils	20.70%	6.47%	2.80%	6.56
Hedgehog pens				
Experimental soils	20.88%	4.44%	2.71%	4.12
Control soils	20.05%	5.58%	4.52%	5.87

TABLE 3

Isolation of keratinophilic fungi

(% presence)

Highfield's swimming pool	cl. A.u	co. A.u	T.t	C.k	M.c	M.g
Experimental soils	1.8	82.2	23.2	32.5	10.5	4.0
Control soils	21.5	92.0	19.7	32.5	25.0	7.5
Hedgehog pens						
Experimental soils	3.9	64.0	28.0	35.0	6.5	0.7
Control soils	7.0	86.0	24.5	40.0	23.5	5.5

cl. A.u = cleistocarpic Arthroderma uncinatum

co. A.u = conidial Arthroderma uncinatum

T.t = Trichophyton terrestre

C.k = Chrysosporium keratinophilum

M.c = Microsporum cookei

M.g = Microsporum gypseum

Apart from the fact that pH values were higher in the control soils, where most keratinophilic fungi were isolated, no other obvious correlations were noted. However, cleistocarpic and conidial A. uncinatum, M. cookei and M. gypseum were isolated more from soils with low water content and higher pH values, whereas for T. terrestre the converse was true. C. keratinophilum was virtually isolated in the same numbers at the swimming pool experimental and control soils, but more in the controls of hedgehog pen soils. In general the distribution of this species resembles that of M. cookei, M. gypseum and both states of A. uncinatum.

Overall the keratinophilic fungi were present in greater numbers in the control soils, with the exception of T. terrestre. M. cookei, M. gypseum and cleistocarpic A. uncinatum were appreciably more in controls than experimental soils.

Individual month recordings

On degreased baits, conidial A. uncinatum was more frequently isolated from the experimental soils than the controls of hedgehog pen soils in December, June, July and August and from the experimental soils of the swimming pool in May, July, August and September. On greasy baits the differences in numbers of isolations were consistently smaller. Cleistocarpic A. uncinatum was isolated more from experimental soils on degreased wool than controls soils at the swimming pool in November, June and August and in the hedgehog pens on degreased wool in December, January, February, April, June, August and September.

With C. keratinophilum however, where more isolations occurred on greasy baits, the increases in experimental soils were greater in November, December, January and July on both degreased and greasy wool, while at the swimming pool isolations were greater in experimental soils in October, January and March.

Numbers of T. terrestre, in general, were higher in experimental soils than controls in hedgehog pens and at the swimming pool, although there were a few exceptional months when the controls yielded more isolates.

In the hedgehog pens M. cookei and M. gypseum were always higher in numbers in controls with the exception of May and January, respectively. At the swimming pool however, M. cookei was isolated more in experimental soils in odd months, showing no particular distribution, and M. gypseum was isolated more from experimental soils in November, January and August. However, both were really isolated so seldomly that detailed comparison was not possible.

Biotic factors

Figures 27 and 28 show the percentage colonisation of degreased and greasy wool baits by the keratinophilic fungi isolated from the swimming pool soils when biotic factors were present.

For all cases of conidial A. uncinatum and C. keratinophilum and all but one of M. cookei, percentage colonisation was the same or greater on degreased wool than greasy wool.

a) degreased wool

In the first 3 samples of soil from the swimming pool, after people had started attending the pool, percentage colonisation of wool by conidial A. uncinatum was lower in the experimental plots (37% - 57%) than the control (55% - 65%). However, in the fourth sample when no people were attending the pool the experimental percentage was greater (57%) than the control (53%). In general, this applied to C. keratinophilum too. Microsporum cookei and T. terrestre had low percentage colonisation throughout and no real trends could be detected.

Figure 27

Percentage colonisation of degreased wool baits on Highfield's swimming pool soils.

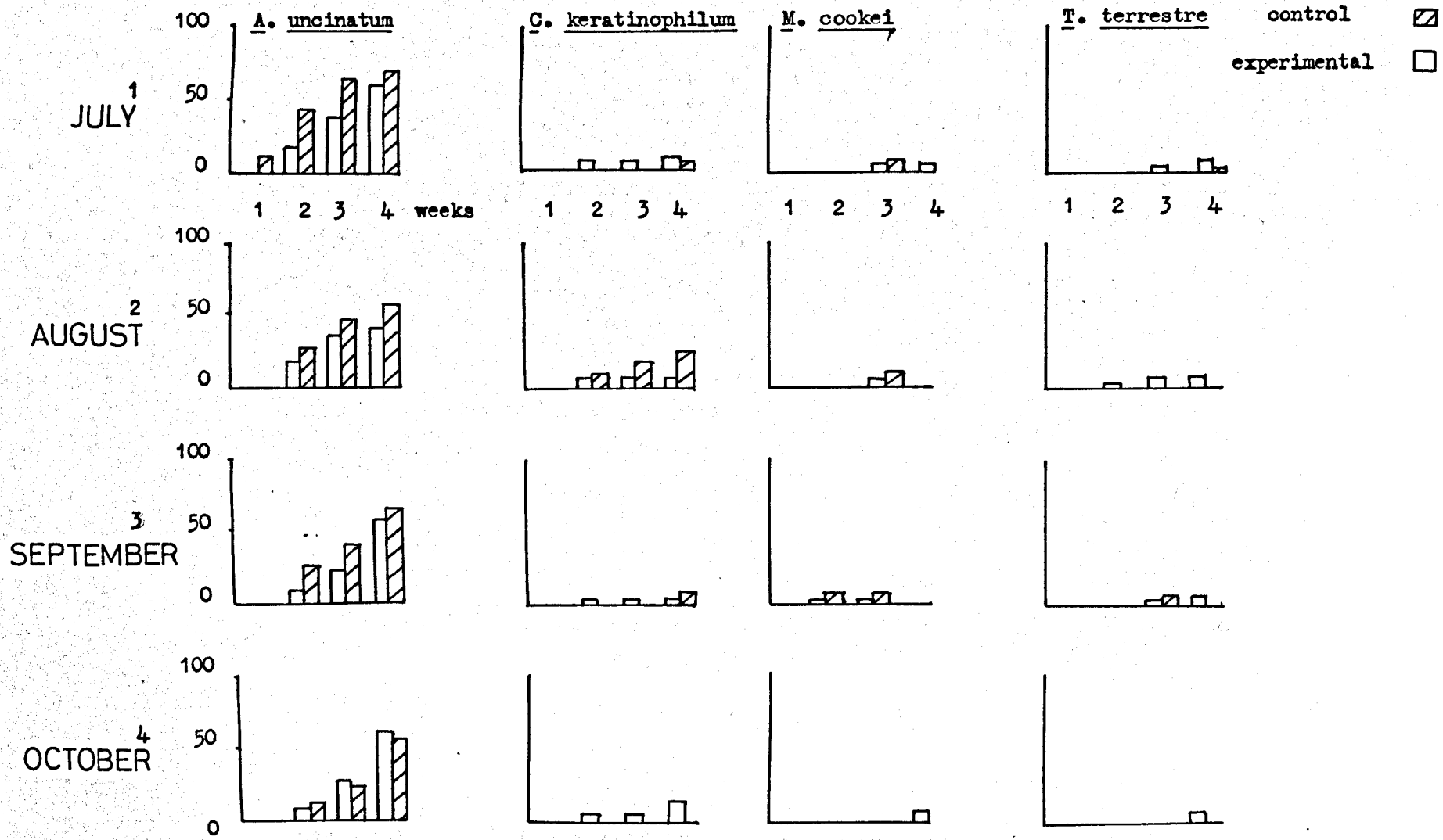
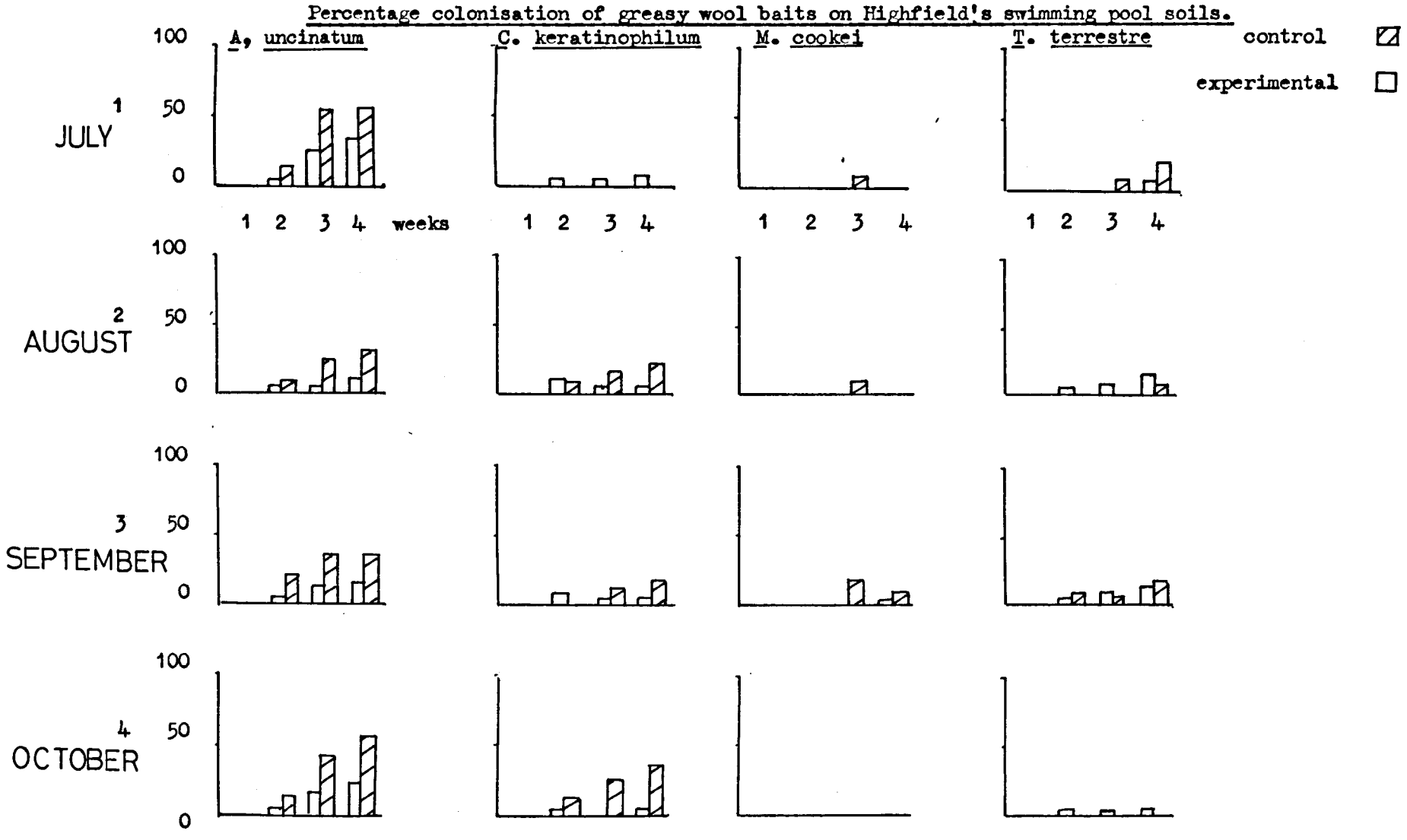


Figure 28


Percentage colonisation of greasy wool baits on Highfield's swimming pool soils.
A. uncinatum
C. keratinophilum
M. cookei
T. terrestre
control
experimental
1 JULY
2 AUGUST
3 SEPTEMBER
4 OCTOBER
100
50
0
1 2 3 4 weeks

Trichophyton terrestre was isolated, in general, more in experimental soils than controls.

b) greasy wool

In the case of greasy wool the percentage colonisation for conidial A. uncinatum and C. keratinophilum was greater in the control with the exception of the first sample where C. keratinophilum was only isolated from the experimental soils.

Microsporum cookei was isolated more from the controls than the experimental soils although in the fourth sample, no evidence of this species was seen.

The percentage colonisation of wool by T. terrestre showed no particular trends on greasy wool, although in the fourth sample it was only found in the experimental soils.

Figures 29 and 30 show that percentage colonisation of greasy wool by keratinophilic fungi from hedgehog pen soils was the same as or less than that of degreased wool for conidial A. uncinatum and C. keratinophilum. This was the case with the swimming pool soils. Percentage colonisation of greasy wool by T. terrestre exceeded that of degreased wool in the second and third soil samples. M. cookei was hardly isolated in the experimental soils baited by either wool type.

a) degreased wool

In all cases but one of baiting hedgehog pen soils, the results of the controls were higher than in the experimental soils. The exception to this was the percentage colonisation of wool by T. terrestre in the second soil sample.

The percentage colonisation of degreased wool by keratinophilic fungi fluctuated little over the four months of sampling with the exception of conidial A. uncinatum. In this instance percentage colonisation fell in the second and third samples, but increased in the fourth.

Figure 29

Percentage colonisation of greasy wool baits on hedgehog pen soils.

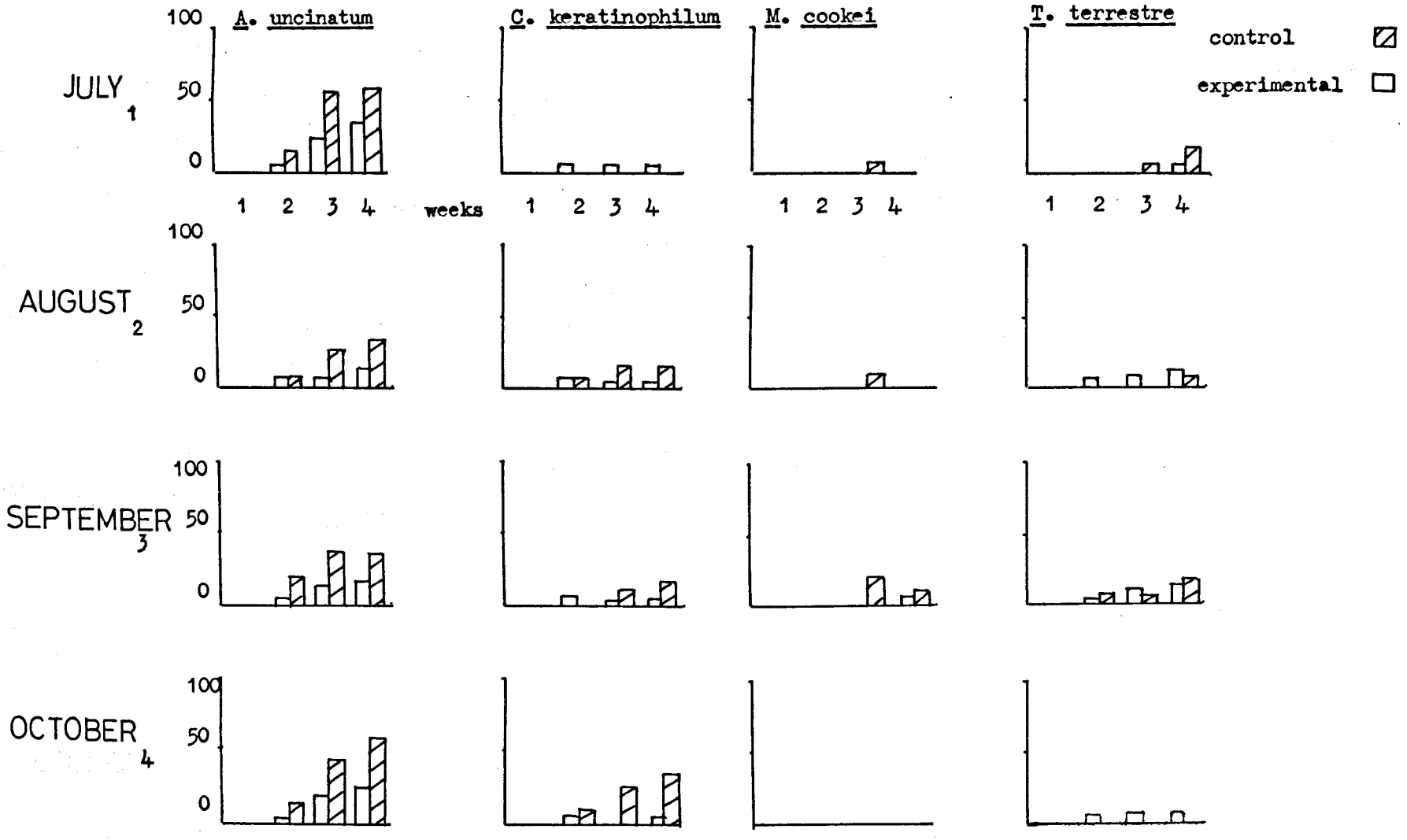
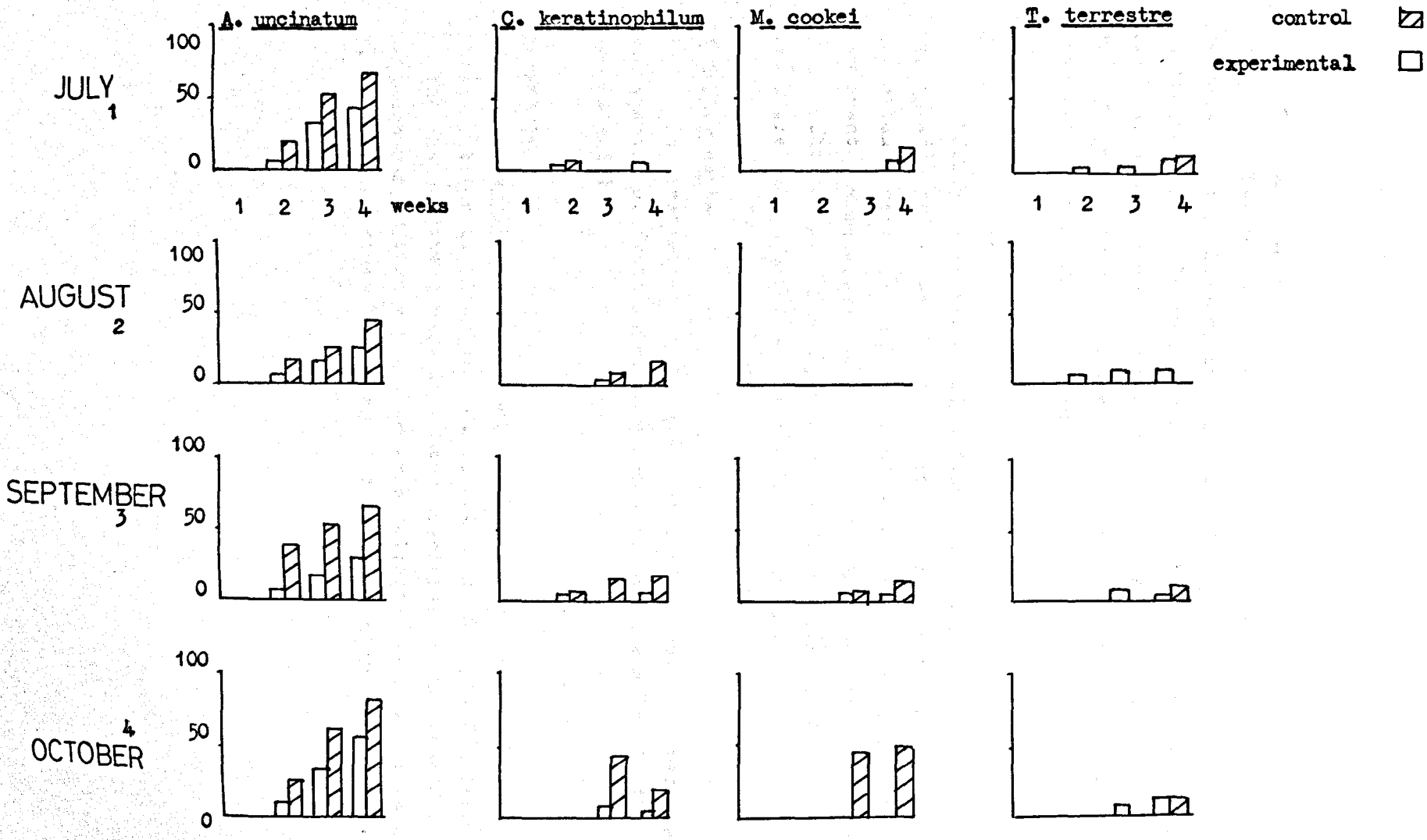


Figure 30

Percentage colonisation of degreased wool baits on hedgehog pen soils.



b) greasy wool

The course of colonisation resembled that of degreased wool, although in this case T. terrestre was isolated in the control soils in the second sample but not in the fourth, i.e. the opposite of degreased wool. In the experimental soils T. terrestre increased in the second and third samples and decreased in the fourth sample.

Soil crumb plates

Growth of keratinophilic fungi on soil crumb plates was rarely observed in any of the soils examined, but the few isolations there were can be seen in Figures 31 and 32, which represent the swimming pool and the hedgehog pens respectively.

The results of isolations on agar of keratinophilic fungi are not comparable with those by baiting.

Hedgehog nests

No keratinophilic fungi were isolated from nest material. The species of fungi which were isolated are presented in the Appendix III

Hedgehogs

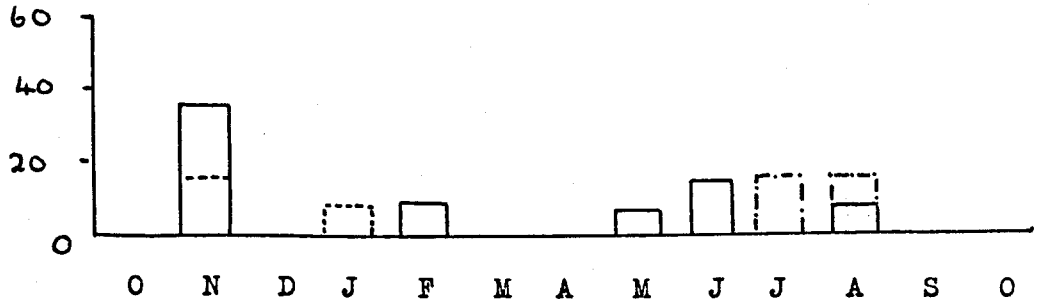
Trichophyton erinacei was isolated from only one hedgehog throughout the sampling period. It was isolated from 2 spines whilst the hedgehogs were in winter quarters. In the summer pens, although many spines and scales were collected, no isolations of T. erinacei or other keratinophilic fungi were made from them.

Seasonal percentage isolation of keratinophilic fungi

on soil crumb plates.

a) Highfield's swimming pool

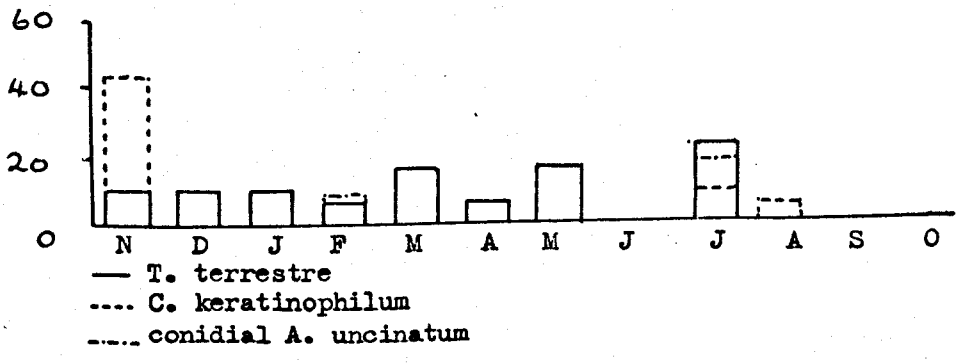
Figure 31



b) hedgehog pens

% isolation

Figure 32



Results of isolations from Rothampsted soils treated with fungicides.

Figures 33 - 36 represent percentage colonisation both of greasy and degreased wool by conidial A. uncinatum before and after treatment of soils with fungicides. With the exception of formaldehyde and greasy wool, an immediate drop in percentage colonisation was seen in all combinations. Thereafter, in general, a return in the degreased wool to approximately the level of the control or less and with greasy wool an increase compared with the control was seen.

Figures 37 - 40 show results for C. keratinophilum. The only initial fall in percentage colonisation by this fungus was with captan and formaldehyde on degreased wool and thiram with greasy wool In all the other combinations initial percentage colonisation increased and after one drop in percentage colonisation the final result showed an increase on greasy wool compared with the control and a fall compared with the control on degreased wool, with the exception of captan and quintozene.

Results of isolations from Sutton Bonington soils treated with fungicides.

The soils at Sutton Bonington were sprayed 2 days before the first sample.

Figures 41 - 44 represent percentage colonisation of degreased and greasy wool by conidial A. uncinatum. Initial percentage colonisation of degreased and greasy wool, with the exception of treatment with Triarimol, was below that of the control. As was the case at Rothampsted, the degreased wool colonisation returned to below the control and the greasy to above, or the same as, the control.

Figures 45 - 48 represent percentage colonisation by

Percentage colonisation of baits by A. uncinatum from soils at Rothampsted treated with fungicides

Greasy wool

Figure 33 - ●----● Formaldehyde

o·····o Captan

×——× Thiram

Figure 34 - Δ----Δ Dicloran

□——□ Quintozene

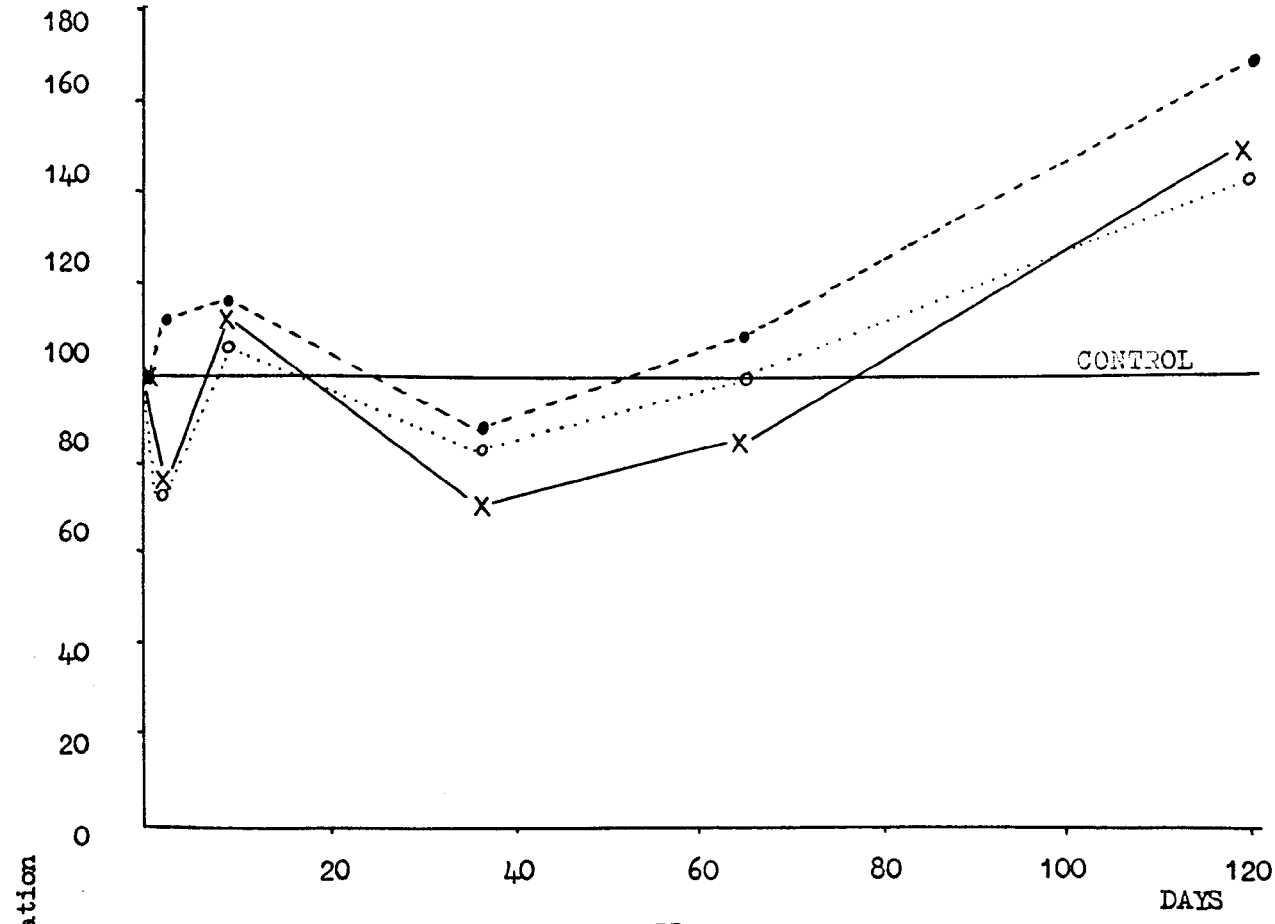


Figure 33

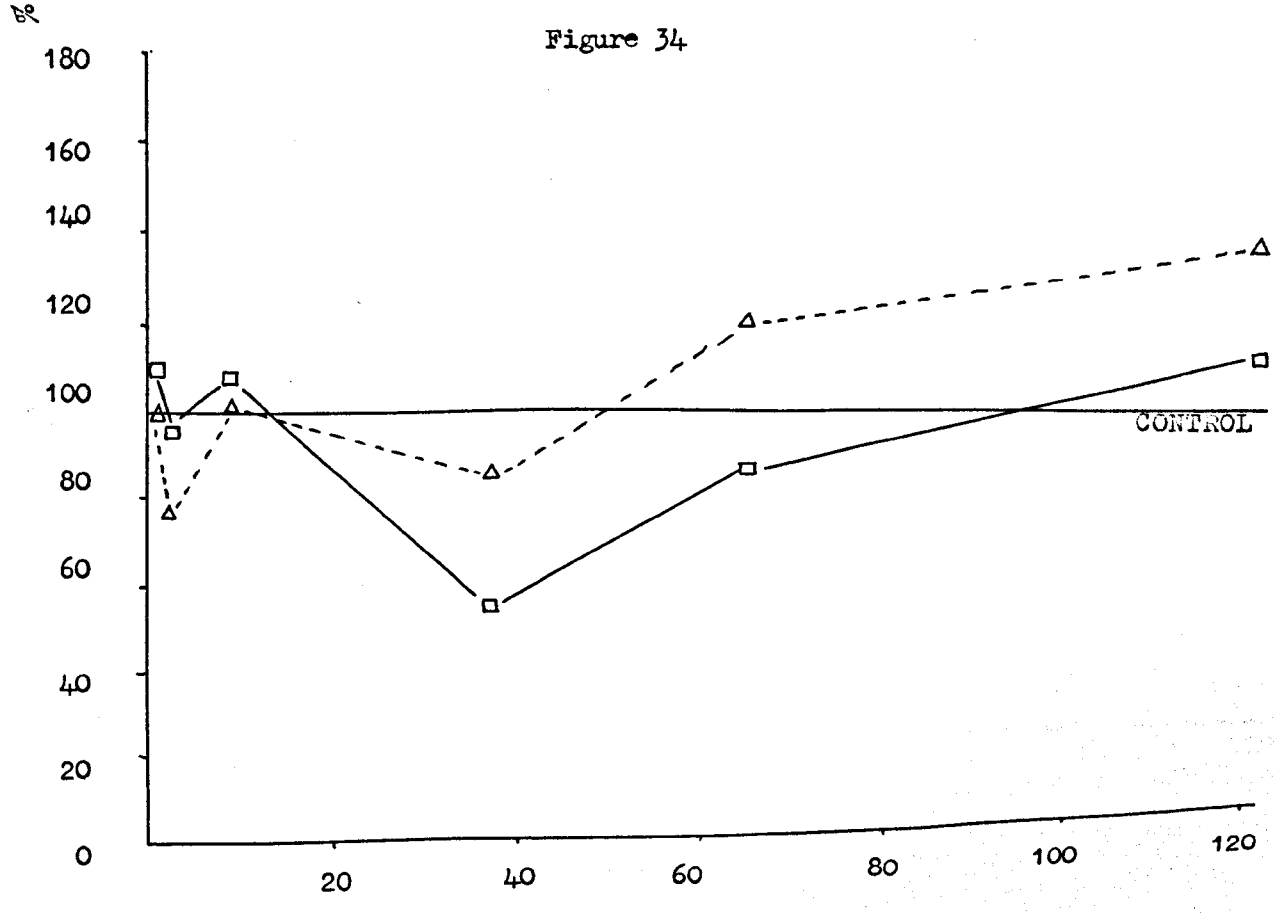


Figure 34

Percentage colonisation of baits by *A. uncinatum* from soils at Rothampsted treated with fungicides

Degreased wool

Figure 35 - ● ----- ● Formaldehyde

o ..... o Captan

x —— x Thiram

Figure 36 - △ ----- △ Dicloran

□ —— □ Quintozene

C = CONTROL

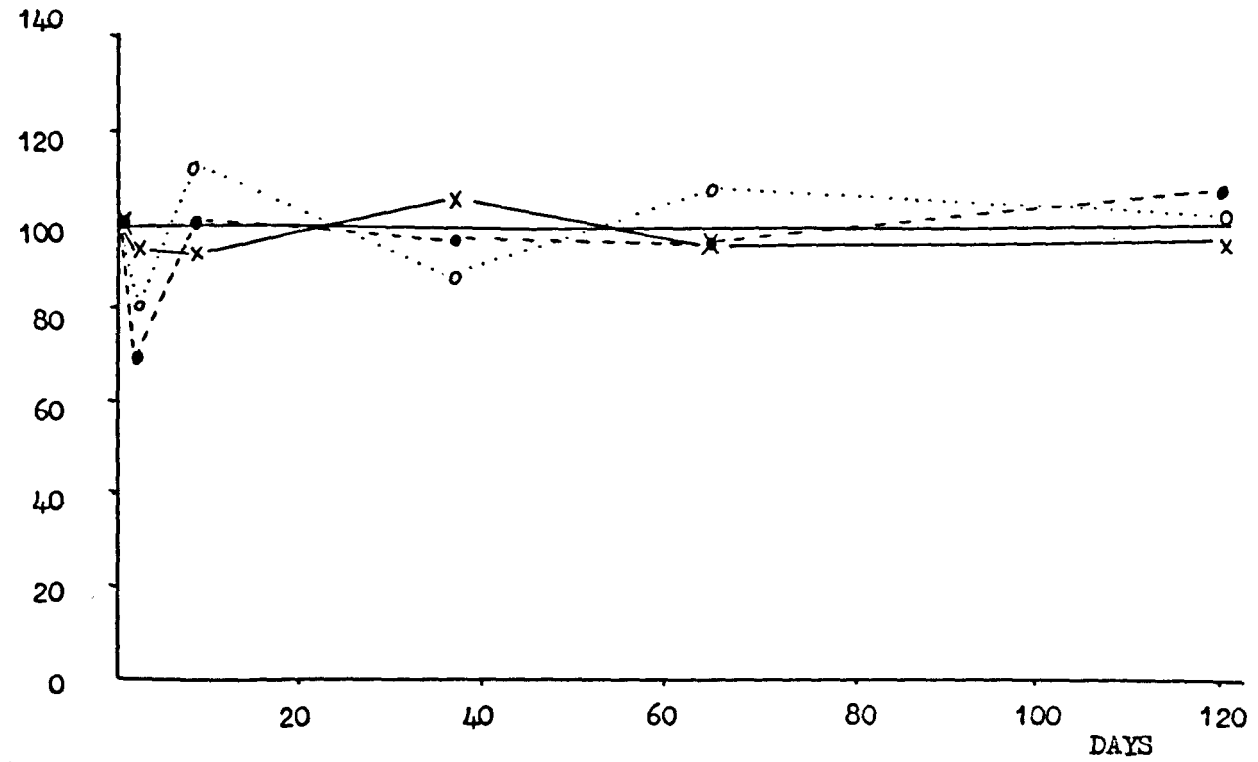
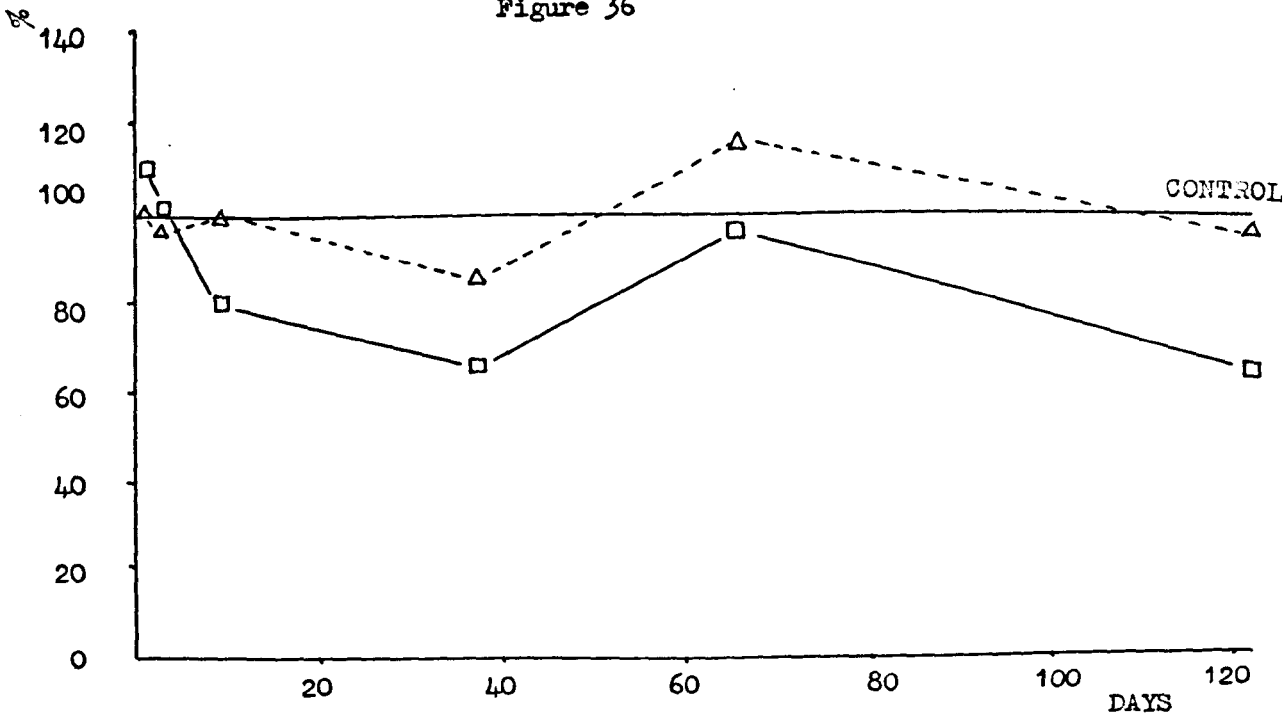


Figure 35

Figure 36



Percentage colonisation of baits by C. keratinophilum from soils at Rothampsted treated with fungicides

Degreasy wool

Figure 37 - o —— o Formaldehyde

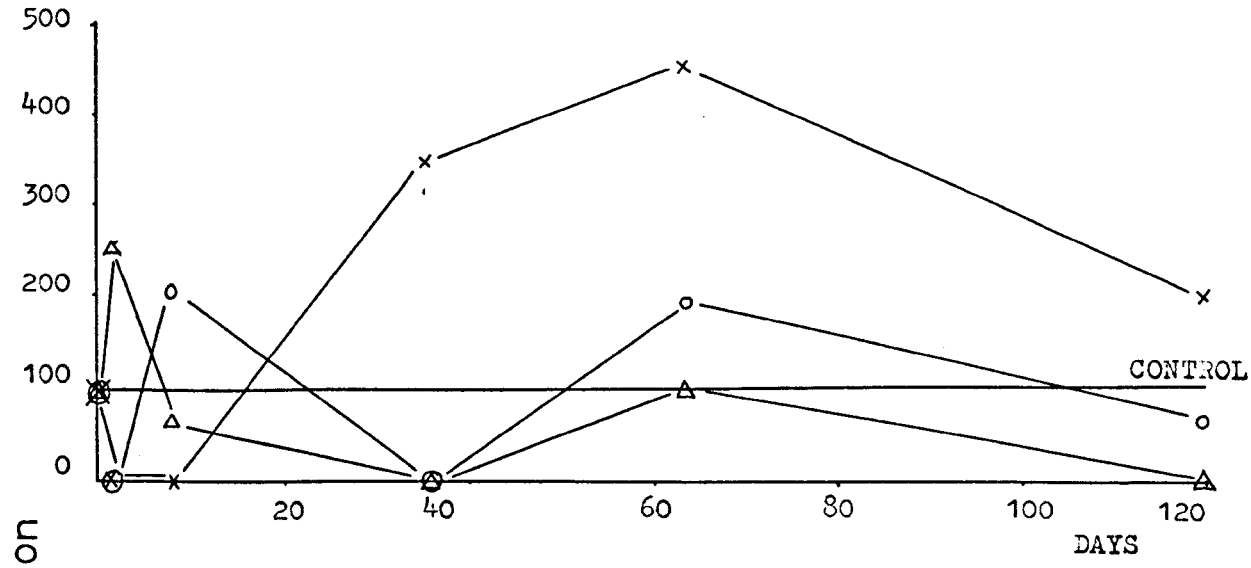
× —— × Captan

△ —— △ Thiram

Figure 38 - □ —— □ Dicloran

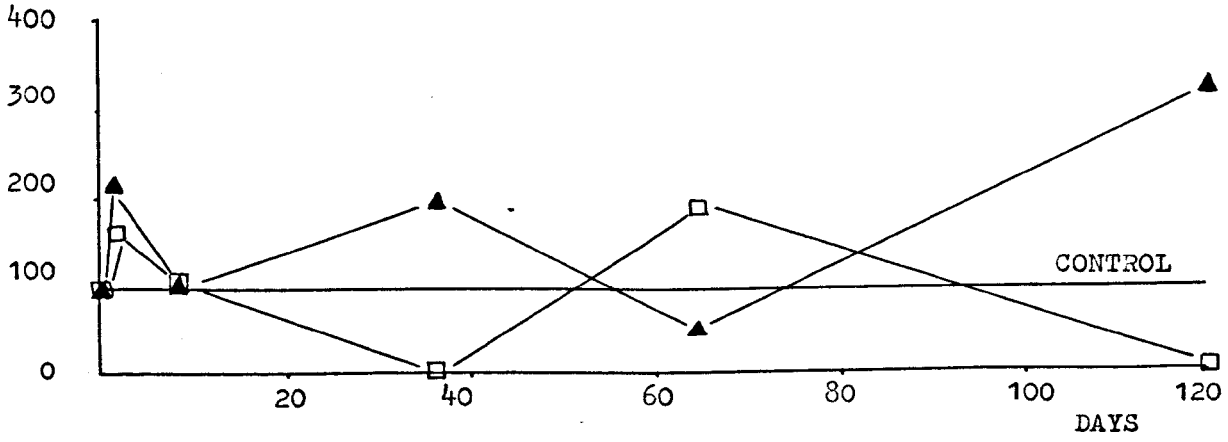
▲ —— ▲ Quintozene

Figure 37



% colonisation

Figure 38



Percentage colonisation of baits by C. keratinophilum from soils at Rothampsted treated with fungicides

greased wool

Figure 39 - o —— o Formaldehyde

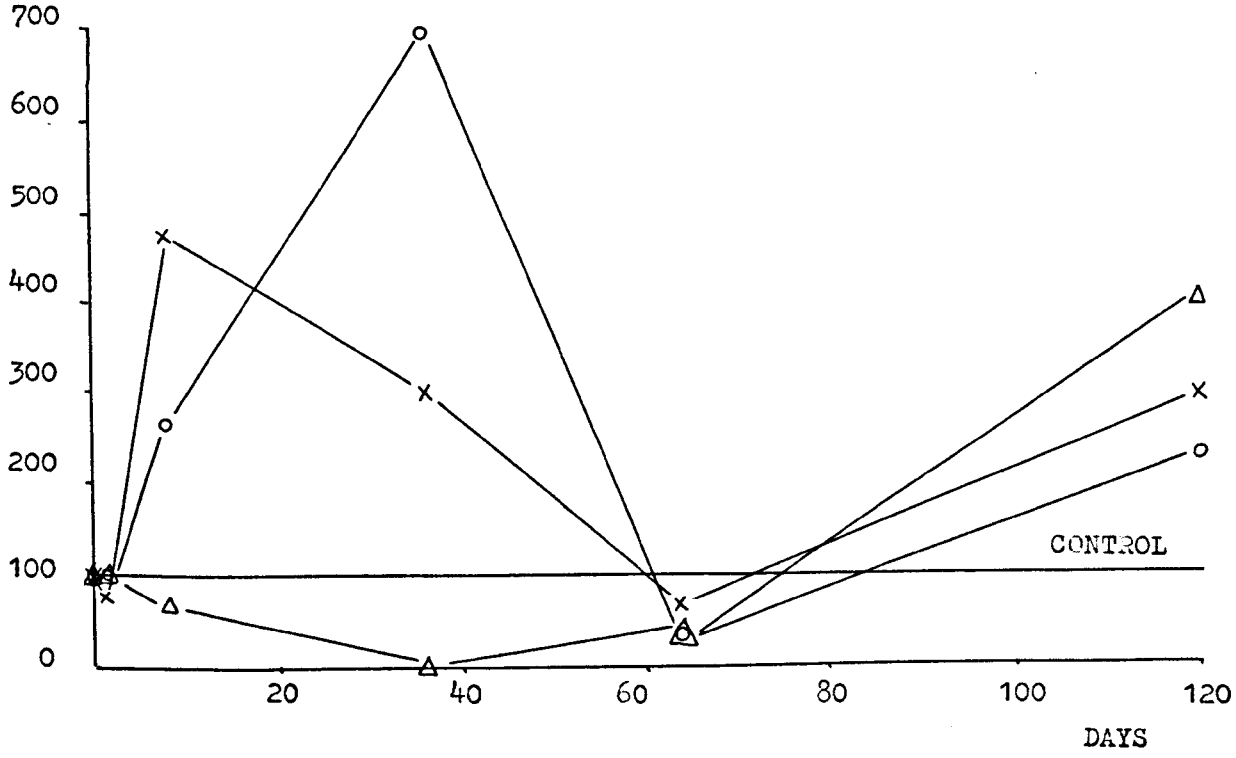
× —— × Captan

△ —— △ Thiram

Figure 40 - □ —— □ Dicloran

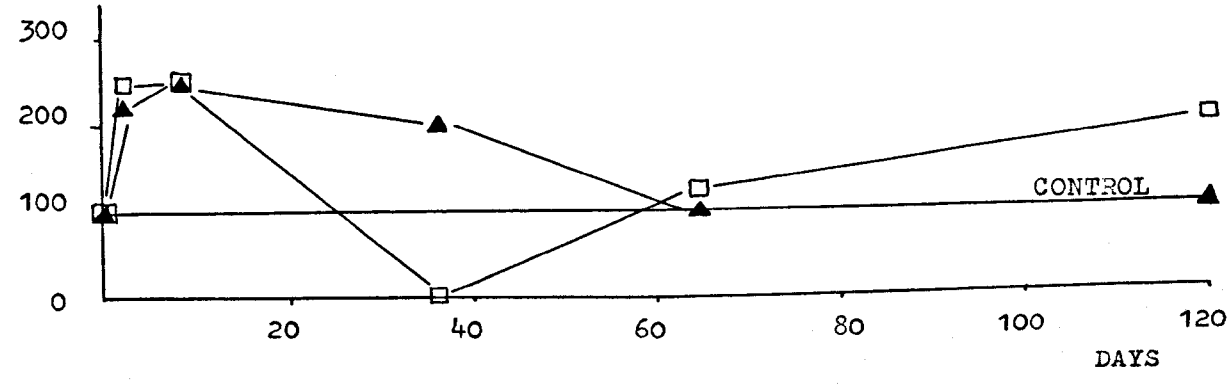
▲ —— ▲ Quintozene

Figure 39



% colonisation

Figure 40



Percentage colonisation of baits by *A. uncinatum* from soils at Sutton Bonington treated with fungicides

Greasy wool

Figure 41 - o ——o Dicloran

Δ ——Δ Milcol

Figure 42 - x - - - - x Captan

● ——● Triarimol

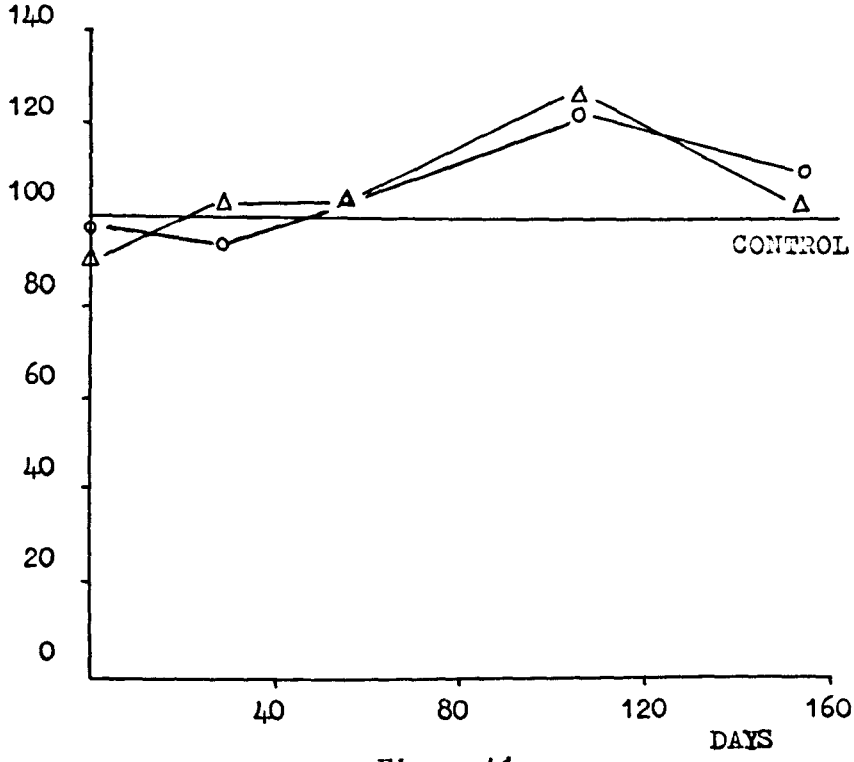
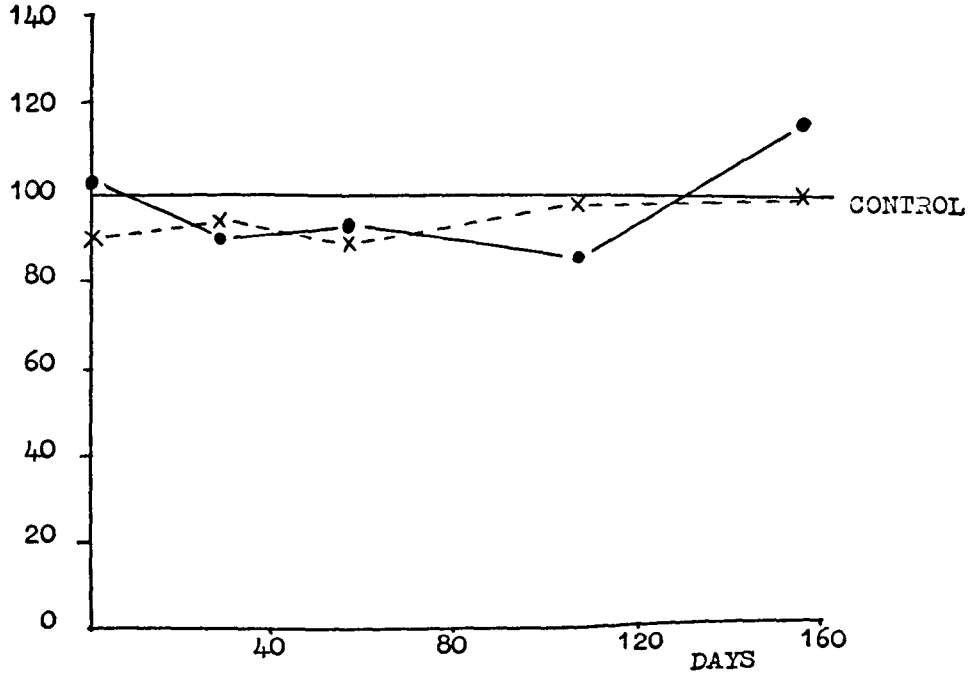


Figure 41

Figure 42



Percentage colonisation of baits by *A. uncinatum* from soils at Sutton Bonington treated with fungicides

Degreased wool

Figure 43 - o----o Dicloran

Δ——Δ Milcol

Figure 44 - x----x Captan

●——● Triarimol

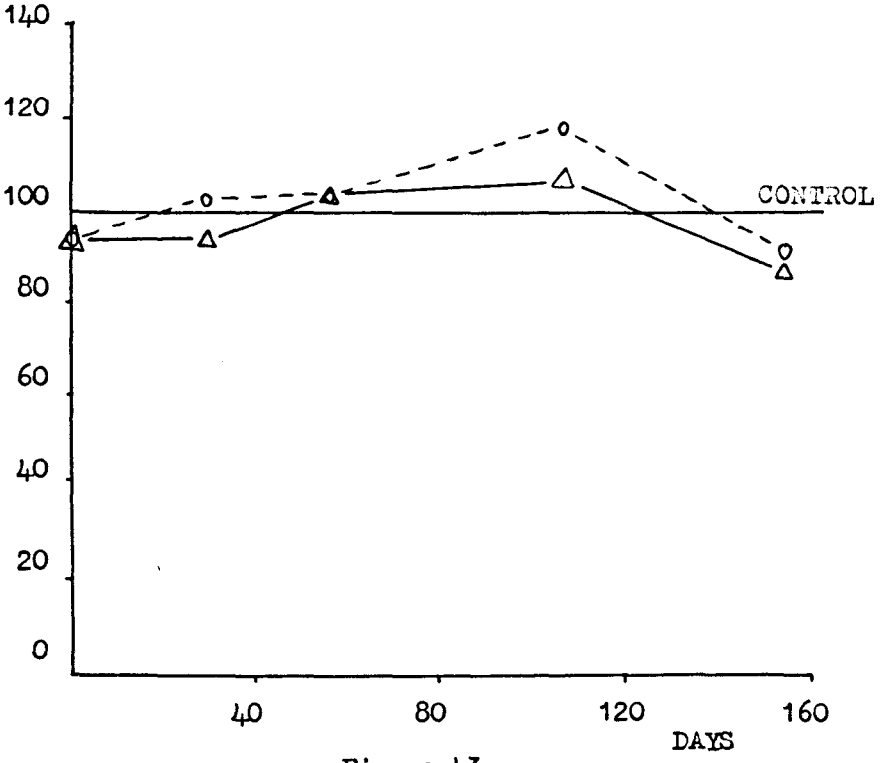
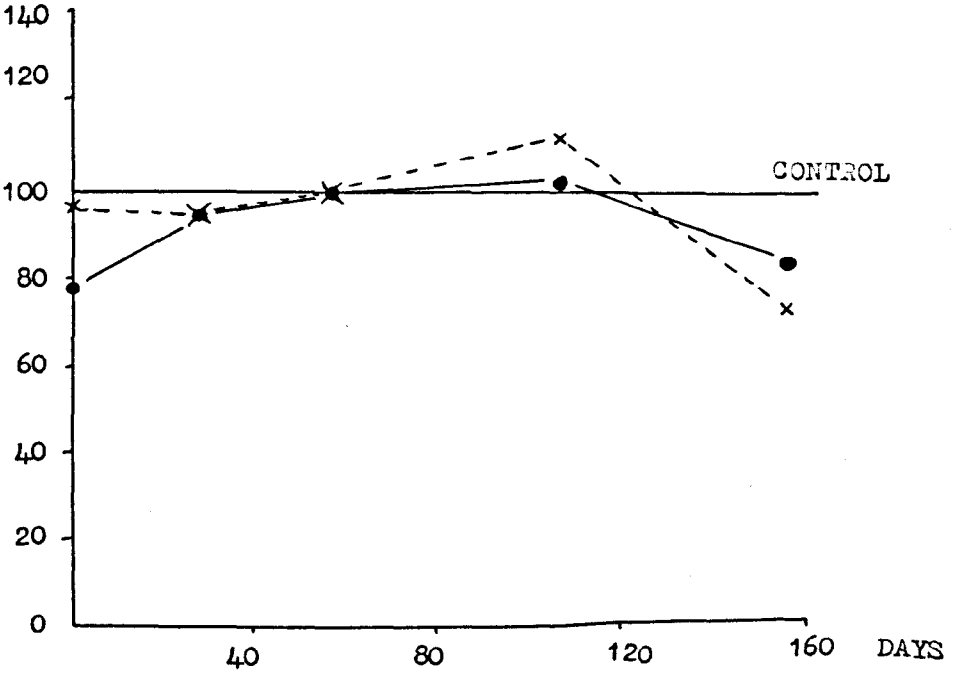


Figure 43

Figure 44



Percentage colonisation of baits by C. keratinophilum from soils at Sutton Bonington treated with fungicides

Greasy wool

Figure 45 - Δ —— Δ Milcol

o —— o Dicloran

Figure 46 - × —— × Captan

● —— ● Triarimol

Figure 45

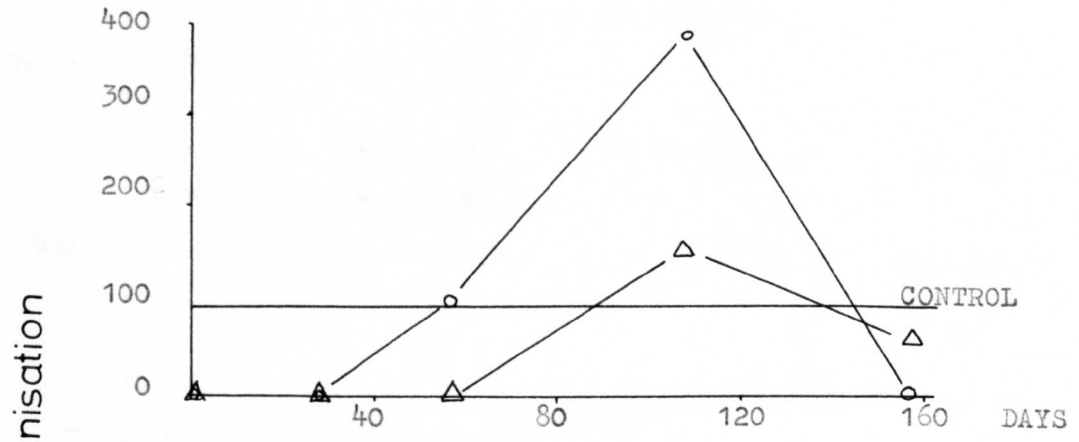
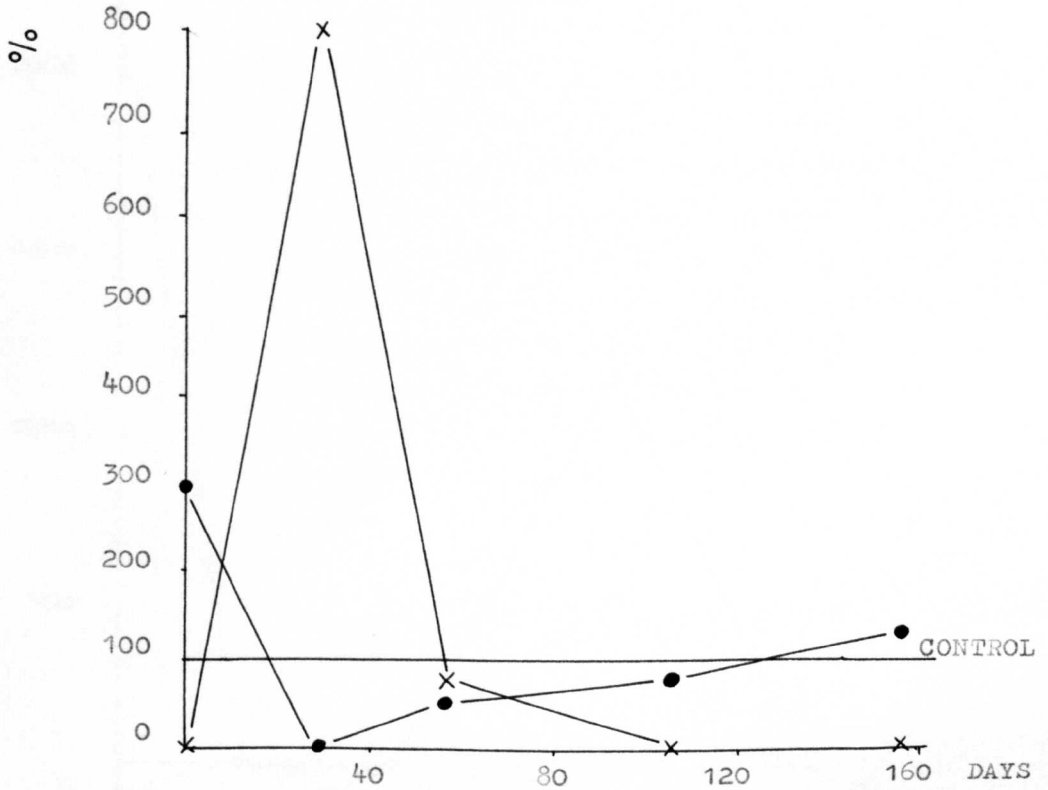


Figure 46



Percentage colonisation of baits by C. keratinophilum from soils at Sutton Bonington treated with fungicides

Degreased wool

Figure 47 - Δ —— Δ Milcol

o —— o Dicloran

Figure 48 - × —— × Captan

● —— ● Triarimol

Figure 47

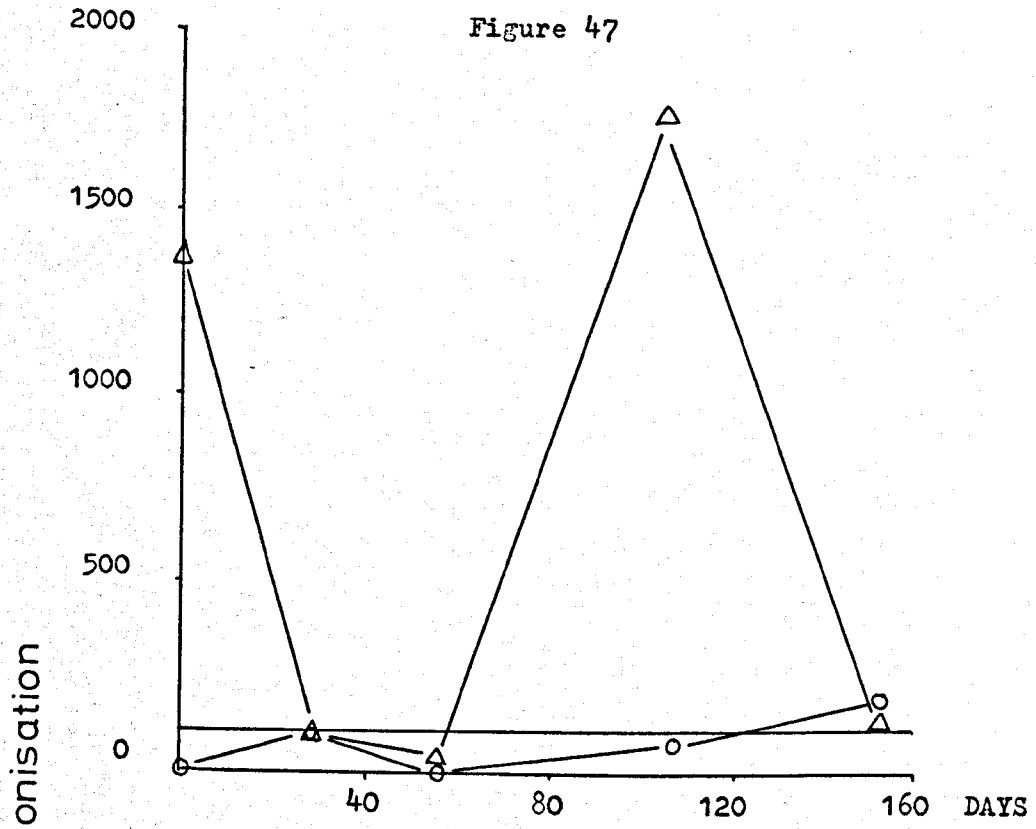
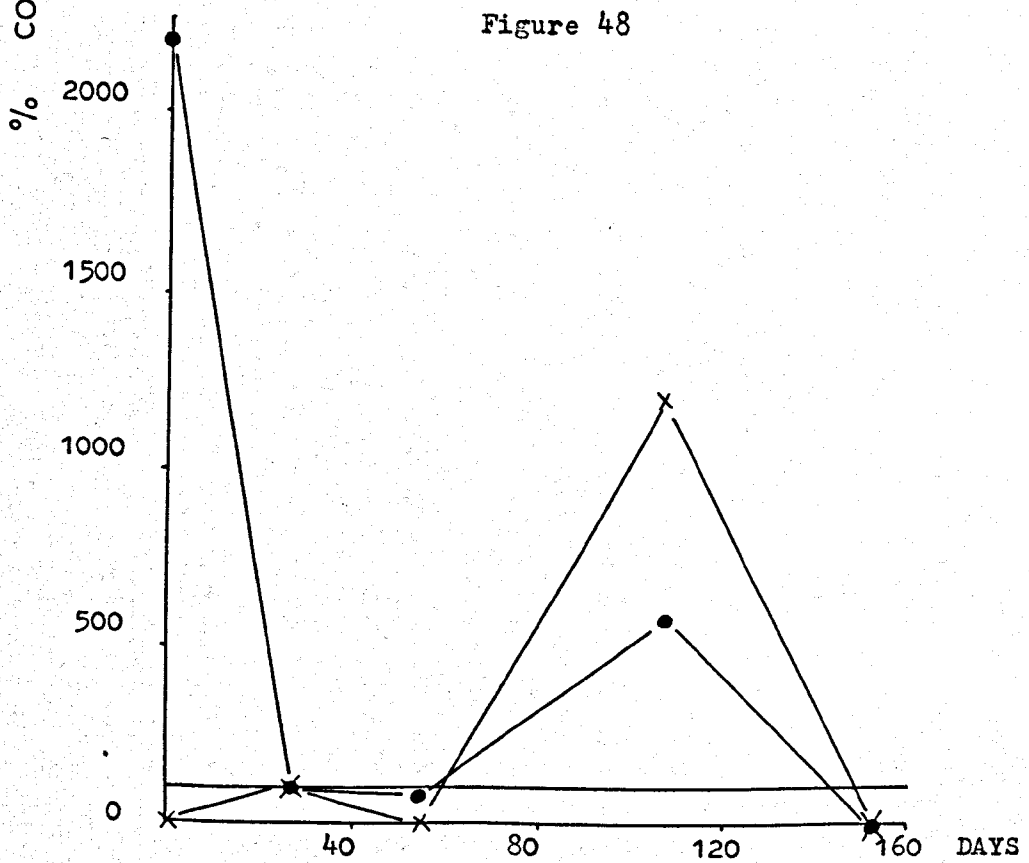


Figure 48



C. keratinophilum. In the case of dicloran and captan, initial percentage colonisation was 0% compared with 100% in the control. This also applied to Milcol and greasy wool. Triarimol, on the other hand produced an increase compared with the control in the first sample on greasy wool, as did Milcol and degreased wool for colonisation by C. keratinophilum. Captan was the only fungicide where this species had 0% colonisation of both types of wool at the end of the sampling period. Milcol was the only fungicide where percentage colonisation was not 0% for one type of wool at the end of sampling.

At both Rothampsted and Sutton Bonington, T. terrestre was rarely isolated from either control or experimental soils.

Comparison of the effect of dicloran and captan at Rothampsted and Sutton Bonington, for A. uncinatum.

Dicloran and captan were the only two fungicides used at both Rothampsted and Sutton Bonington. A comparison of their behaviour at the two places shows that dicloran behaved in a similar manner on both greasy and degreased wool. On greasy wool the percentage colonisation rose above the control at 45 days and on degreased wool it rose above the control at 18 days at Sutton Bonington and 50 days at Rothampsted. The figures fell below the control again at 140 and 110 days respectively. Captan, on the other hand, showed a distinct inhibitory effect upon colonisation of degreased wool at Sutton Bonington, but not at Rothampsted, and a distinct stimulatory effect at Rothampsted but not Sutton Bonington on percentage colonisation of greasy wool. Captan and degreased wool at Rothampsted and captan and greasy wool at Sutton Bonington showed neither an inhibitory nor a stimulatory effect after 120 days. There appeared to be a quicker reaction at Rothampsted by conidial A. uncinatum to captan.

No similarities were seen between percentage colonisation by C. keratinophilum at Rothampsted and Sutton Bonington when soils had been treated with dicloran and captan and no clear-cut contrasts were seen either.

Comparison of pretreatment and post-treatment percentage colonisation, at Rothampsted.

Table 4 shows the observations made for percentage colonisation of degreased and greasy wool at pre and post treatment and the percentage change after treatment both immediately and after 120 days, for conidial A. uncinatum.

Formaldehyde had the greatest initial inhibitory effect on percentage colonisation by conidial A. uncinatum of degreased wool, but the least for greasy wool. Captan and thiram gave the greatest initial inhibitory effect on colonisation of greasy wool. On degreased wool quintozene had the greatest inhibitory effect after 120 days. The effect of formaldehyde and captan was not noticeable at 120 days and dicloran and thiram showed no change in percentage colonisation at 120 days from 1 day.

On greasy wool, however, the effect of the fungicides was shown after 120 days as having a stimulatory effect upon colonisation by conidial A. uncinatum. Formaldehyde had the greatest effect both with degreased and greasy wool after 120 days.

Comparison of the pretreatment and final percentage colonisation differs in degreased and greasy wool. The former results show, in general, a decrease compared with the pretreatment, whereas the latter shows an increase compared with the pretreatment.

TABLE 4

Percentage colonisation of wool by A. uncinatum

in soils from Rothampsted.

	Pretreatment	Post-treatment (1 day)	Initial % change	Final % (120 days)	Final % change
Degreased wool					
Quintozene	110	103	-6	64	-42
Dicloran	100	96	-4	96	-4
Formaldehyde	100	68	-32	106	+6
Captan	100	80	-20	100	0
Thiram	100	94	-6	94	-6
Greasy wool					
Quintozene	110	96	-14	110	0
Dicloran	100	78	-22	136	+36
Formaldehyde	100	108	+8	170	+70
Captan	100	76	-24	140	+40
Thiram	100	76	-24	146	+46

Comparison of pretreatment and posttreatment percentage colonisation at Sutton Bonington.

(At Sutton Bonington the treatment of soils was 2 days before the first sample, therefore the pretreatment was considered to be the same as the control, i.e. 100%).

Table 5 shows that Triarimol had the greatest initial inhibitory effect on percentage colonisation by conidial A. uncinatum of degreased wool but least for greasy wool. Milcol and captan gave the greatest initial inhibitory effect on colonisation of greasy wool. All the fungicides produced an initial inhibitory effect except Triarimol with greasy wool.

On degreased wool, captan had the greatest effect after 120 days on percentage colonisation. Dicloran and Milcol showed little difference between the immediate percentage change and final change after 120 days and Triarimol showed a slight decrease in the degree of inhibition after 120 days.

On greasy wool the final percentage change showed an increase (with the exception of captan) compared with the pretreatment result, as was the case at Rothampsted.

Triarimol and formaldehyde at Sutton Bonington and Rothampsted respectively behave in a similar manner to one another in the general overall percentage inhibition.

TABLE 5

Percentage colonisation of wool by A. uncinatum

in soils from Sutton Bonington

Degreased wool	Pretreatment	Post-treatment (2 days)	Initial % change	Final % (150 days)	Final % change
Dicloran	100	94	-6	91	-9
Milcol	100	94	-6	86	-14
Captan	100	98	-2	70	-30
Triarimol	100	78	-22	86	-14
Greasy wool					
Dicloran	100	96	-4	110	+10
Milcol	100	90	-10	102	+2
Captan	100	90	-10	100	0
Triarimol	100	103	+3	116	+16

Results of isolations from soils of three different pH values.

Conidial A. uncinatum (Figures 49 and 50) had the highest percentage colonisation on degreased wool at pH 6 (68%), followed by greasy wool at pH 6 (63%). This species colonised 55% degreased wool on soils at pH 8, but at pH 4 it only colonised 17% of degreased wool. Greasy wool was not colonised at all at pH 4.

Trichophyton terrestre (Figures 51 and 52) had the highest percentage colonisation on degreased wool at pH 8 (64%), followed by 53% at pH 6 on degreased wool. At pH 4 only 3% colonisation of either degreased or greasy wool was seen.

Microsporum gypseum (Figures 53 and 54) was isolated mostly from pH 8 (62%) on degreased wool, followed by pH 6 on greasy wool (46%). The lowest percentage colonisation of either degreased or greasy wool was at pH 4.

Microsporum cookei (Figures 55 and 56) was never isolated from soils at pH 4 and only colonised degreased and greasy wool at pH 6 and 8 below 25%.

Chrysosporium keratinophilum colonised degreased wool 1% at pH 6 and 3% of greasy wool at pH 6 and these were the only times this species was observed from these soils.

The reactions of the soils were pH 4.0, 6.7 and 8.2.

Table 6 shows the isolations from all soils in relation to the pH of each sample.

Results of isolations from Gibraltar Point.

Figure 57 represents the percentage colonisation by various keratinophilic fungi on degreased and greasy wool at seven sites sampled.

No keratinophilic fungi were isolated from the bare mud. Trichophyton terrestre was the species isolated most i.e. from

Figures 49 - 56

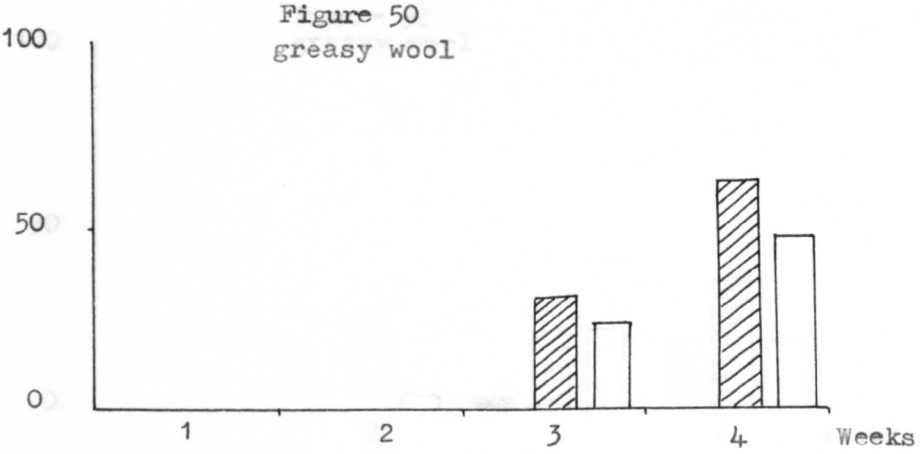
Histograms representing reisolation of keratinophilic fungi from soils of different pH values.

A. uncinatum

Figure 49
degreased wool

% colonisation

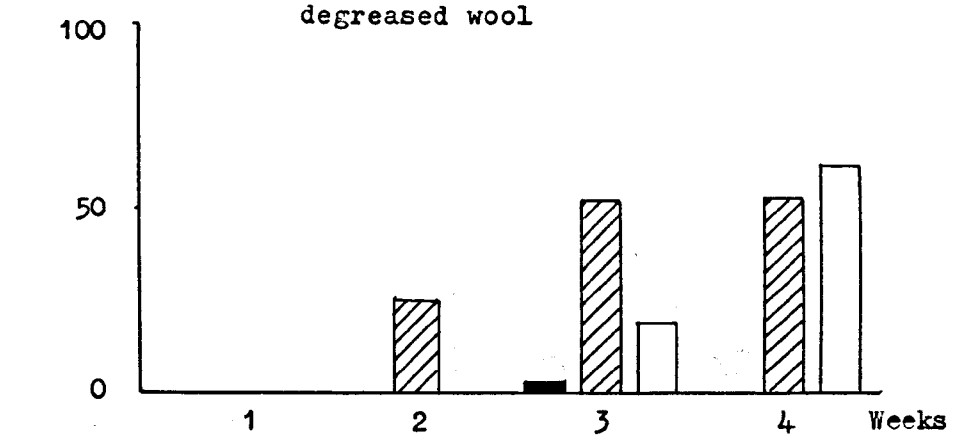
Figure 50
greasy wool



pH
4
6.7
8.2

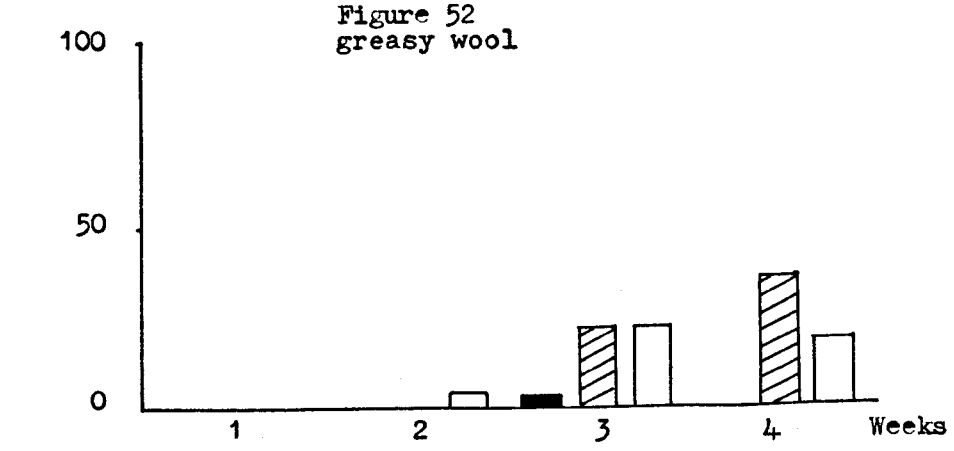
T. terrestre

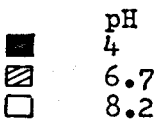
Figure 51
degreased wool



% colonisation

Figure 52
greasy wool

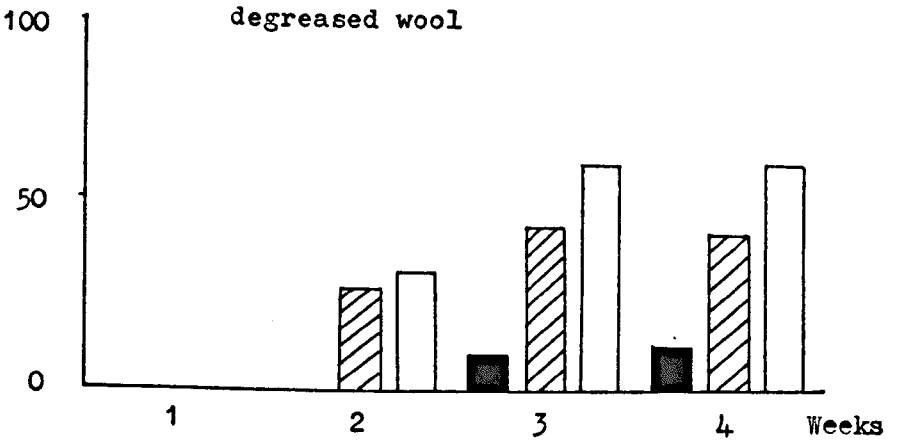




M. gypseum

Figure 53

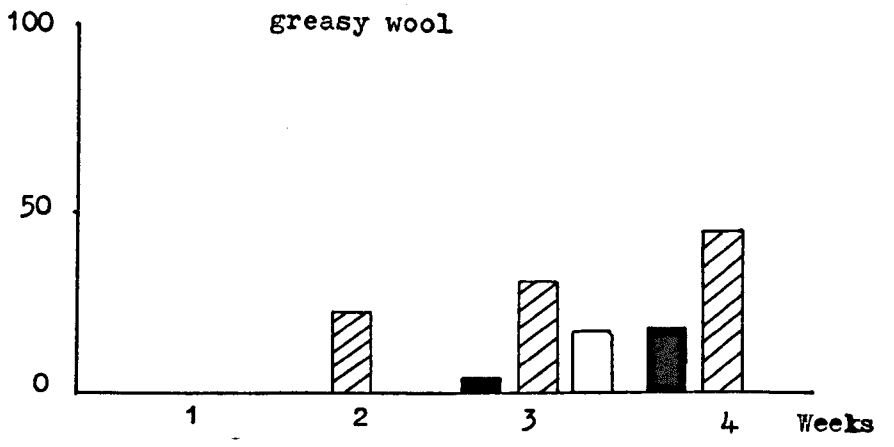
degreased wool



% colonisation

Figure 54

greasy wool



pH

4

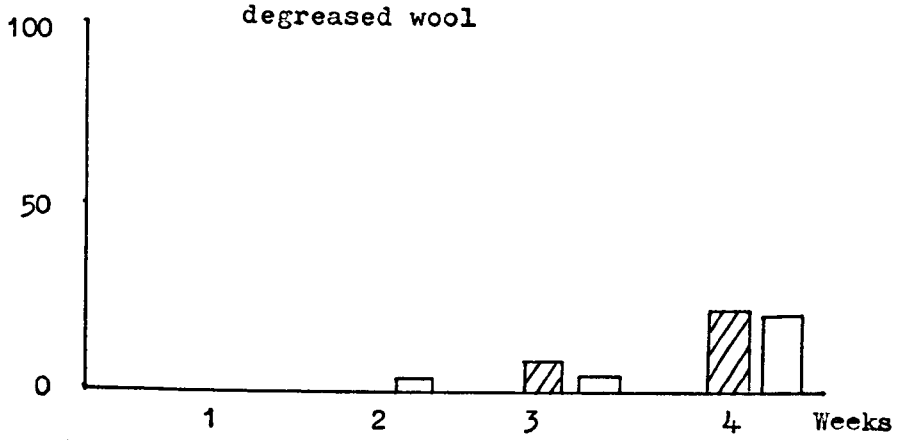
6.7

8.2

M. cookei

Figure 55

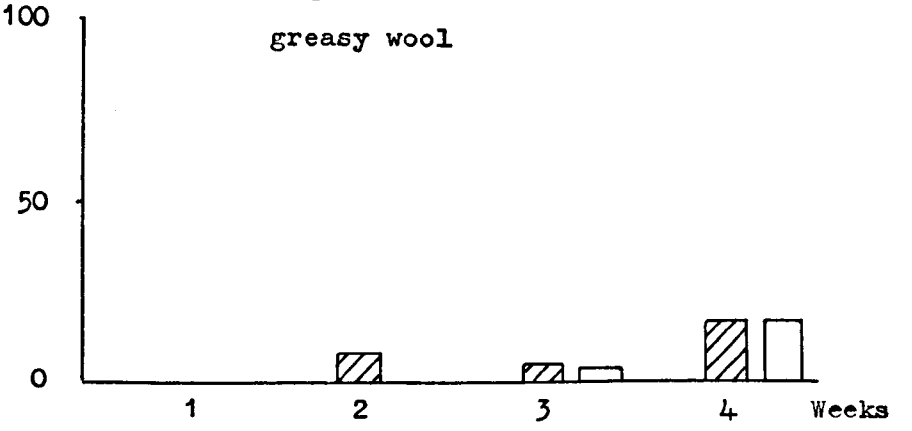
degreased wool



% colonisation

Figure 56

greasy wool



pH

4

6.7

8.2

TABLE 6

Isolation of keratinophilic fungi from soils of different pH values

(% presence)

pH of soil	No. of soils	co. A. u		C. k		T. t		M. g		M. c	
		No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%	No. of isolates	%
3.1 - 4.0	43	32	73	29	67	27	63	0	0	5	11
4.1 - 5.0	72	67	92	52	72	43	60	2	3	13	18
5.1 - 6.0	41	34	83	23	56	17	41	2	5	11	26
6.1 - 7.0	75	74	99	54	72	45	60	15	20	29	38
7.1 - 8.0	45	45	100	38	84	35	77	13	28	20	44

co. A. u = conidial Arthroderma uncinatum

C. k = Chrysosporium keratinophilum

T. t = Trichophyton terrestre

M. g = Microsporum gypseum

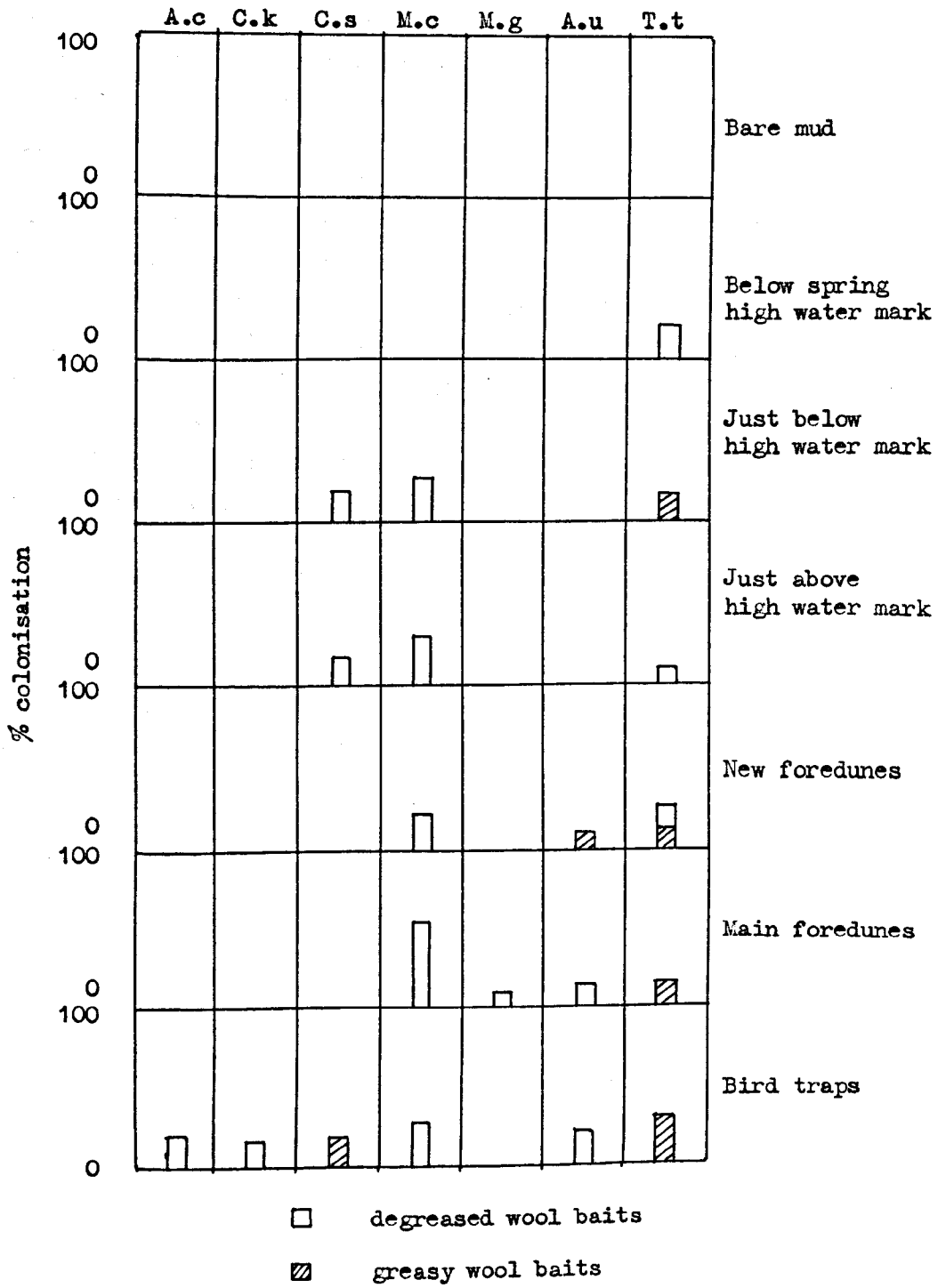
M. c = Microsporum cookei

KEY

A.c	Arthroderma curreyi
C.k	Chrysosporium keratinophilum
C.s	Ctenomyces serratus
M.c	Microsporum cookei
M.g	Microsporum gypseum
A.u	Arthroderma uncinatum
T.t	Trichophyton terrestre

Figure 57

Histograms representing isolations of keratinophilic fungi from Gibraltar Point



all sites, other than bare mud, followed by M. cookei which was not isolated from the two sites which came into contact with sea water the most. Conidial A. uncinatum was only found where the soils were not covered by the sea. Microsporum gypseum was isolated only on the foredunes, where the soil was driest. Arthroderma curreyi and C. keratinophilum were only found in the bird traps, whilst Ctenomyces serratus was found just above and below high water mark and in the bird traps.

The highest incidence of T. terrestre, Cs. serratus, A. curreyi, C. keratinophilum and conidial A. uncinatum was in the bird traps. Only M. cookei occurred more elsewhere, viz, the foredunes.

The reaction of the soils at Gibraltar Point was between pH 6.9 and 8.2.

Results of the tinea pedis survey

Table 7 shows the incidence of tinea pedis in the 1972 student intake sampled on three occasions and in the 1973 student intake. There was very little difference between the incidence in October 1972 and October 1973 of the two lots of new students ($p => 0.50$).

The sample in May 1973 shows a statistically highly significant increase compared with October 1972 ($p =< 0.001$). However, these students showed a return in the following October to almost the initial incidence which was not significantly different from the initial incidence ($p => 0.20$).

The second survey involved 181 students, i.e. 75% of the original sample of 244 were successfully recalled. (Several people of the 244 did not want to take part again and a few had left the university to go abroad for the third term). The third survey consisted of 93 people. The low number of students who repeated the survey was due to poor response and difficulty in contacting people no longer in halls of residence. The policy of the University Accommodation Officer did not permit new addresses to be supplied.

The number of men with tinea pedis was, with the exception of October 1973, nearly always double the number of women with the infection. The overall total shows this was the case.

One male yielded Trichophyton mentagrophytes var interdigitale and Epidermophyton floccosum on one foot, hence the discrepancy between 23 isolates but only 22 people suffering from tinea pedis.

Table 8 shows some of the results from the questionnaire regarding hygiene. In addition to these points, several other aspects were of interest:-

TABLE 7

Distribution of tinea pedis in 1972 student intake sampled on three occasions, and in 1973 student intake.

Date of sample		Total sample	Positive : Total	%	Total female	Positive : Total	%	Total male	Positive : Total	%
I	1972 October	244	22	9.0	97	5	5.1	147	17	11.5
II	1973 May	181	44	24.0	71	12	17.0	110	32	29.0
III	1973 October	93	11	11.8	33	4	12.1	60	7	11.6
IV	1973 October	240	26	10.8	93	5	5.3	147	21	14.3
Total		758	103	13.6	294	26	8.8	464	77	16.6

TABLE 8

Factors which may have some influence on the difference of incidence of tinea pedis in men and women

	MEN (% mean of all samples)	WOMEN (% mean of all samples)
Use of talcum powder after bathing	25.7	57.3
Showers after sports	84.0	52.9
Changed hosiery daily	39.0	71.0
Discard hosiery in summer	34.0	92.0
Dry carefully between toes after bathing	58.0	76.0

a) The majority of students lived in halls of residence.

	MEN	WOMEN
I	99%	87%
II	99%	97%
III	89%	69%
IV	94%	94%

b) The number of men and women treated by a doctor for tinea pedis before coming to university was more or less the same.

	MEN	WOMEN
I	13.5%	14.5%
IV	13.0%	14.0%

c) The number of men and women who used medication for prevention of tinea pedis was more or less the same.

	MEN	WOMEN
I	11.0%	11.0%
II	6.5%	8.3%
IV	7.5%	9.6%

d) The number of men who took communal showers, with the exception of those in III, was more than the number of women who took showers. When 61% women took showers, a greater percentage of women had tinea pedis than did men.

	MEN	WOMEN
I	92.5%	74.4%
II	94.0%	69.0%
III	56.0%	61.0%
IV	95.0%	66.0%

e) More women were suffering from maceration and burning in the interdigital spaces, but more men were suffering from scaling and itching.

Maceration

	MEN	WOMEN
II	5.8%	7.1%
III	4.5%	6.0%

Scaling

	MEN	WOMEN
II	14.0%	10.0%
III	11.0%	6.0%

Itching

	MEN	WOMEN
II	5.8%	4.2%
III	11.0%	0%

Burning

	MEN	WOMEN
II	0%	1.4%
III	0%	0%

f) Only 3 people had suffered from ringworm before coming to University (i.e. 2.7% of the men) and only 2 had ringworm since May 1973, again both men; one on the elbow and one in the groin.

g) More men than women had a combination of wool and nylon hosiery, but more women wore nylon hosiery than did the men.

	MEN	WOMEN
Woollen	6.5%	8.4%
Nylon	30.0%	50.0%
Mixture	55.0%	41.6%

Table 9 shows the dermatophytes which were isolated. Trichophyton mentagrophytes var interdigitale was by far the most common dermatophyte. Epidermophyton floccosum and Trichophyton rubrum were only isolated once each. These values represent the lower limit of the true frequency for what is commonly called athlete's foot, since the students who took part in the survey were only screened for dermatophytes and not for Candida or bacteria. However, the term tinea pedis is considered not to include these last two types of organism.

Some students were found to harbour a dermatophyte but showed no clinical signs of infection.

Table 10 shows the ratio of dermatophytes on right and left feet. There was more one foot involvement than two foot involvement ($p = <0.001$), and more isolations were made from the right foot than the left. The percentage of men with two foot involvement was greater than that of women (28.2 : 19.3 %), but more women than men had left foot involvement (30.8 : 38.4).

Table 11 shows the ratios of infected feet to the sports played by the students.

TABLE 9

Fungi isolated and their distribution on male and female students.

	I		II		III		IV		Total	% samples
	w	m	w	m	w	m	w	m		
Trichophyton mentagrophytes var interdigitale	5	17	12	31	4	7	5	21	102	13·5
Trichophyton rubrum				1					1	0·13
Epidermophyton floccosum		1							1	0·13
Total	23		44		11		26		104	

w = women, m = men.

TABLE 10

Ratios of fungi on left and right feet of male and female students.

	Both feet	Right foot	Left foot	One foot
	m : w	m : w	m : w	Right : Left
I	7 : 1	3 : 3	6 : 1	6 : 7
II	8 : 1	15 : 3	9 : 8	18 : 17
III	3 : 2	4 : 2	1 : 0	6 : 1
IV	4 : 1	9 : 3	8 : 1	12 : 9
Total	22 : 5	31 : 11	24 : 10	42 : 34
	27	42	34	76
% ratio	28.2 : 19.3	41.0 : 42.3	30.8 : 38.4	

m = men, w = women.

TABLE 11

Numbers of infected students in relation to the sports played by the students.

Sport		I October 1972	II May 1973	III October 1973	Total sampled
Rugby	(♂)	1 : 16	1 : 2.5	1 : 3.5	83
Tennis		1 : 12	1 : 4	1 : 4	137
Athletics	(♂)	1 : 7	1 : 2	1 : 2	28
Football	(♂)	1 : 6.5	1 : 3.7	1 : 5	111
Badminton		1 : 8	1 : 4.3	1 : 7.5	121
Swimming		1 : 12	1 : 6	1 : 5	59
Squash		0 : 0	1 : 5	1 : 10	70

♂ = men only

Ratio 1:16 = 1 person infected in 16

As a generalisation, among the students who played these sports there was a higher incidence of tinea pedis after one year at the university. In the case of those students who played rugby, football, badminton and squash there was a decrease in the incidence of the disease after May.

Of those 22 with tinea pedis in October 1972 (I):

15 had tinea pedis in May 1973 (II)

3 did not have tinea pedis in (II) and

4 were not sampled again.

There were 29 new cases of tinea pedis in May 1973 (II) who did not have tinea pedis in October 1972.

Of the 44 who had the infection in II:

24 returned to be sampled

20 did not return (one had died).

Of those 24:

8 still had tinea pedis in October 1973, (4 of whom had tinea pedis in October 1972)and there were

3 new cases of tinea pedis in October 1973 (1 of whom had tinea pedis in October 1972).

Therefore only 4 students had tinea pedis all the way through the survey, i.e. of those who were sampled each time.

DISCUSSION

Five species of keratinophilic fungi were isolated from the soils at Highfield's swimming pool and from the hedgehog pens: Arthroderma uncinatum, Trichophyton terrestre, Microsporum cookei, M. gypseum and Chrysosporium keratinophilum. Kunert (1965, 1966) found Trichophyton ajelloi to be the most abundant keratinophilic fungus isolated in Czechoslovakia. Otčenášek, Dvořák and Kunert (1967) said that T. ajelloi is probably the most abundant keratinophile in the temperate regions of the world. In the present work conidial A. uncinatum has been the most frequently isolated species from both sampling sites. The percentage presence of conidial A. uncinatum was higher on degreased wool than greasy wool, which would be expected from work carried out by Evans (1969) and Pugh and Evans (1970b), who found that conidial A. uncinatum was inhibited by pigeon feather fats and by animal wool fats over quite a range of concentrations. Baxter and Trotter (1969) found that this species was inhibited by adult hair fats, squalene and to a certain extent by boys' hair, pigeon feather fats, guinea pig hair fats and horse hair fats, but not at all by animal wool fats at 0.2% concentration. Most of the keratinophilic fungi were isolated more on degreased wool than greasy wool, but C. keratinophilum was isolated more frequently on greasy wool. Pugh (1971) discovered that in the majority of cases keratinophilic fungi grew faster or the same on defatted feathers than fresh feathers, with the exception of conidial A. uncinatum on pheasant feather fats and A. curreyi on blackbird feather fats. Pugh and Evans (1970b) found C. keratinophilum was stimulated slightly by olive oil and by wool fats up to a concentration of 0.1% and hardly affected by any bird feather fats. They also found T. terrestre was only stimulated by olive oil at high concentrations and was unaffected by bird

feather fats and wool fats. Baxter and Trotter (1969) reported T. terrestre and 5 strains of T. ajelloi as being the only keratinophilic fungi unaffected by animal wool fat at the concentration used (they only mention a concentration of 0.2%), but they did not study C. keratinophilum. Their analysis supported observations that the shorter chain acids were more important fungistatic agents. However, Grunberg (1947) considered that fungistatic and fungicidal actions of fatty acids in the series from formic acid to undecylenic acid increase with length of carbon chain and that the change was regular. Bose (1964) determined that M. gypseum produced more perforating organs in hair if the fat had been removed. It would seem that the fat content of keratin baits can determine the course of colonisation of bait in soil. The present work endorses that of Bose (1964), Evans (1969), Pugh and Evans (1970b) and Pugh (1971).

At Highfield's swimming pool the incidence of conidial A. uncinatum fell after May until September. This decrease coincided with the opening of the swimming pool to the public. Since keratin, in the form of skin, hair and nails, would have been deposited by the visitors to the pool who sunbathed on the grassy area, one might expect the isolations of conidial A. uncinatum to increase, but this was not so.

In the hedgehog pens both conidial and cleistocarpic A. uncinatum increased in frequency when the hedgehogs were present, as did C. keratinophilum, M. gypseum and M. cookei. These fungi, with the exception of cleistocarpic A. uncinatum, decreased in numbers when the hedgehogs were absent. The spines and scales of the hedgehogs provided the source of keratin in the pens for the keratinophilic fungi in the soil. Whereas at the swimming pool no obvious source of keratin could be seen by the naked eye, in the hedgehog pens many spines could clearly be seen. This source of keratin, because it came into direct contact with

the soil, was probably utilised sooner by the keratinophilic fungi, whereas at the swimming pool, since the soil was grassed over, incorporation of the keratin into the soil was possibly slower and the increase in frequency of conidial A. uncinatum was delayed until September. Chlorinated water from the pool may have had an adverse effect upon conidial A. uncinatum. When the swimmers went onto the sunbathing area, they would have taken some of the pool water onto the grassy areas. Samples of chlorinated water were collected and an attempt was made to determine the effect of this water upon the isolation of keratinophilic fungi. Unfortunately the chlorinated water is not stable, and after watering the hair-baited soils, the chlorine was liberated, so that strictly this was no longer chlorinated water, since the chlorine was released. The results indicated that this water had no inhibitory effects upon the keratinophilic fungi. An alternative method would be needed to test the hypothesis that the chlorine in the water was the reason conidial A. uncinatum decreased in isolations from the swimming pool soils. Some explanation is necessary for the observed pattern of occurrence of conidial A. uncinatum with its decrease in the summer. None of the other keratinophilic fungi showed any correlation with presence of people at the swimming pool. Cleistocarpic A. uncinatum was always more abundant in control soils away from the swimming pool than in swimming pool soils, and the chlorinated water may be the reason why the perfect state was less abundant.

Otčenášek , Dvořák and Kunert (1967) reported that T. ajelloi was more frequently isolated from temperate zones, T. terrestre in temperate and subtropical zones and M. gypseum in subtropical and tropical areas. They stated that the "geographical distribution of geophilic dermatophytes is probably in the first place determined by the influence of temperature" and emphasised this suggestion by citing the work of Dawson, Gentles and Brown (1964).

These researchers considered that cleistothecial formation of T. terrestre (A. quadrifidum) was inhibited at temperatures above 24°C whereas temperatures of over 24°C and up to 30°C can be used for production of cleistothecia of M. gypseum. The results of isolations of T. terrestre and conidial A. uncinatum from the swimming pool and the hedgehog pens agree with the classification of Otčenášek, Dvořák and Kunert (1967) that T. terrestre and conidial A. uncinatum prefer temperate regions.

Chrysosporium keratinophilum was, like T. terrestre, isolated most in winter and least in summer from hedgehog pens and Highfield's swimming pool soils, but in control soils for these 2 sites, i.e. soils from outside the sites, there was a minimum of C. keratinophilum in the winter with maxima in spring and autumn. However, it does appear to be a fungus which prefers temperate zones. It would also seem that, since it grows well in winter inside the hedgehog pens and the swimming pool soils, the keratin source provided by the quills and scales from the hedgehogs or skin and hair from bathers is sufficient to overcome the effects of temperature.

Kunert (1966) found a minimum for T. terrestre in autumn, which agreed with the results from soils inside the hedgehog pens and swimming pool, and a maximum in spring and summer in Czechoslovakia (see Table 11a), which contrasted with the present work. On the other hand, in the soils taken from outside the swimming pool a minimum was seen in winter and summer and a maximum in spring and autumn, whereas outside the hedgehog pens the reverse was found. It can also be seen from the table that Chmel, Hasilíková, Hraško and Vlačílíková (1972) showed a maximum peak in mid spring for T. terrestre (and in mid autumn), but a minimum in spring-summer, in contrast to Kunert's results and those in the present work in the "experimental" soils.

In the case of conidial A. uncinatum, the results of the

TABLE 11a

Seasonal maxima and minima of T. terrestre and A. uncinatum reported in the literature : comparison with the present work.

	Kunert (1966)	De Vroey (1970)	Chmel et al. (1972) a	Chmel et al. (1972) b	Present work a	Present work b
A. uncinatum						
maximum peak	autumn	-	spring	autumn	spring	summer
minimum peak	spring	-	summer	spring	winter	winter/ spring
T. terrestre						
maximum peak	spring/summer	autumn	summer	autumn	summer	winter
minimum peak	autumn	spring/summer	autumn/ winter/ spring	winter/ spring/ summer	winter/ autumn	spring/ autumn

Chmel et al. (1972) a = carbonate meadow soil, b = chernozem soils

Present work a = Highfield's swimming pool soils, b = hedgehog pen soils

seasonal isolations from the swimming pool and hedgehog pens contrast with those of Kunert (1966) who discovered a minimum of this species in spring and a maximum in autumn, as did Chmel, Hasilíková, Hraško and Vláčilíková (1972). Outside the swimming pool (control soils) a minimum was seen for conidial A. uncinatum in July, August and September. Therefore, the decrease of conidial A. uncinatum in soils from inside the swimming pool may just be a seasonal change and not due to the effect of chlorine which, if released from soils in petri dishes may also be released in vivo, and therefore have no effect. The seasonal decrease in conidial A. uncinatum in summer was also seen in the "control" soils for the hedgehog pens (June, July, August and September) with an additional decrease in December.

Microsporum cookei showed an almost complete fall off in winter months and it would seem to have a preference for warmer temperatures, i.e. like M. gypseum. Otčenášek, Dvořák and Kunert (1967) found that although M. cookei was rarely isolated, it showed greatest resemblance to M. gypseum.

From all these results it is difficult to come to any definite conclusions regarding seasonal changes.

Microsporum gypseum was isolated very little at all at either Highfield's swimming pool or the hedgehog pens and this is in accordance with the fact that M. gypseum prefers warmer soils, i.e. tropical zones. Chmel, Hasílíková, Hraško and Vláčilíková (1972) thought that temperature did not seem to influence the occurrence of any keratinophilic fungi.

The ecology and transmission of M. gypseum from soil to man was discussed by Chmel and Buchvald (1970) who found that cultivated fertile soils were the main reservoir of this fungus. Chmel, Hasílíková, Hraško and Vláčilíková (1972) reported that M. gypseum was isolated almost exclusively from carbonate meadow soil with the highest humus content. The soils from the swimming pool and hedgehog pens were not cultivated and had quite low

humus content and this may account for the fact that M. gypseum was rarely isolated. Ěivazov and Martsishevskaya (1966) found M. gypseum and T. ajelloi showed no preference for any particular soil type. Zeidberg and Ajello (1954) isolated M. gypseum from soils in barns, barnyards and around places where there was a concentration of animals. Chmel, Hasíliková, Hraško and Vlačílíková (1972) also found that the number of isolations of T. ajelloi rose with an increase in humus, whilst those of T. terrestre fell with an increase in humus. Otčenášek, Dvořák and Kunert (1967) discovered T. ajelloi to be a fungus of wide distribution, which was found most frequently in medium humus soils. T. terrestre was the opposite of M.gypseum and was found in light sandy soils and sand on beaches. It has been suggested by English (1964) that T. terrestre is unable to compete against T. ajelloi for a bait and that T. terrestre only increases in numbers when other keratinophilic species fall in numbers (Pugh and Evans, 1970a). The results of this study, both in control and experimental soils endorse this suggestion, although no antagonism has been seen between T. terrestre and other keratinophilic fungi when grown on the same agar plate.

Trichophyton terrestre was the only species which decreased in numbers when more hedgehogs were present in the pens, and although this may indicate that a source of keratin is unimportant for the presence of T. terrestre in soil, it may be that because the other species increased, the competition was too great for T. terrestre to proliferate. This species was isolated more from experimental than control soils possibly because it is more tolerant of lower pH values, or that the soils were poorer in the experimental plots. Conidial A. uncinatum and C. keratinophilum both showed very similar trends overall in control and experimental soils and no definite conclusions were drawn about the influence of chlorine, pH or biotic factors upon their isolation.

Cleistocarpic A. uncinatum, M. gypseum and M. cookei were appreciably more in controls than experimental soils from both sites, indicating that the presence of biotic factors had little effect upon increasing the isolations of the 3 species in the experimental soils compared with controls. The effect of lower pH or chlorinated water may be the important factors which determine the low occurrence of these fungi in experimental soils, since all the other edaphic factors studied showed no definite correlations. Ajello (1953) found a correlation between positive samples of M. gypseum and the presence of animals at the collecting site.

More isolations were made at Highfield's swimming pool than at the hedgehog pens and this was probably because of the reaction of the swimming pool soils (6.30) which was higher than that of the hedgehog pens (4.21).

Chmel, Hacílíková, Hraško and Vlácílíková (1972) found that with increased humidity isolations of T. ajelloi increased, whereas with T. terrestre isolations decreased when there was an increase in humidity of soils, i.e. there was an increase in T. terrestre in months with low water content of soil.

It would seem that the soil at both experimental sites plays only a minor role in being a reservoir of infection, since the only keratinophilic fungi isolated are rarely pathogenic, with the exception of M. gypseum. In the case of the hedgehog pens none of the hedgehogs were infected with any of the species isolated. Those people who do become infected with M. gypseum are, in many cases, closely associated with the soil, e.g. gardeners (Whittle, 1954; Alsop and Prior, 1961; Sonck and Lundell, 1962; Kaben and Moldenhauer, 1963; Bensch and Gemeinhardt, 1966; Gip and Hersle, 1966; Rakhmanov, Fedorova and Yashkul', 1969; Steinerová and Dubrovova, 1969; Alteras and Cojocaru, 1970; Kadlec and Podivínska, 1970; Baran, 1971; Simordova, 1971 and Percebois and Vadot, 1972).

These infected people had long contact with soil, whereas sunbathers at the swimming pool, because they are lying on grass and are not in direct contact with soil, are not easily infected. Evolceanu and Alteras (1967) isolated T. ajelloi, M. gypseum, T. evolceanui, T. indicum, M. canis and C. keratinophilum from a grassy bathing place at a lakeside in Roumania and M. gypseum, C. keratinophilum and conidial A. uncinatum have also been isolated from the soil at the swimming pool. It appears however, that although the grassy areas of swimming pools are of little importance epidemiologically, the floors surrounding outdoor and indoor swimming pools are important. Gentles (1957) found that T. mentagrophytes could exist for long periods on concrete surfaces and can grow on moist crushed asphalt if soap were present. Ajello and Getz (1954) isolated T. mentagrophytes, T. rubrum and Epidermophyton floccosum from shower stalls. Gentles (1956) isolated T. rubrum and T. mentagrophytes var interdigitale, Gip (1967) and Gip and Aschan-Åberg (1968) isolated the same species, from floor surfaces of open-air pools. Drouhet, Marcel and Labonde (1967) found the greatest number of fungi (T. mentagrophytes, T. rubrum and E. floccosum) around a pool in shower cubicles where the ground was always wet. Adamson and Annan (1949) were the first to isolate T. mentagrophytes from skin scales on floors of a bath-house. Discussion on the importance of floors in epidemiological studies is continued in the section on the tinea pedis survey.

Böhme and Ziegler (1969) discovered that it was difficult or impossible to isolate keratinophilic fungi from soil by means of agar dilution plates used for quantitative determination of other soil microorganisms and this was found to be the case with soil crumb plates in this present work. It is possible that the conditions provided by the agar substrate were too severe for

the keratinophilic fungi to compete against other soil "sugar" fungi, which in many cases were able to grow much more quickly.

Other species of fungi were isolated by the hair baiting technique and the results can be seen in the Appendix. Griffin (1959, 1960) described the sucession of fungi colonising hair in great detail. Although these species are not all keratinophilic, but use the non-keratinaceous substances, some like Monodictys levis (Acrospeira levis) are able to utilise keratin (Anglesea and Swift, 1971). Gentles (1967) commented on the fact that some Chrysosporium species were keratinophilic, but appeared to be less closely related to the ringworm fungi than A. uncinatum, T. terrestre or M. gypseum. He also proposed a classification where M. cookei, T. terrestre and T. mentagrophytes were thought to predominate on animals and man, but were capable of survival and / or sexual reproduction in soil. Dawson (1964) found that T. terrestre was isolated from a higher proportion of animals than could be explained solely by contamination and Marples and Smith (1962) first suggested T. terrestre was a natural inhabitant of the animal body (a hedgehog) rather than a soil contaminant. Microsporum cookei and T. terrestre are both usually considered non-pathogenic, but Shik (1965), Mariat and Tapia (1966) have isolated M. cookei from man and animals, while Alteras, Nesterov and Ciolofan (1968) have isolated T. terrestre from the same. Microsporum gypseum, T. ajelloi and M. nanum were the species in Gentles' (1967) classification which predominated in soil and were capable of at least transient survival on animals' bodies. Now it is thought that T. terrestre, A. uncinatum, M. cookei and M. gypseum are true soil inhabitants with at least the capacity for transient survival on animal bodies, whereas, M. nanum and T. mentagrophytes are considered to be zoophilic, but can survive for a short time in soil (Ajello, 1960; Dvořák and Otčenášek, 1964).

Although anthropophilic fungi may be present in soil, because of their low keratinolytic ability they are never isolated from soil by the hair baiting technique (Bieżunska, 1963).

In the Appendix, a table of geophilic fungi with their animal and human hosts is presented, whether the fungi were pathogenic or not.

English and Morris (1969) isolated Trichophyton erinacei from 14 hedgehog nests, but no dermatophytes were isolated from the nests of the Nottingham hedgehogs. The winter nests studied by English and Morris (1969) were made of grass and leaves, whereas those in this study were composed of hay alone. English and Morris stated that in the summer months wild hedgehogs do not use nests, but may make a flimsy shelter. This was not the case in this study, because the animals were not free to roam, but were caged and used the nests as a means of shelter. English and Morris (1969) found an exact correlation between infection of hedgehogs and their nests and it would appear that this was the case in this study, i.e. no hedgehogs were infected when sampled from summer nests and no nests harboured any dermatophytes. English and Morris (1969) considered summer nests as unimportant in the transmission of ringworm. At Nottingham, the hedgehogs sampled were kept in separate cages overwinter and remained healthy.

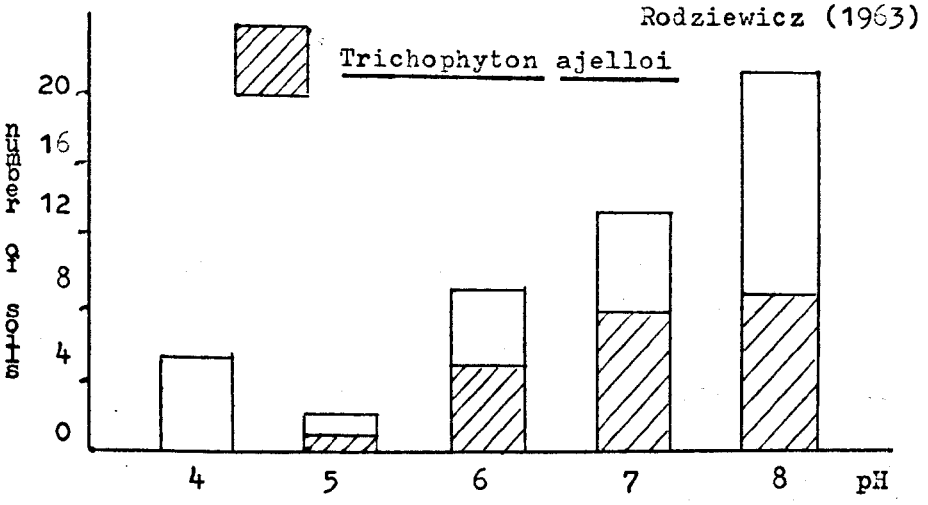
Morris and English (1973) suggested that T. erinacei is a weak pathogen and although they thought that hedgehogs living in close quarters were more likely to be infected than those in low densities, they found that transmission was still relatively slow. Morris and English (1969) found 20 - 25% of British hedgehogs to carry ringworm and more males were affected than females. However, although T. erinacei has been isolated from

hedgehogs by Marples and Smith (1960), Hellier and La Touche (1962), La Touche and Forster (1963), English, Evans, Hewitt and Warin (1962), Smith and Marples (1963), English, Smith and Rush-Munro (1964) and Morris and English (1969) and T. terrestre has been isolated by Marples and Smith (1962), only one hedgehog (of 40) harboured T. erinacei in this present work and on just one occasion. It may be that the hedgehogs kept at Nottingham University were only retained if in good condition and if they appeared to be infected they were discarded, hence the fact that none seemed to permanently harbour any dermatophytes.

Morris and English (1973) discussed in detail the behaviour of the hedgehog in its natural state which helps to minimise the infection from dermatophytes.

Böhme and Ziegler (1969) state that T. ajelloi is almost evenly distributed in soils with more or less acid pH. Marples (1965) and Pugh (1966) also found this to be the situation. Böhme and Ziegler said that the percentage distribution of T. ajelloi is even in soils with pH lower and higher than 6.0, and they considered that this work contradicted Marples' discovery that T. ajelloi frequently occured in soils with pH <6, but significantly less in soils with pH >6. However, the average pH of soils yielding T. ajelloi recorded by them (5.7) fully agreed with values which Pugh (1966) found for birds' nests (5.7). Pugh (1966) considered that his work corroborated Marples' results, but conflicted with that of Rodziewicz (1963) who found that T. ajelloi most frequently grew in soil of pH 8 and 7, although it was not isolated from soil of pH 4. On close inspection, the work of Rodziewicz gives a false impression. Since he obtained more soils at pH 7 and 8 than pH 4, 5 or 6,it is not surprising that most T. ajelloi was isolated at pH 7 and 8. If the percentage of T. ajelloi in soil samples of different pH values

is studied, a more meaningful result is obtained:-



4	5	6	7	8	
0/5	1/2	5/9	8/13	9/21	= isolations / no. of soil samples
0	50	55	61	42	= % isolation

It can be seen in this interpretation that the distribution of T. ajelloi is even from pH 5 to pH 7. At pH 8 the percentage of soils from which T. ajelloi was isolated is only 42%. Therefore, it seems that despite the apparent contradictions in the literature, most of the documented work seems to favour a distribution of T. ajelloi on the acid side of neutral. Chesters and Mathison (1963) found that in pure culture work, T. ajelloi grew in pH 7.5 up to 8.7 medium. Competition from other soil organisms in vivo may counteract this.

The results of the present work indicate that conidial A. uncinatum is evenly distributed in soils with pH values lower and higher than 6. (See Table 6).

The results of percentage colonisation of animal wool by keratinophilic fungi in the soils of 3 different pH values, collected from Nottingham University Agricultural School, Sutton Bonington, indicated that the optimum pH for conidial A. uncinatum was on the acid side of neutral (6.7). However, no soil pH values between 4.2 (the minimum pH studied) and 6.7 were used, so that the average pH of soils yielding most conidial A. uncinatum could be lower than 6.7.

Trichophyton terrestre showed highest percentage colonisation in soils of pH 8. The isolations from all other sites also showed highest percentage frequency in soils of pH value 7.1 - 8.0 (77%). Between pH 3.1 and 7.0 the average percentage isolation was 55%. This was similar to the result for C. keratinophilum, for all other sites besides Sutton Bonington. Chrysosporium keratinophilum was virtually absent from the 3 soils from this particular site. Pugh and Mathison (1962) found that C. keratinophilum preferred weakly alkaline soils, and Bohme and Ziegler (1969) reported both T. terrestre and C. keratinophilum more from soils of pH > 6 than pH <6 and this was also true for M. cookei.

In this present work M. gypseum and M. cookei were both isolated from the observational soils studied, mostly in the range pH 6.1 and 8.0, with M. cookei also being isolated between pH values 5.1 and 6.0. The percentage colonisation results from the 3 soils from Sutton Bonington are in full agreement with the results from all other soils, i.e. M. gypseum was isolated in greatest numbers at pH 8 and M. cookei at both pH 6.7 and 8.0 equally. Somerville and Marples (1967) could find no correlation between the occurrence of M. gypseum and pH of the soil habitat within a range from 5.5 to 8.5. Although Pugh (1966) isolated little M. gypseum, he suggested that it preferred acid conditions, in agreement with Mohapatra and Gugnani (1964). Steinerová and Buchvald (1967) also showed that this species was found in soils with an average pH of 5.2. Their results

do not agree with those in the present work for M. gypseum, but their observations of T. terrestre in soils of average pH 6.7 do agree.

Trichophyton terrestre was the keratinophilic fungus which was isolated almost ubiquitously in the coastal ecosystem. Schönfeld, Rieth and Thianprasit (1960), Pugh and Mathison (1962), Gip and Paldrok (1966) and Orrù, Pinetti and Aste (1968) also found T. terrestre in sand and beach samples from the Baltic Sea, Gibraltar Point, Lincolnshire, Sweden and Sardinia, respectively. Dabrowa, Landau, Newcomer and Plunkett (1964) isolated T. terrestre from sea-bird feathers and sea urchin spines on the sands of tide-washed areas on the California coast. They suggested that these results indicate that the intertidal zone may constitute an additional reservoir of potentially pathogenic fungi and that certain fungal diseases may be acquired by exposure in those areas, although no dermatophytes have been isolated. No dermatophytes have ever been found under sea water (Schönfeld et al., 1960; Gip and Paldrok, 1966 and Dabrowa et al., 1964), but Kishimoto and Baker (1969) isolated E. floccosum, M. gypseum, M. canis, T. rubrum and T. mentagrophytes from beach sands. More pathogenic and potentially pathogenic fungi were isolated from areas where the swimmer density was high, and few were isolated from low density areas. The recovery of dermatophytes from beach sands is of epidemiological importance because, even if the fungi remain there for only a short time they are still a potential source of infection for bathers.

No dermatophytes were isolated from the bare mud at Gibraltar Point, i.e. from the area most frequently inundated with sea water. These findings agree with those documented in the past by Pugh and Mathison (1962), although they did isolate a Chrysosporium species described as conidial A. curreyi. This fungus was regularly isolated

on birds (Pugh, 1964), and while it is not pathogenic, it may grow on their feathers while still attached to the bird. The isolation of this species by Pugh and Mathison (1962) was at high water mark rather than bare mud.

Just above and below high water mark, in addition to T. terrestre, M. cookei and Ctenomyces serratus were isolated, but in low numbers. Pugh and Mathison (1962) found C. serratus more commonly in bird traps than dunes. However, at high water mark there was an abundance of debris which included feathers and dogfish egg cases. The reason so few keratinophilic fungi are isolated from tide-washed areas may be because keratin sources are removed by tides more frequently than from sands not washed by the sea. In addition, Pugh (1961, 1962) suggested that the increased fungal colonisation up the shore was a reflection of the greater amount of organic matter present as a result of increasing cover by higher plants and also because of reduced periods of inundation by the sea.

Microsporum cookei and T. terrestre appear to be able to tolerate the infrequent washing with sea water, since they were found below high water mark. A combination of lack of keratin and the inability to tolerate sea water would appear to determine the dermatophytic flora of coastal soils.

The highest incidence of all species except M. cookei was in the bird traps, suggesting that the source of keratin was very important for these fungi. Since some of them were isolated from sea-washed areas, the sea water plays a secondary role to the source of keratin in determining the distribution of these fungi.

The isolations of conidial A. uncinatum from Gibraltar Point from foredunes and bird traps disagree with the results of Pugh and Mathison (1962). Since the results reported here were only low percentages, it has been suggested that the isolations of this fungus were contaminants from the laboratory. However, more than 12 years have lapsed since Pugh and Mathison studied

Gibraltar Point and the pattern of distribution may have changed. Pugh (1971) suggested that either the pH of the soil or the influence of sea water determined the absence of conidial A. uncinatum at Gibraltar Point. However, the pH (of average 7.6) was in the range for growth of conidial A. uncinatum and it may be that neither pH or sea water are the limiting factors.

Pugh and Mathison (1962) found that M. gypseum and conidial A. uncinatum were not isolated from salt marshes or sand dunes which are alkaline soils, but in the present work it has been found that these 2 species are capable of growth in alkaline soils, therefore, pH does not seem to be the limiting factor.

Arthroderma curreyi and Ctenomyces serratus were most frequently isolated (Pugh and Mathison, 1962) and the latter has been found in bird nests of pH 7. Since C. serratus has also been isolated below high water mark and mostly where there is an abundance of keratin, the keratin would seem to influence the occurrence of C. serratus rather than pH or sea water. A. curreyi, on the other hand, was found at high water mark, but infrequently in birds' nests. Therefore, although it can tolerate alkaline pH and salt concentrations, it is unable to compete for keratin in the bird trapping chambers against such a keratinophilic fungus as C. serratus.

Trichophyton terrestre was found in salt conditions and in alkaline soils and this is perhaps why it is found ubiquitously, in addition to the possibility that it can grow better when no other species are there for it to compete against.

When the results are related to those from the hedgehog pens at Nottingham, it can be seen that some species, excluding T. terrestre, seem to be dependent upon a keratin source for their increase, e.g. Chrysosporium keratinophilum, M. cookei and conidial A. uncinatum, but for T. terrestre and M. gypseum the lack of keratin does not appear to be a limiting factor.

- In general, an immediate drop in percentage colonisation of wool baits by conidial A. uncinatum was seen after the fungicide treatment at Rothampsted. This is the same sort of observation made for true soil fungi,e.g. Penicillium, Trichoderma and Gliocladium, whereas bacteria showed an initial increase and then fell dramatically when nutrients were depleted (Williams, 1973; Wainwright,1974). Corden and Young (1965) found that in soil treated with 5 fungicides which they studied, the fungal population decreased rapidly and then increased due to reproduction of certain fungi. The fungicides are obviously effective for an initial "kill off", but Newhall (1958) found that several fungicides were leached from soil very quickly (e.g. formaldehyde and captan) and failed to kill below one inch of soil. They were also reported to be rendered ineffective on the way down through the soil. This means the effect of the fungicides may not be long-lasting and the fungi can recover quickly, especially if the compounds are fungistatic rather than fungicidal. It is also possible that the fungicides can cause genetic mutations, creating fungi which are resistant to the chemicals.

Some fungi are able to adapt to captan (Parry and Wood,1959a), but no strains of fungi so far tested have been found to be resistant to thiram (Parry and Wood, 1959b). These investigations did not include keratinophilic fungi. Dekker (1972) reports that Webster et al.(1970) found Botrytis cinerea acquired resistance to dicloran. Dekker also considered that acquired resistance may be due to detoxification, e.g. some fungi are able to convert quintozene into much less fungitoxic compounds i.e. p-chloro-aniline and p-chloro-anisole.

After the initial killing of organisms, percentage colonisation recovered to be either virtually the same as the control or more. It may be that the keratinophilic fungi find conditions for growth better when there is less competition from other soil

fungi and that when the soil fungi increase again, the numbers of keratinophilic fungi fall.

Percentage colonisation at the end of sampling was higher on greasy wool than degreased wool at both Rothampsted and Sutton Bonington. As this was the reverse of the normal isolation pattern, it raises the question of whether the grease plays any part in the deactivation of the fungicide in the baited soil. It may be that the grease is utilised better by any strains of conidial A. uncinatum which may survive fungicide treatment, i.e. conidial A. uncinatum has its ability to use the grease increased.

At Rothampsted, C. keratinophilum was affected by captan and formaldehyde on degreased wool and by thiram on greasy wool. In the other combinations of fungicide and baits, there was no effect. Captan and formaldehyde are fungicides which would be leached from soil quickly and although they did have an immediate effect, it would not be expected to be long-lasting.

At Sutton Bonington, the final results for C. keratinophilum resembled those for conidial A. uncinatum for degreased and greasy wool, i.e. an increase in percentage colonisation on greasy wool and a decrease compared with the control on degreased wool was seen. Since C. keratinophilum is known to be isolated more on greasy wool than degreased, this is not unexpected (Pugh and Evans, 1970b).

Munnecke (1961) found that the movement and penetration of fungicides through soil in columns were related to effects due to soil type, to various soil treatments and to inherent fungicide factors. Treatments of soil which made it more porous increased fungicide penetration, especially of those applied as suspensions. The fungicides in the present work were applied in suspension, with the exception of formaldehyde, which was in solution. Fungicides prepared as solutions penetrate soil more readily than those prepared as suspensions. Therefore, one would expect that

formaldehyde would take effect fairly rapidly, but because it can be leached from soil quickly, it would only have a short-lived effect. The action of the other fungicides in suspension might be prolonged. This appeared to be the case for degreased wool at Rothampsted. The initial fall in percentage colonisation of conidial A. uncinatum after application of fungicides was greater for formaldehyde than any other fungicide studied and yet the recovery of conidial A. uncinatum was quickest for formaldehyde. However, no initial decrease in percentage colonisation of greasy wool by conidial A. uncinatum was seen, and this may be because of the possible part played by the grease in the deactivation of the fungicide. A delayed decrease was seen at 38 days, but it was not prolonged. Finally, conidial A. uncinatum in soils treated with formaldehyde increased the most after 120 days, i.e. in these soils the fungus was quick in recovering.

Fluctuations of C. keratinophilum in the soil are so great, that it is not possible to determine any such pattern of frequency due to the difference in the physical properties of the fungicides.

The important times to sample the soils treated with fungicides would appear to be near the days of treatment, rather than over a period of time. This makes it possible to study small differences rather than gross ones, more clearly.

Tinea pedis survey

Communal life offers more opportunities for dissemination of organisms causing tinea pedis; because of this the study of such communities can reveal much about the epidemiology of the disease. Groups of people have been studied in the forces (Ajello, Keeney and Broyles, 1945; Sanderson and Sloper, 1953; Marples, 1958; Ohm and Skogland, 1968 and Davis, Garcia, Riordon and Taplin, 1972), in boarding schools (English, Gibson and Warin, 1961), at universities (Legge, Bonar and Templeton, 1929 a, b; Gould, 1931; Muskatblit, 1933; Marples and di Menna, 1949; Doby-Dubois, Berthault and Doby, 1961), in the mining industry (Adamson and Annan, 1949; Gentles and Holmes, 1957; Holmes, 1959; Koch and Schippel, 1971). and in families (English and Gibson, 1960).

Unfortunately it is not possible to say how the present results compare with those from outside the university environment. A university gathers people from a variety of backgrounds and with varying interests, but when they are living in halls of residence, people who would normally not come into contact, do associate in such a way that cross infection is more probable than outside the university. English and Gibson (1960) found that the number of baths taken per week increases the chances of cross infection in families. At the university halls of residence, the supply of hot water is virtually endless and therefore the number of baths or showers taken is probably considerably higher than if the same people lived at home or in a flat.

Many people have discussed susceptibility factors in fungus infections (Legge, Bonar and Templeton, 1929; Hopkins, Hillegas, Camp, Ledin and Rebell, 1944; Schwartz, 1947; Rothman, 1949; Rosenthal, Baer, Litt, Rogachevsky and Furnari, 1956; Gentles and Holmes, 1957; Vanbreuseghem, 1958; English, 1961; Pan, 1964;

Il'in, 1966; Rosenthal and Baer, 1966; Marples, 1968; Bhutani, Mohapatra and Kandhari, 1971; Koch and Schippel, 1971; Davis, Garcia, Riordon and Taplin, 1972; and Tronnier, 1968), and possible sources of infection (Baer, Rosenthal, Rogachevsky and Litt, 1955; Gentles and Holmes, 1957; Fichtenbaum, 1966; Alteras, Cojocaru and Hontaru, 1967; Alteras and Cojocaru, 1973; Gentles and Evans, 1973). Rosenthal, Baer, Litt, Rogachevsky and Furnari (1956) found that viable fungi must be constantly and readily shed from clinically and subclinically infected feet and therefore exposure and reexposure to these fungi is practically inescapable and unpreventable.

It would seem that on arriving at Nottingham University, very few students have tinea pedis. The percentage incidence of the 2 new intakes of students was virtually identical at 10% of the sample. Although the numbers of students sampled is considered by some to be low and possibly not statistically viable (Everall, 1974, personal communication) the results are sufficient to indicate possible trends and allow at least tentative suggestions.

1. Seasonal occurrence

The sample in May showed an increase from 9% to 24%. This type of seasonal increase has been reported before by English, Warin and Gibson (1961) in a boys' boarding school visited in October and June, when the same boys were studied. Legge, Bonar and Templeton (1929), Il'in (1966) and Padhye and Sekhon (1973) reported a similar occurrence and the latter found an even bigger increase later in the summer, from August to October, although these were not necessarily the same group of people studied over the year. At this time of year, Nottingham students were on vacation and were therefore not studied. However, these students showed a return in the following October to the initial incidence. More outdoor swimming would have taken place between

June and October, but at some time during the vacation the students either cured their tinea pedis or it disappeared of its own accord. This may be due to better hygiene standards at home or attention brought to the disease by more knowledgeable parents. Many students were unaware that they had tinea pedis and Gentles and Evans (1973) also found that few infected bathers were aware of their infection and not many people knew of the dangers of contamination in communal bathing. Even those who knew of the risks did not always take precautions. Few of the students at Nottingham knew what the symptoms of the infection were, and this has been reported before by Holmes and Gentles (1956), English and Gibson (1959b).

The increase in May could be associated with warmer weather as suggested by English, Gibson and Warin (1961). Padhye and Sekhon (1973) considered humidity and warmer temperatures more favourable than dry and low temperatures of winter, Vanbreuseghem (1958) suggested that dermatophytoses of feet were probably favoured by warmth, i.e. spring and summer, and a generally hot climate favours their development. Il'in (1966) considered that the increase in incidence of tinea pedis which he reported was probably due to increased humidity and precipitation during the summer, i.e. when the increase took place. Gentles and Holmes (1957) considered that hot working conditions in deep hot pits did the aggravating. Vanbreuseghem (1967) reported that high temperatures, where miners or blast furnace workers were, cut down their natural resistance.

Many students wear plimsolls all day regardless of whether they played sports or not. Since rubber shoes prevent good aeration of feet, the atmosphere inside the shoe is warm and moist. Pan (1964) found that in Taiwan, the highest rate of infection in men was in those who wore shoes (office workers and students) whereas in women, it was those whose feet were in

wet surroundings (housekeepers and workers). Although Peck, Rosenfeld, Leifer and Bierman (1939) considered sweat probably to have fungicidal powers, little sweat is produced by feet (Marples, 1968). Weidman,(1927) thought that moisture, especially from sweating, was conducive to infection, especially in intertrigous places.

However, as more people started to remove their socks and stockings in warmer weather, it was suggested that exposure to the air would help to keep feet cooler and drier and therefore less prone to developing tinea pedis.* This may explain the fall in October 1973, although May might have been too early for removal of hosiery. From the questionnaire it was found that 3 times as many women (90%) discarded their hosiery in summer as men (30%). Since this was the case one might expect the incidence of tinea pedis in women to decrease more than in men in October, but this was not so. The incidence of the disease in women was twice that of the initial incidence, but that of the men had virtually returned to the initial incidence. Therefore, it seems that the women either came into greater contact with tinea pedis than did men in summer, or that they are exposed to tinea pedis for a longer period of time than are men. Vanbreuseghem (1967) wondered whether higher prevalence is due to larger numbers of contacts with pathogens or to a progressive breakdown of resistance of skin or of some other unknown barrier.

* (Flowers - personal communication)

2. Male : female ratio

Overall the incidence of tinea pedis was found to be twice as much in men as women. The fact that more men suffer from tinea pedis has been found by several other workers (See table overleaf). On the other hand, Rosenthal and Baer (1966) found that there was no difference in susceptibility to T. rubrum and T. mentagrophytes of males and females. Vanbreuseghem (1958)

Table of documented male : female infection ratio.

	male : female
Legge, Bonar and Templeton, 1929a	53.3% : 15.3%
1929b	78.6% : 17.3%
Gould, 1931	50-90% : 4-15%
Marples, 1958	17.8% : 5.9%
English and Gibson, 1959	4 : 1
English, 1961	6.5% : 1.6%
Marples, 1968	16.3% : 2.3%
Cordonnier, Lundy-Mahieu, Parent and de Beer, 1971	2 : 1
Gentles and Evans, 1973	21.5% : 3.3%
	6.3% : 0.9%

reported that tinea pedis occurred as frequently in men and women, but appeared to acquire a real importance with men more often than with women. Pan (1964) discovered that the rate of tinea pedis infection was the same in men and women in Taiwan, while Marples and Bailey (1957) found no differences between infection in men and women.

Legge, Bonar and Templeton (1929) stated that the reason women have less tinea pedis than men did was because their habits were cleaner and they observe a much higher type of personal hygiene; they perspire less than men and wear lighter shoes, which are better ventilated and changed more often. They also reported that at California University, women students had to wear rubber bathing shoes and were not to walk barefoot

in showers, pools or gymnasia, whereas the men constantly walked in their bare feet. In addition, there was a lack or non-use of bathing shoes and inferior sanitary facilities. Cordonnier, Lundy-Mahieu, Parent and de Beer (1971) also considered women to have higher hygiene standards than men did. The results from the questionnaire endorse this suggestion. The use of talcum powder, whether containing a medicinal preparation or not, after bathing helps to keep feet dry by absorbing moisture and a change of hosiery daily means that if any skin particles which are infected, are inside the socks or tights, continual contact from the same sock is prevented.

The numbers of students who had been treated by a doctor for tinea pedis before coming to university were very similar in both men and women, and in I and IV. This also applied to the numbers who used medication for prevention. Rosenthal and Baer (1966) said presence of blistering encouraged infection.

All these factors were discussed by Schwartz (1947). By use of a) talcum powder, b) wearing shoes in showers c) thorough drying of skin, d) changing to clean dry hosiery frequently and e) wearing shoes in gymnasia, elimination of tinea pedis is helped even in the presence of heat, humidity, local trauma and communal living (Schwartz, 1947; Davis, Garcia, Riordon and Taplin, 1972 and Legge, Bonar and Templeton, 1929.) However, Marples (1968) found that in shoe-wearing populations, the interdigital spaces of feet, especially the 4th space, appears to provide a particularly favourable habitat which supports a rich and varied flora. "Desquamated skin tends to remain in place, the opposed surfaces reduce loss of water, absence of sebaceous glands reduces supplies of inhibitory components in surface film and the skin temperature, although variable, remains at a level which is generally lower than on the head or trunk."

Bhutani, Mohapatra and Kandhari (1971) also said that, in

an urban group which they studied, footwear was a major contributary factor. This may be true for people who harbour a dermatophyte on the skin in the interdigital spaces, but for those who do not have tinea pedis or harbour a fungus, this probably is not so. Because Bhutani, Mohapatra and Kandhari (1971) found that shoes contributed to their groups infection, it does not mean that going around barefoot necessarily prevents one from contracting tinea pedis, but the better aerated the shoes, the less the situation will be exacerbated. Nickerson, Irving and Mehmert (1945) have suggested that reduction of humidity of the feet by wearing open-toed sandals is beneficial in the prevention of foot infections.

Legge, Bonar and Templeton (1929) noticed the persistence of tinea pedis in those wearing woollen socks. Wool is a keratin source and it may be that the dermatophytes can utilise this as a food source. Broughton (1955) isolated T. mentagrophytes var interdigitale even from laundered socks of infected patients. Legge, Bonar and Templeton (1929) thought that wool produces heat and moisture, but it is more likely to absorb moisture than nylon is. More women (50%) than men (30%) wore nylon hosiery, whereas men wore mixtures (55%) more than did women (41.6%).

Apart from the question of hygiene, fewer women took showers after sports. They were inclined to take baths at their halls of residence, where the number of girls allotted to one bath (4) was less than the number of men (12), athletes and non-athletes, using showers in the mens' halls. This would help explain the higher incidence in men and why non-athletes contracted the disease.

	% infected
Men who used showers	18.6
Men who did not use showers	6.1
Women who used showers	8.0
Women who did not use showers	12.8

Therefore showers appear to be the main source of cross infection.

Trichophyton mentagrophytes var interdigitale was by far the most common dermatophyte isolated. Many people have reported the relative incidence of T. mentagrophytes and T. rubrum, but no definite conclusions have been reached yet. Some people found T. rubrum to be the most common dermatophyte isolated (Kornbleet, 1960; Lavalle, 1966; Ohm and Skogland, 1968; McCaffree, Fethière and Blank, 1969; Shetsiruli, 1969a,b; Galler, 1971 and Hantschke, 1971) and others found T. mentagrophytes to be the more common. Those who reported this as being so were: Burke and Bumgarner, 1949; Marples and di Menna, 1949; Hall, 1956; Gentles and Holmes, 1957; Cremer, 1959; Holmes, 1959; Marples, 1959; English and Gibson, 1959, 1960; Alteras, Cojocaru and Hontaru, 1967; Koch and Schippel, 1971; Panfilis and Vidoni, 1971 and Gentles and Evans, 1973. On the other hand, some people have discovered that although T. mentagrophytes is most common, the incidence of T. rubrum is increasing (Bienias, 1963; Meier, 1967 and English and Turvey, 1968), while Rosenthal and Baer (1966)and Blaschke-Hellmessen (1968) found no differences in the relative incidence of the two species. The reason for the discrepancy may just be that although T. mentagrophytes is common, T.rubrum infections are more serious and more difficult to cure and therefore more persistent.

Epidermophyton floccosum is quite rarely found in large numbers (Ajello, Keeney and Broyles, 1945, Marples, 1958; Holmes, 1959; Koch and Schippel, 1971.)

3. Left : right foot ratio.

The incidence of tinea pedis in relation to right and left feet is of some interest. One might expect that half as many people would have an infection on both feet as on one foot, but this was not the case. Both feet were much less infected than one foot ($p = < 0.001$). One wonders why there is so little cross

transfer. Many wives, according to Weidman (1927), were free from dermatophytes whereas their husbands were notably infected. Immunity was given as the reason for this, but this does not explain why more men have more 2 foot involvement than women. Some people are able to acquire immunity if they have been infected before (Jones, Reinhardt and Rinaldi, 1974). Rothman, Knox and Windhorst (1957) thought that a T. rubrum infection they found was transferred from husband to wife and Greenbaum (1924) quotes Sabouraud as saying that differences in susceptibility could be attributed to differences in reaction of sweat. It is thought that women are less susceptible to disease than are men, but it is also possible that socks can be put on either foot, aiding cross transfer, whereas with tights (some brands) they are put on the same feet each time, or it may just be that they are washed more frequently. Since tights are a fairly new innovation this would not account for results found in 1929 by Legge, Bonar and Templeton.

It was found that there was more infection on the right foot than on the left. It is not known why there should be this difference. It has been suggested (Pugh, personal communication) that right-handed people may be able to dry their left foot more easily than the right. When sampling took place, it was noticeable that some people were more sensitive on one foot than the other, but at the time no note was made of which foot this was. If the right foot were more sensitive, it may be possible that these people could not bear to dry that foot as carefully as the other.

4. Influence of sports.

The sports that the students played can give an idea of where infection may be transmitted. Those students who played all sports documented showed a higher incidence of tinea pedis after one year at university. This may be because they took more showers when at university rather than at school, or it could be that

because so many more people played sports, the opportunity for infection becomes greater, as does cross transfer. In the case of those students who played rugby, football, badminton and squash, there was a decrease in the incidence of the disease after May, probably because these sports were played less in summer, in contrast to tennis, athletics and swimming. The students who played these sports had an increase in tinea pedis by October 1973. Legge, Bonar and Templeton (1929) reported an increase in the incidence of tinea pedis from 53% to 78.9% in men and 15% to 17.3% in women after 2 semesters, when they had been engaged in physical exercise and showers. In addition, the men went round in bare feet in the gymnasium and the women did not.

Fichtenbaum (1966) demonstrated tinea pedis in 64% of swimmers, 87% athletes and 40% bath attendants. Alteras, Cojocaru and Hontaru (1967) found 62% of swimmers and trainers were infected. Gentles and Evans (1973) considered that infection was clearly spread within the swimming baths that they studied and Alteras and Cojocaru (1973) thought swimming pools, beaches and public baths were the main sources of infection, as did Percebois and Chevalier (1972). However, Baer, Rosenthal, Rogachevsky and Litt (1955) found what they termed "convincing evidence", that exposure to fungous material in swimming pools, showers stalls and other such places plays a minor role in eliciting acute infection of the feet. Several workers have found floors a source of infection and even if not very important, they still exist as reservoirs of infection. Adamson and Annan (1949) were the first to isolate T. mentagrophytes from a skin scale on the floor of a bathhouse. English and Gibson (1959) isolated T. mentagrophytes from the floors of swimming pools. Gentles (1956) isolated T. mentagrophytes and T. rubrum from floors of communal bathing places and Gip and Aschan-Aberg (1968) cultured T. terrestre,

T. mentagrophytes and E. floccosum from floor surfaces of an open-air public bath in Sweden. Trichophyton mentagrophytes, T. rubrum, E. floccosum, T. terrestre, T. ajelloi, M. cookei, M. nanum and M. gypseum have been found on floors of army barracks (Gip, 1964) and the first 4 have been isolated from floors of a bath (Gip, 1967). Bocobo and Curtis (1958) and Bocobo, Miedler and Eadie (1963) isolated T. mentagrophytes from schoolhouse floor sweepings and Ajello and Getz (1954) recovered from shower stalls and shoes, T. mentagrophytes, T. rubrum and E. floccosum. These 3 species have been isolated from samples around a swimming pool and in shower cubicles where the floor was always wet (Drouhet, Marcel and Labonde, 1967). Ajello and Getz (1954) cite the work of Grenecser (1937) who reported T. mentagrophytes from a bath mat. Cordonnier, Lundy-Mahieu, Parent and de Beer (1971) isolated T. mentagrophytes, T.rubrum and E. floccosum from a swimming pool edge. Two-thirds of the isolations were from men's cubicles, sides of pools and on steps. The other third of the isolations were from men's showers, women's cubicles and showers. Very little was isolated from water, but twice as many isolations were from men's areas than women's.

Gip (1967) found that the isolations of dermatophytes could be cut down if feet were washed before walking across floors, especially when numbers of bathers were large. Bonar and Dreyer (1933) discovered that T. interdigitale could grow readily on floor material which was covered by a coating of slime or algal growth. Gould (1931) reported that ringworm fungi could be isolated from wooden floors, runways, benches, diving boards and rubber and canvas mats at swimming pools, inaddition to being found on towels and soap.

Doby-Dubois, Eon and Doby (1961) reported that 33% who played sports were infected, whereas only 3.8% of non-players were infected. Legge, Bonar and Templeton (1929) found 85% of men

who were required to take gymnastics at California University, were infected. Vanbreuseghem (1958) said that tinea pedis is supposed to be more common amongst those who use swimming pools and shower baths, but that the part played by these 2 places, in transmission of disease, is very debatable.

Baer, Rosenthal and Furnari (1955) and Baer, Rosenthal, Litt and Rogachevsky (1956) considered from experimental infection, that in most people the skin of the feet has a remarkable capacity for ridding itself of pathogenic fungi. This may help to explain why the incidence of tinea pedis decreased in October 1973, and may be the factor which causes the elimination of the disease in some students. Certainly, since so few students had been treated for tinea pedis (3%) and few took any form of preventive measures (9%), cures by professional treatment did not appear to be the reason.

Comments on methodology

Friedman, Derbes, Hodges and Sinski (1960) commented on the fact that investigators carried out microscopic studies less frequently than cultural studies. This being partly due to the fact that if cultural studies are to be carried out, then microscopic ones are unnecessary and time-consuming, because cultural studies not only tell whether a fungus is present, but the species can be identified too. However, they comment on the fact that contamination from the air in the laboratory can result in false recordings. Friedman, Derbes, Hodges and Sinski (1960) found Microsporum audouini and Trichophyton tonsurans occurred as airborne contaminants in the environment of a dermatology clinic and Clayton and Noble (1963) isolated dermatophytes and Candida albicans from air when examining patients with groin infections. Midgley and Clayton (1972) found that, although dermatophytes were rarely present in the microflora of air or skin of healthy individuals, when patients suffering from mycotic infections of the skin were examined in confined spaces, the dissemination of dermatophytes was demonstrated by recovery from air and normal areas of the body.

The fungi isolated on the ink blue-supplemented malt extract agar all turned the agar colourless. Baxter (1965) found that 24 keratinophilic fungi that he studied had the ability to do this, including T. mentagrophytes, T. rubrum, T. terrestre, T. ajelloi, M. gypseum, M. cookei and Ctenomyces serratus. In this present work, Chrysosporium keratinophilum has also been found to possess the ability to decolourise the ink blue medium. In the appendix are some photographs of those keratinophilic fungi which, in the present work , were able to change the ink blue medium to colourless.

SECTION II
EXPERIMENTAL

INTRODUCTION

There appeared to be a correlation between the occurrence of some keratinophilic fungi and the soil pH and temperature at Highfield's swimming pool and in the hedgehog pens. Further experiments were carried out to elucidate this situation for conidial Arthroderma uncinatum and other keratinophilic fungi.

The effect of temperature on growth has been studied before by other workers, (Kadisch,1929; Gabrielsen, 1943; [illegible]; Robbins and Ma, 1945; Giblett and Henry, 1950; Stockdale, 1953; Gentles, Dawson and Brown, 1964; Evans, 1969; Pugh and Evans, 1970b; Saëz and Battesti, 1970 and Engelhardt-Zasada and Prochacki, 1972.). This study provides a comparison of growth of six keratinophilic fungi at different temperatures.

At Highfield's swimming pool and the hedgehog pens some of the keratinophilic fungi did not seem to be influenced by the presence or absence of keratin, viz skin and hair from swimmers or spines and scales from hedgehogs. In fact, some keratinophilic fungi appear to have an even distribution throughout the year. These observations pose questions on the status of these fungi in the soil. Do they form survival structures,or are they able to grow through the soil utilising carbon and nitrogen sources in the form of simple sugars and amino acids,known to be present in the soil? Flaig (1971) said that according to published results, about 20 - 50 % of the organic-bound nitrogen is in the form of amino acids in the soil. Among those amino acids found in soil are histidine, arginine and lysine (Schreiner and Shorey, 1909, 1910a and b). Aspartic acid, serine, threonine, glutamic acid, hydroxyproline, glycine, alanine, valine, proline, isoleucine, leucine, ornithine, tyrosine and phenylalanine have also been found in

soil hydrolysates by Stevenson (1956) and Young and Mortensen (1958).

Gupta (1967) listed the different carbohydrates which have been found in soils, which included glucose, fructose and sucrose. Nagar (1962) indicated that common sugars e.g. glucose, exist in a free condition in soil organic matter.

Since conidial Arthroderma uncinatum, Trichophyton terrestre and Chrysosporium keratinophilum were the most abundant fungi isolated from all soils, it was decided to study the nutrition of these three fungi. Trichophyton ajelloi has been studied by Hejtmánek (1961), Mathison (1961), Ragot (1966a and b) and Evans (1969) from various angles, but T. terrestre and C. keratinophilum have been little studied. Kuehn, (1961) studied the nutritional requirements of Arthroderma tuberculatum and although this has been referred to as the perfect state of C. keratinophilum it has not been validated. Microsporum cookei and M. gypseum were the other two species isolated but they have been studied in some detail by Mandels and Shotts (1947), Johnson and Grimm (1951), Bereston (1952), Bereston, Robinson and Williams (1958) and Koehne (1962) and therefore it was thought unnecessary to study them further. This study was a new line of investigation to find the best combination of carbon and nitrogen source for optimum growth of each of the chosen species.

The effects of fungicides have been studied in vivo at Rothampsted and Sutton Bonington. Experiments to determine their effect in vitro upon keratinophilic fungi were carried out to help explain the results in vivo. For the same reasons, the effect of sea water upon these fungi was studied, i.e. to determine which factors influence the isolation of keratinophiles from different regions at Gibraltar Point. Orrù, Pinetti and Aste (1968) studied the effect of NaCl and sea water on some

dermatophytes, including Trichophyton, Microsporum and Epidermophyton species. On solid media 4 - 6% NaCl hindered growth and 8 - 13% inhibited it. Adding NaCl to garden soil or beach sand did not affect fungal growth but inhibited microconidial formation and the ability to attack hair bait. McGinnis and Hilger (1972) made a study of the ability of Trichophyton mentagrophytes to grow through soil because it has rarely been isolated from soil (Lurie and Borok, 1955; Rodriguez, 1958; Puri, 1961; Evolceanu, Alteras and Cojocaru, 1962, 1963; Rogers and Beneke, 1963; Shome, 1963; Campos and Vilella, 1964; Baxter, 1965, 1969 and Rioux, Jarry, Jarry and Bourelly, 1965). Since little is known about the ability of A. uncinatum, T. terrestre and C. keratinophilum to grow through soil it was thought that such a study would provide some useful information about these three commonly occurring keratinophilic fungi. The ability of these species to compete for colonisation of an agar plate was studied by the method of Wastie (1961) and their ability to compete with saprophytes in soil for a keratin bait was studied using soil colonisation tubes (Evans, 1955). For A. uncinatum this was complementary to the use of the fluorescent antibody technique.

The basic principle of the fluorescent antibody technique is an immunological staining procedure which allows the rapid detection of the specific fungus under investigation, in an antigen-antibody reaction. The use of fluorescein dyes achieves this end. Two advantages which this technique has are

a) that fluorescing antigen-bearing cells with distinct morphological characters can be seen, and

b) that even if the antigen is present in a small amount or in a mixture with other organisms it can easily be detected against a dark background.

The technique has been widely used in medical mycology

for the study of deep mycoses and yeasts. This work has been reviewed by Kaplan (1970) and Kaplan and Kaufman (1961). However, there are few reports on the application of the technique to dermatophytes. Although Grappel, Blank and Bishop (1967, 1968 a,b, 1969), Grappel, Buscavage, Blank and Bishop (1970), Evolceanu, Alteras and Nicolau (1966), Noguchi, Hattori, Shimonaka and Ito (1971), Basarab, How and Cruickshank (1968) and Andrieu, Biguet and Laloux (1968) have all carried out immunological studies on dermatophytes, only Miura (1968) and Walzer and Einbinder (1962) have made immunofluorescent studies on this group of fungi. These authors used the indirect fluorescent antibody technique to study Trichophyton rubrum, T. interdigitale and T. mentagrophytes antibodies in the sera of patients with dermatomycosis and they both found a high rate of positive reactions occurring with sera. However, strong cross-reactions were seen and no direct relationship was found between the intensity of the reaction and that of infection.

Apart from this work no other applications have been reported for either zoophilic or geophilic keratinophiles.

One reason for this is that some keratinophilic fungi were found to be autofluorescent, e.g. Microsporum species. Stuka and Burrell (1967) found that young mycelia of T. rubrum were more antigenic than old and so gave varying degrees of fluorescence.

Despite these difficulties there seemed to be an obvious potential for the use of the technique to determine the competitive saprophytic ability in soil of A. uncinatum, which is the most abundant keratinophile in the soils of temperate regions. Some dermatophytes are considered to be good competitive saprophytes, e.g. Ctenomyces serratus, because in colonisation tubes it grew through non-sterile soil(Pugh,1964), but there is little evidence to support this other than his work. Pugh also found

that Arthroderma curreyi was not a good competitive saprophyte but was capable of survival in soil in the form of cleistocarps. It was hoped that the form in which A. uncinatum survived could be determined, and also to discover if it actually does grow through soil. The normal methods for isolating fungi from soil are unsuitable for determining the form of the fungus in soil.

A study of the imperfect and perfect structures of several dermatophytes was made with the scanning electron microscope.

MATERIALS AND METHODS

All soils used in the experimental section were passed through a 2 mm sieve before use.

Effect of temperature on the growth of keratinophilic fungi.

Discs from 2-week-old cultures of Trichophyton terrestre, T. mentagrophytes, Arthroderma uncinatum, Microsporum cookei, M. gypseum and Chrysosporium keratinophilum were cut with a sterile cork borer and plated out onto Sabouraud dextrose agar. A set of 5 plates for each fungus was incubated at 5°, 10°, 15°, 20°, 25°, 35° and 40°C. The diameter of each concentric colony was measured every day for 2 weeks and recorded as the mean of the readings, but if the colony was eccentric, 2 diameters were measured and the mean used as the result.

Effect of pH on growth of keratinophilic fungi.

a) liquid culture

Sabouraud's liquid medium was buffered to give pH values of 4, 5, 6, 7 and 8. For pH 8, the 1N NaOH used to make the medium alkaline was added aseptically after autoclaving. To make the medium acid 1N HCl was added. The medium was dispensed in 250 ml Erlenmeyer flasks in 50 ml quantities, autoclaved at 120°C for 20 min and when cool inoculated with 5 mm discs from 2-week-old cultures of T. terrestre, T. mentagrophytes, A. uncinatum, M. gypseum, M. cookei and C. keratinophilum. There were 3 replicates for each pH. The flasks were placed on a reciprocating shaker at 25°C for one week and then the contents of the flask were harvested, dried at 50°C for 48 h and then dry weight determinations were made. Growth was expressed as mgm mycelium/ 100 ml medium.

b) solid medium

The same method as for liquid culture was used to give

a range of pH values in solid media. However, the range could only be made from pH 5.0 to 7.1 because the agar would not solidify out of this range. Various other solutions had been considered to create the different pHs, but some, e.g. acetates and phosphates, may be toxic and others, e.g. citrates can be used as carbon sources, with consequent displacement of the apparent pH optimum (Cochrane, 1958).

The same source of inoculum as in liquid culture was used and the discs were plated onto S.D.A. and incubated at 25°C. Radial extension of growth was recorded every day for 2 weeks. Results are given as the mean of 3 colony diameters.

Effect of pH of soil on reisolation of keratinophilic fungi.

The soils of 3 different pHs from Sutton Bonington were autoclaved at 120°C for 1h on 4 successive days and then the sieved samples were placed in petri dishes and inoculated with 4×10^6 spores of T. terrestre, T. mentagrophytes, A. uncinatum, M. gypseum, M. cookei and C. keratinophilum. Half the plates were baited with 1 cm pieces of degreased wool and half with greasy wool. The soils were kept moist with sterile distilled water. Three replicates of each experiment were set up. The plates were incubated at 25°C for 3 weeks. Each week 10 pieces of wool were removed and percentage colonisation for each fungus was determined. The pH was measured before and after autoclaving to determine any alterations.

Carbon and nitrogen requirements of 3 keratinophilic fungi.

The fungi studied were T. terrestre, C. keratinophilum and A. uncinatum. They were grown on S.D.A. for 2 weeks. A sterile cork borer was used to cut discs 5.5 mm diameter from the colony periphery.

A basal medium was made containing:

K_2HPO_4	0.4 g
$MgSO_4 \cdot 7H_2O$	0.05 g
NaCl	0.01 g
$FeCl_3$	0.01 g
distilled water	1000 cc

This non-nitrogenous basal salt solution with additional nitrogen and carbon sources was dispensed into 250 ml Erlenmeyer flasks in 50 ml quantities and autoclaved at 120°C for 20 min.

All glassware was washed in acidified potassium dichromate.

Amino acids were added to the basal salt solution using asparagine as the standard (2 g of asparagine give 0.3731 g nitrogen).

Amount of nitrogen source added to the medium

(g / l)

DL-Alanine	2.3719
NH_4NO_3	1.0665
$(NH_4)_2SO_4$	1.7608
L-Arginine	1.1606
L-Asparagine	2.0000
L-Hydroxyproline	3.4946
DL-Aspartic acid	3.5474
L-Cysteine	3.2289
L-Glutamic acid	3.9210
Glycine	2.0006
DL-iso-Leucine	3.4960

DL-Methionine	3.9765
DL-β-Phenylalanine	4.4023
L-Proline	3.0682
DL-Serine	2.8006
L-Tryptophan	2.7214
L-Valine	3.1221
Urea	0.8003

Using a ratio of carbon : nitrogen of 1 : 1, the amount in g / litre of carbon source (one of six) was calculated:-

glucose	10.00 g
fructose	10.00 g
maltose	10.00 g
sucrose	9.47 g
lactose	9.47 g
starch	9.00 g

Several controls were also set up without carbon sources, without nitrogen sources, without both carbon and nitrogen and the third control was Sabouraud's liquid medium

Each flask was inoculated with one 5.5 mm disc when the media had cooled. The flasks were incubated at 25°C on a reciprocating shaker for 2 weeks. The contents of the flasks were then harvested and dried at 50°C for 48 h and then dry weight determinations were made.

Each experiment was carried out in triplicate and several experiments were repeated.

The growth from three flasks was calculated in terms of the average weight of dry mycelium / 100 ml of medium.

The pH of each medium was measured before and after 2 weeks growth of each fungus.

Effect of fungicides on growth of keratinophilic fungi.

Measured quantities of distilled water in 30 medical flats were autoclaved at 120°C for 20 min and when cool, appropriate amounts of fungicide were added aseptically to 5 flats to give concentrations of 100, 50, 25, 10 and 5 ppm of total fungicide (when diluted in media) for each of the 6 fungicides used. The fungicides were formaldehyde, dicloran, captan, thiram, Milcol and Triarimol.

Formaldehyde was used as a 40% solution. Dicloran is 4% active ingredient and called Allisan D.D. Captan is N- (trichloro -methylthio) tetrahydrophthalimide and is composed of 50% active ingredient. Thiram is tetramethyl thiuram disulphide; the active ingredient comprises 50% of the compound. Milcol is 4-(2-chlorophenyl - hydrazone) -3-methyl-5-isoxazolone. Triarimol is -(2,4-dichlorophenyl)-α-phenyl-5-pyrimidine methanol.

These 5 concentrations of 6 fungicides were then used to make up both liquid and solid media to determine their effect on the growth of the 5 fungi T. terrestre, A. uncinatum, M. cookei, M. gypseum and C. keratinophilum.

a) liquid medium

Sabouraud's liquid media was dispensed in 50 ml quantities into 250 ml Erlenmeyer flasks and autoclaved at 120°C for 20 min. When the medium was cool, 1 ml of the appropriate sterile fungicide solution was added to each of 3 flasks to give 100, 50, 25, 10, or 5 ppm. No fungicide was added to the controls of pure Sabouraud's liquid medium. The flasks were inoculated with a 5 mm disc from the periphery of a 2-week-old culture of one of the 5 keratinophilic fungi. After 1 week incubating at 25°C on a reciprocating shaker the contents of the flasks were harvested and dry weight determinations made. Growth was expressed as mg mycelium / 100 ml of medium.

b) solid medium

Measured quantities of Sabouraud dextrose agar were

autoclaved at 120°C for 20 min. When the agar had cooled to 50°C, the correct amount of sterile fungicide solution was added aseptically to give the concentrations 100, 50, 25, 10 or 5 ppm. Plates were then poured immediately and when the agar had set, they were inoculated with 5 mm discs of the keratinophilic fungi. The plates were incubated at 25°C and the diameter of the colony was measured every day for 2 weeks. The results were compared with the control of S.D.A made up with no fungicide. The result was the mean of 5 colony diameters.

Effect of sea water upon keratinophilic fungi.

a) isolation of fungi from three soils watered with different concentrations of sea water.

The three soils were the two collected from the Pembrokeshire farm and one collected from the Botany Department, Nottingham University.

Thirty petri dishes per soil type were half-filled with sieved soil. Half were baited with 1 cm pieces of degreased Animal Wool B.P. and half with greasy wool. The soils were watered with 4 concentrations of sea water and controls with sterile distilled water. Three replicates were set up for each concentration and were incubated at 25°C for 4 weeks.

Examination was made studying 10 pieces of hair picked at random from the soil surface and percentage colonisation was calculated.

b) reisolation of keratinophilic fungi inoculated into sterile soil.

One hundred and fifty petri dishes were half-filled with sieved soil which had been sterilised by autoclaving on 4 successive days for 1 h at 120°C. For each fungus 30 petri dishes were inoculated with 4×10^6 spores of T. terrestre, A. uncinatum, M. cookei, M. gypseum or C. keratinophilum.

Seventy-five plates were baited with degreased and 75 with greasy wool. Using 3 replicates for each combination, the soils were watered with 4 concentrations of salt water and sterile distilled water as the control. Percentage colonisation of the wool bait was calculated as before.

An identical set of experiments was set up using sterile sand obtained from Gibraltar Point and autoclaved on 4 successive days for 1 h at 120°C.

c) growth of keratinophilic fungi in solid media containing different concentrations of sea water.

The five fungi, M. cookei, M. gypseum, A. uncinatum, T. terrestre and C. keratinophilum, were cultured on S.D.A. After 2 weeks growth, 15 cores of agar per fungus were cut from the periphery of the colony and plated out onto S.D.A with sterile sea water of the experiments being substituted for sterile distilled water in the control. Four concentrations of sea water were used; $\frac{1}{4}$ strength, $\frac{1}{2}$ strength, full strength and double strength, as in experiments a) and b).

Plates were kept at 25°C and the diameter of each colony was measured every day for a fortnight.

d) growth of keratinophilic fungi in liquid media containing different concentrations of sea water.

Five sets of Sabouraud's liquid medium were made up using 4 concentrations of sea water and sterile distilled water which acted as the control. The medium was dispensed in 50 ml quantities into 250 ml Erlenmeyer flasks and autoclaved at 120°C for 20 min. When cool, the flasks were inoculated with the appropriate fungus. Three replicates per fungus per concentration were set up. After 1 week on a reciprocating shaker at 25°C the cultures were harvested, dried for 2 days at 50°C and then dry weights determined. Growth was expressed as g mycelium / 100 ml medium.

A study of Arthroderma uncinatum and Trichophyton mentagrophytes using the fluorescent antibody technique.

Preparation of inoculum for rabbit (antigen preparation)

Arthroderma uncinatum was cultured on S.D.A and 5 mm diameter discs were cut from the periphery of a 2-week-old colony of either A. uncinatum or T. mentagrophytes. One disc was used to inoculate each of 3 Erlenmeyer flasks containing 50 ml of Sabouraud's liquid medium which had been autoclaved at 120°C for 20 min. The mycelium was harvested after incubation for 2 weeks at 25°C in stationary culture. The equivalent in wet weight to 5 mg (15 mg for T. mentagrophytes) dry weight was weighed and suspended in 15 ml physiological saline after grinding to give 1 mg (3 mg for T. mentagrophytes) dry weight mycelium/3 ml saline.

A test was made for contamination of the prepared inoculum on S.D.A.

Production of antibodies

A 1 ml volume of the fungal suspension was injected into a sterile bijou bottle and an equal volume of Freund's complete adjuvant was added and thoroughly mixed with the spore and hyphal suspension. This mixture was used to inoculate a male New Zealand white rabbit, weighing 3.5 Kg, subcutaneously in the flank once a week for four weeks. After 2 months the rabbit, which had been inoculated with A. uncinatum, was inoculated again every other day for a week in alternate flanks. The rabbit was rested for one week and then given a test bleed to obtain about 10-15 ml of blood. The ear of the rabbit was shaved to remove hair at a point where a prominent vein could be seen. The area was bathed in 70% alcohol and a small nick was made in the vein and the blood flowed fairly freely into welled bottles. After obtaining the blood the rabbit's ear was prevented from bleeding by adding adrenaline to a wad of cotton wool and pressing this hard onto the ear.

The blood collected was left for ½ h to clot and then placed in a refri gerator at 4°C overnight. The serum separated and was decanted from the clot and centrifuged at 1500 RPM for 10 min. The serum was again decanted and respun at 2500 RPM for 30 min to spin off platelets.

Agar gel diffusion plates were set up (prepared as described by Gooding, 1966) with 0.8% agar and sodium azide as a bacteriostat, to test the response of the rabbit to the antigen.

A crude fungal extract was made by grinding a dry mycelial mat of the fungus in question in a pestle and mortar and then made up to 10 ml with phosphate-buffered saline (pH 7). Sodium deoxycolate (1%) was added to this extract to precipitate the particulate matter. This was not entirely successful so an ultrasonicator was used to solubilise the crude fungal extract in 1 min.

The outer wells of the double diffusion plates were filled with fungal extract and the inner wells contained dilutions of serum. The plates were refri gerated and left for a few days. The wells were kept topped up. Ten days after the onset of this test, precipitin lines were seen at 3 of the wells: undiluted, 1:2 and 1:4 diluted serum for A. uncinatum and at the first 2 wells for T. mentagrophytes. (Figure 5?)

Final bleedings were made from the ear vein a month after the ultimate injection. The serum was collected and stored without preservative at -20°C. The γ globulin fraction was prepared using the method of Hill and Gray (1967).

Equal volumes of antiserum and 3.9 M ammonium sulphate were mixed at 2°C. The resulting precipitate was centrifuged at 3000 g for 15 min, washed with 1.95 M ammonium sulphate several times and dissolved in a minimal volume of distilled water. The solution was dialysed in 17.5 mm diameter tubing against 0.85% NaCl at 2°C until all the ammonium sulphate had been

removed (tested with barium salt). The γ globulin was stored at 2°C.

Experimental slides of 4 kinds were examined. They were:-
a) those which were placed in a transverse manner in sandwich boxes half-filled with non-sterile soil. Two portions of 1 cm pieces of degreased Animal Wool, B.P. were placed 3.5 cm apart on the surface of the soil adjacent to the slide face. One portion was inoculated with either A. uncinatum or T. mentagrophytes and then the slides and wool were completely covered with more nonsterile soil. The contents of the box were kept moist at 60% of the water-holding capacity.
b) Those which were set up in a similar manner to a) but only uninoculated bait was used so that growth on the bait could only have been from the soil.
c) Those which were smeared with soil taken from close proximity to degreased, uninoculated wool baits buried in the field for six to seven weeks.
d) Slides which were placed in sterile and non-sterile soil inoculated with a spore suspension of either A. uncinatum or T. mentagrophytes, with no keratin bait.

Control slides were set up using the slide culture technique of Preece and Cooper (1969), which was an adaptation of a technique by Riddell (1950). Slides of A. uncinatum, A. curreyi, C. keratinophilum, Ctenomyces serratus, M. gypseum, M. cookei T. terrestre were set up by this method to determine the specificity of this fluorescent antibody technique.

Other controls used to determine the reaction of normal serum and unlabelled immune serum were set up. Serum was collected from the experimental rabbit before it was inoculated, to give "normal" serum. The slide cultures were stained with

a) labelled normal serum
b) unlabelled normal serum
c) unlabelled immune serum

in addition to a set being treated in the following manner.

Control and experimental slides were fixed in acetone for 30 min and then placed in a moist chamber at 35°C for 1 h with a drop of unconjugated γ globulin. The slides were then rinsed for 30 min and then again for 15 min in phosphate-buffered saline (Nairn, 1969) and replaced in the moist chamber. A drop of fluorescein-labelled antirabbit globulin (produced from a goat by Nordic Diagnostics) was added and left at 35°C for 1 h and then rinsed in phosphate-buffered saline for 30 min and 15 min again. Glycerol saline (Preece and Cooper, 1969) was used as the mounting medium because normal immersion oil is autofluorescent.

The slides were examined under a Leitz Ortholux microscope, with a mercury vapour UV source, using blue light plus Schott BG12 transmission filters (3 mm and 5 mm) and a K510 supression filter. Photographs were taken using a Leitz Orthomat camera with either Ilford HP4 or Kodak high speed Ektachrome film.

Growth of A. uncinatum, T. terrestre and C. keratinophilum on natural substrates.

Twenty g of a sandy loam from the Botany Department, Nottingham University, was placed in each of 36 250 ml Erlenmeyer flasks: 18 contained non-sterile soil and the other 18 contained sterile soil which had been autoclaved at 120°C for 1 h on 4 consecutive days. Nine of each set of 18 contained 100 mg of human hair sterilised by autoclaving at 120°C for 20 min. To 3 flasks from each of the 4 experiments was added 10^5 spores for each species of keratinophilic fungus being studied. A spore suspension of each fungus was made by pipetting sterile physiological saline onto the surface of a 2-week-old culture on S.D.A and dislodging spores and mycelium with the aid of a sterile seeker. The mixtures were then poured into sterile McCartney bottles containing 4 mm glass beads and shaken for 30 min on a bench shaker. The spore suspensions were filtered through glass wool in the barrel of a Millipore filter extension,

and then centrifuged at 27000g for ½ hr at room temperature. The resulting supernatants was decanted and the spores washed three times with sterile physiological saline and finally diluted with the same to give 10^5 spores / ml. This is an adaptation of the method of McGinnis and Hilger (1972).

The soil in the flasks was kept moist throughout at 60% water-holding capacity at room temperature. On the day of inoculation and every 3-4 days after for 31 days, thorough mixing of the contents of the flask took place for every flask. One g of soil was withdrawn, placed in a sterile test tube and diluted serially to 10^5 with sterile water. Samples of 1 ml of each dilution were pipetted into each of 3 petri dishes. Ten ml of modified DTM according to Taplin, Zaias, Rebell and Blank (1969) was added and thoroughly mixed. Colonies of each fungus were counted on each plate after 10 days incubation at room temperature. DTM was modified because, although Merz, Berger and Silva Hutner (1970) found DTM superior to Ink Blue because it supressed bacteria, gentamycin sulphate was unavailable.

Dermatophytes changed the medium from acid to alkaline, thereby producing a colour change in the medium from yellow to red. Colonies which produced a colour change were counted.

Certain fungal contaminants turned the medium red, e.g. a red strain of Penicillium, but it was easily distinguished from dermatophytes by its appearance.

Growth tube experiments to determine the growth rate of keratinophilic fungi in soil.

Large quantities of soil were sieved, air dried and autoclaved for 1 h at 120°C. The soil was then poured into soil colonisation tubes (Evans 1955), packed evenly and re-autoclaved for 1 h at 120°C on 3 successive days. The final autoclaving was with wool bait in the side arms. The tubes had been weighed without soil, with soil and the water-holding capacity was calculated.

The amount of water added was 60 - 90% water-holding capacity. A terminal side arm was inoculated with one of six keratinophilic fungi (A. uncinatum, T. terrestre, T. mentagrophytes, M. gypseum, M. cookei or C. keratinophilum) The other terminal side arm was used for watering so that spores were not washed from the inoculum along the tube. Four tubes per fungus were used. Two contained sterile soil and two were reinoculated with all fungi, except keratinophilic, which were present in the soil before autoclaving. The soils were reinoculated with a spore suspension which was produced by adding a crumb of soil to a sterile medical flat containing molten cellulose agar. The flat was revolved in cool water (with the screw top securely closed) until an even coat of cellulose covered the inner surfaces of the flat. By this method soil particles were distributed throughout the agar. When fungal growth had taken place on the cellulose agar, sterile water was added to remove aerial spores. This suspension was used as the inoculum free of keratinophilic fungi to reinoculate 2 growth tubes to produce non-sterile soil (Pugh, 1964). Soil in growth tubes could have come from molehill soils or soil from woodland (Dawson & Gentles, 1959) so long as it was known not to contain any keratinophilic fungi. The growth tubes were examined regularly for growth over a period of four months. (See Figure 57b).

Experiments to determine competitive ability to colonise an agar plate. (Wastie, 1961)

Preparation of agar plates.

Plates of S.D.A. and plates of S.D.A. plus cycloheximide and chloramphenicol were poured. Once the agar had set, the plates were inverted and left for 24 h to allow evaporation of the surface film of moisture to discourage excessive

Figure 57a

Precipitin lines on a double diffusion plate. Inner wells contain extract of A. uncinatum. Outer wells contain serum from a rabbit which had been injected with A. uncinatum.

Figure 57b

Soil colonisation tube

Figure 57a

Precipitin lines on a double diffusion plate. Inner wells contain extract of A. uncinatum. Outer wells contain serum from a rabbit which had been injected with A. uncinatum.

Figure 57b

Soil colonisation tube

FIGURE 57a

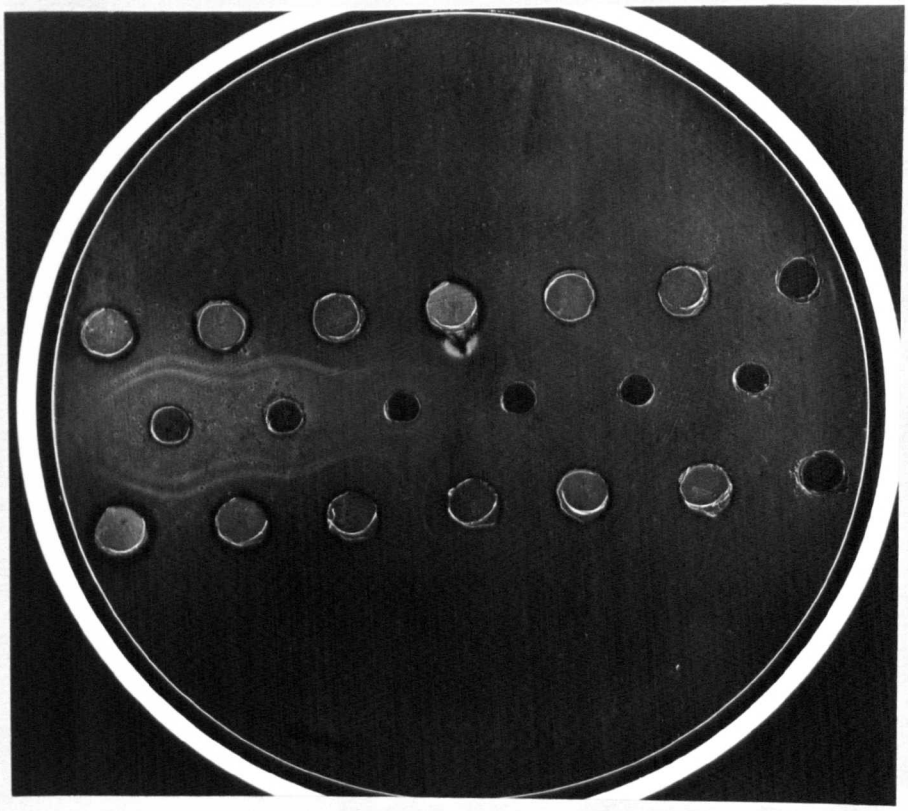
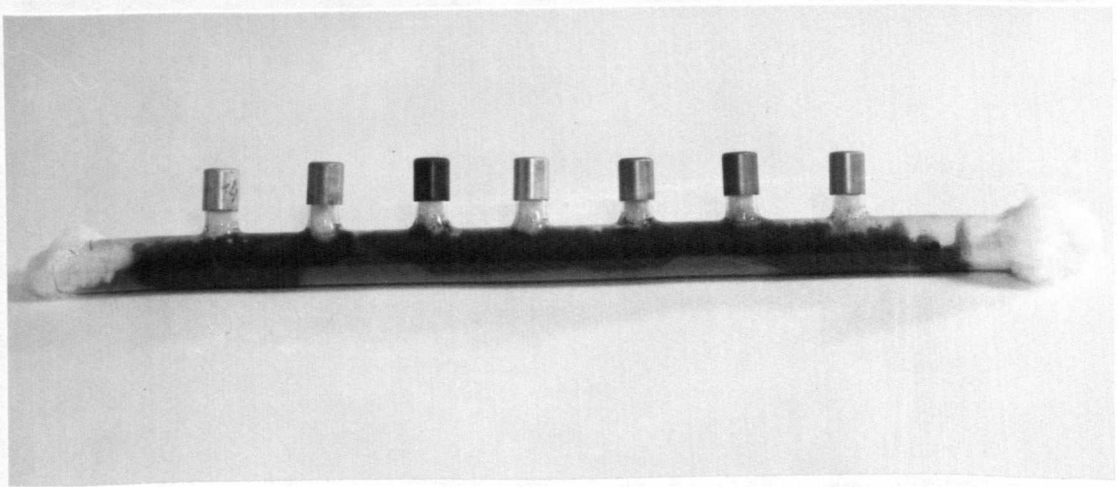


FIGURE 57b



bacterial development after inoculation of the plates with soil.

Preparation of Cellophane discs.

Cellophane discs were cut from commercial Cellophane film, to a size which would fit inside a 9 cm diameter petri dish. The discs were placed in boiling water for as long as necessary to remove the plastic covering from the Cellophane. The discs were then placed under water and autoclaved at 120° C for 20 mins and remained under water until required.

On the bottom of the petri dish four loci were marked so that they were each approximately in the centre of one quarter of the petri dish. At each locus the agar was inoculated by lightly touching it with the end of a glass rod (4 mm diam) whose wet end had been dipped into soil. A set of plates was inoculated with a 4 mm diam circle of sterile soil and another with non-sterile soil. After being inoculated, the agar surface was covered with a sterile Cellophane disc, placed carefully to avoid air pockets between it and the agar surface. The Cellophane disc was then immediately inoculated at each of the loci with a 4 mm diam agar disc cut from the periphery of a 2-week old colony of one of 6 keratinophilic fungi. Thus, the agar disc was immediately above the inoculum of soil, separated only by the Cellophane. Two petri dishes with 4 inocula were set up for each fungus in each experiment, i.e. 8 replicates in all. The diameter of the radial extension of each colony was measured every day for a week.

Scanning electron microscopy.

Cleistothecia of A. uncinatum, A. benhamiae, A. tuberculatum, Nannizzia gypsea, N. incurvata and N. cajetani were obtained by crossing + and - strains of the fungi on soil and hair plates.

Rivets with double sided Sellotape on their surface were placed lightly on the surface of the cleistothecia on the hair

bait. The cleistothecia adhered to the top of the rivet, having come away undamaged. The aluminium rivets were attached to a "stub" with the adhesive Dag 915 (Acheson Colloids). The adhesive was allowed to dry otherwise the rivets might have come loose or the evacuation of the specimen chamber might have taken longer. The rivets and specimens were first covered with aluminium and then gold by evaporation under vacuum at 1×10^{-5} Torr. Aluminium reduces thermal damage and comes off slower so that the specimens can be rotated more slowly giving a better chance for the metal to penetrate the network. Gold is used because it is a good electron emitter.

Treated specimens were viewed with a Cambridge Stereoscan Mark 2 microscope operated at 20Kv.

Photographs were taken using Ilford HP4 film.

RESULTS

Effect of temperature on the growth of keratinophilic fungi.

It can be seen from Figures 58 - 63 that maximum growth for all fungi studied was at 25°C, although M. gypseum grew at maximum efficiency at 20, 25 and 30°C. T. terrestre and C. keratinophilum grew better at 10°C than any of the other 4 fungi. Only T. mentagrophytes, T. terrestre and C. keratinophilum grew at all at 5°C, but only to a small extent, i.e. diameter of the colony after 14 days was 6 - 7 mm compared with 4 mm at the inoculation date. C. keratinophilum and M. gypseum were the only 2 fungi which grew at all at 35°C, producing diameters of 8 and 12 mm respectively, after 14 days.

Effect of pH on the growth of keratinophilic fungi.

a) liquid medium

Figures 64 and 65 represent the g dry weight of mycelium of keratinophilic fungi per 100 ml liquid medium at the pH values of 4, 5, 6, 7 and 8. A. uncinatum, M. cookei and T. mentagrophytes had maximum growth at pH 6 ; M. gypseum and C. keratinophilum at pH 5 and T. terrestre did not change greatly between pH 6 and pH 8.

Most of the fungi, with the exception of M. gypseum, had least growth at pH 4. At and above pH 5, growth increased compared with that at pH 4, but in most cases there was a plateau from pH 5 to pH 8. The growth of M. gypseum decreased from pH 5 down to pH 8, but was always producing 0.5 g mycelium / 100 ml of medium which was higher than the majority of the highest dry weights for A. uncinatum, T. mentagrophytes and M. cookei.

At pH values of 4 and 5, M. gypseum was always the fungus which produced most growth. At pH values of 6, 7 and 8, C. keratinophilum produced most growth. At all pH values, M. cookei

Graphs representing growth of keratinophilic fungi at different temperatures

Figure 58 A. uncinatum

Figure 59 C, keratinophilum

Figure 60 T. terrestre

Figure 61 T. mentagrophytes

Figure 62 M. cookei

Figure 63 M. gypseum

Graphs representing growth of keratinophilic fungi at different temperatures

Figure 58	A. uncinatum
Figure 59	C, keratinophilum
Figure 60	T. terrestre
Figure 61	T. mentagrophytes
Figure 62	M. cookei
Figure 63	M. gypseum

Figure 58

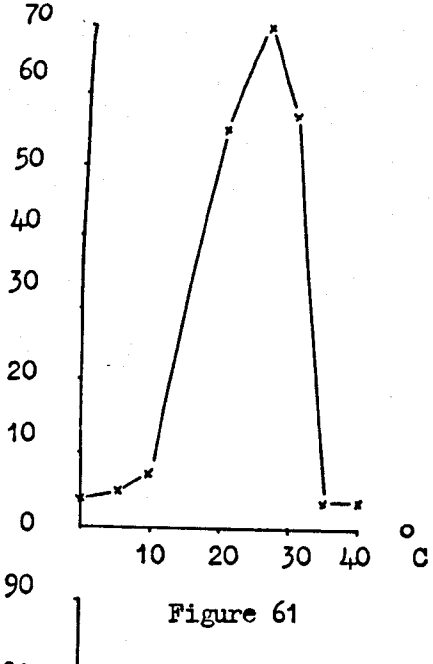


Figure 59

Figure 60

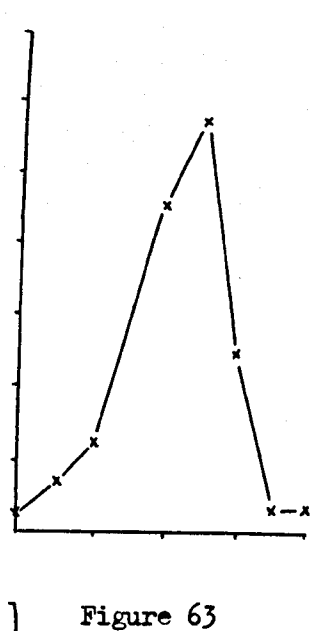


Figure 61

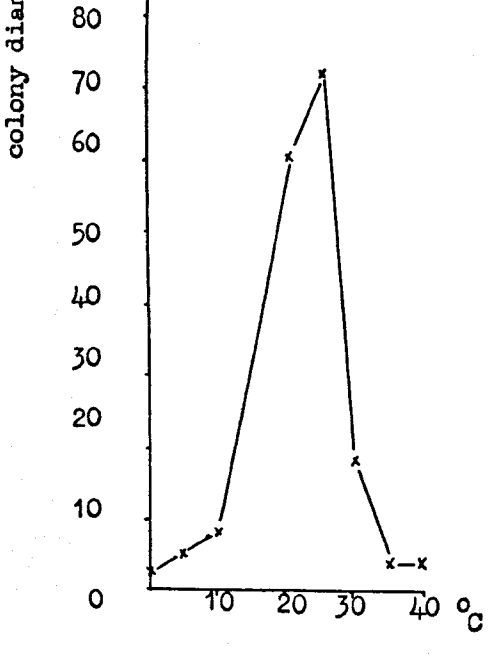


Figure 62

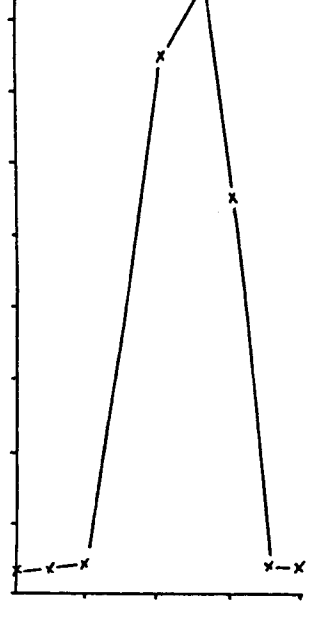
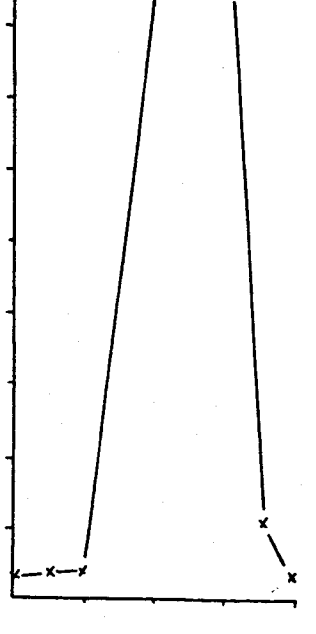
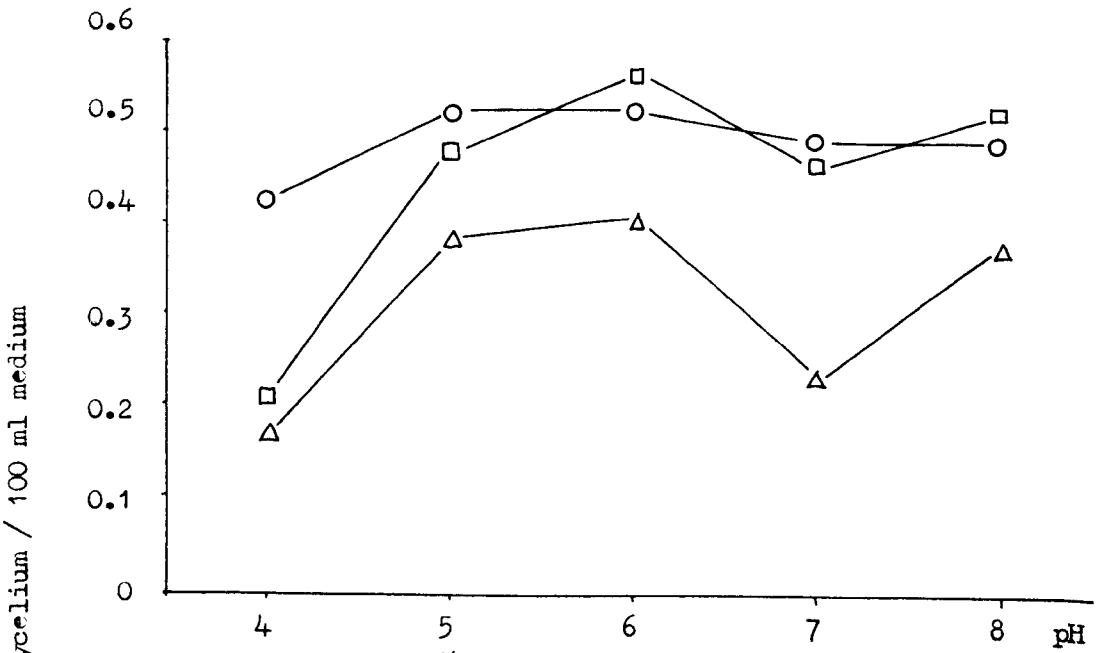


Figure 63



Graph representing growth of keratinophilic fungi in liquid media of different pH values

Figure 64



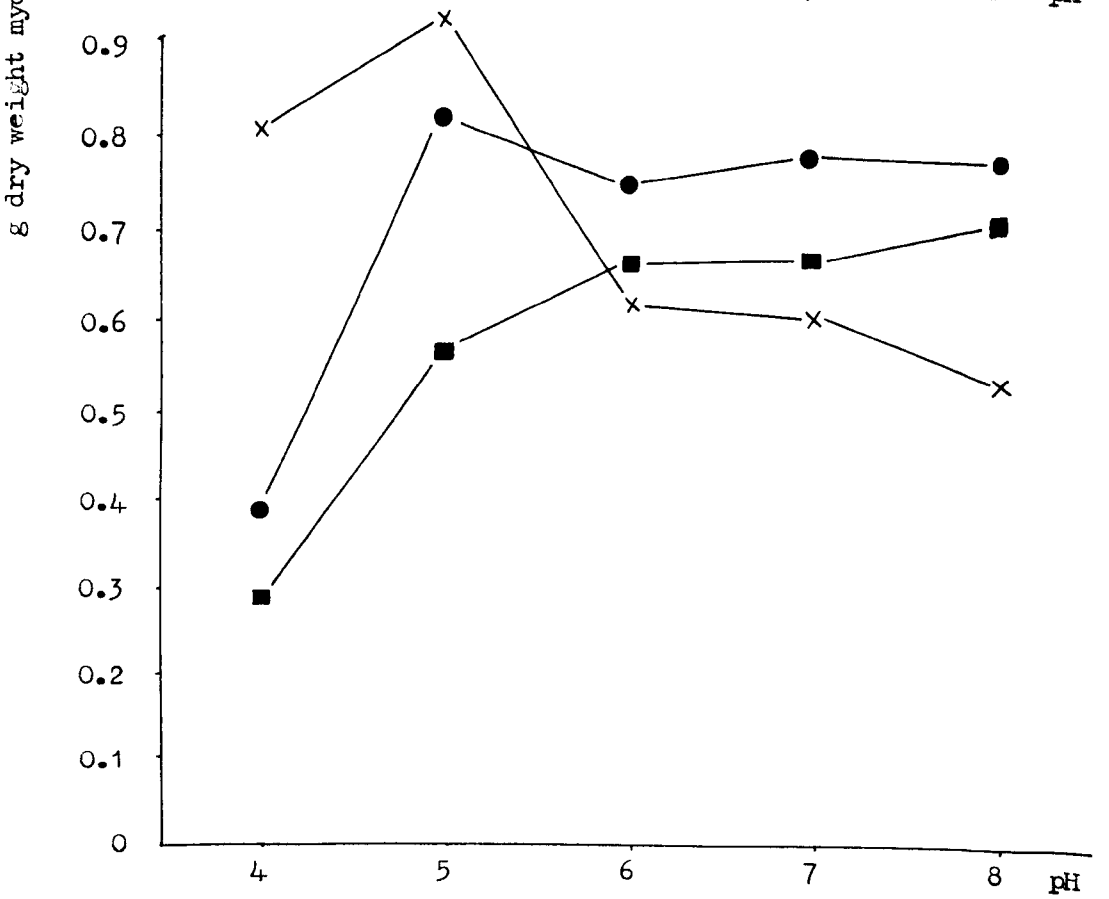


Figure 65

FOR KEY SEE FIGURE 66

produced least growth and A. uncinatum and T. mentagrophytes were, on the whole, inclined to produce less growth than T. terrestre.

b) solid medium

At pH values of 5 and 7, the maximum growth was produced by M. gypseum, (as seen in Figure 66), followed by C. keratinophilum and then M. cookei. The two Trichophyton species and A. uncinatum produced least growth at these two pH values. Maximum growth for each individual species was at pH 6.5 for T. mentagrophytes, C. keratinophilum, M. cookei and M. gypseum, and 7.1 for T. terrestre and A. uncinatum. However, on the whole, growth between values pH 5 - 7.1, was so little that differences were not clearly seen.

Because the agar would not solidify beyond this small range of values of pH, this method was not considered to be of any use in determining the effect of pH on growth of keratinophilic fungi. Liquid medium, on the other hand, showed differences more distinctly and over a wider range of pH values.

Effect of pH of soil on reisolation of keratinophilic fungi.

All the fungi, with the exception of C. keratinophilum and M. gypseum at pH 6 and 4, colonised degreased wool more than they colonised greasy wool. At pH 8, maximum percentage colonisation by M. cookei, T. terrestre, T. mentagrophytes and A. uncinatum took place; at pH 6, by C. keratinophilum, and at both pH 6 and 8 by M. gypseum. (See Figure 67).

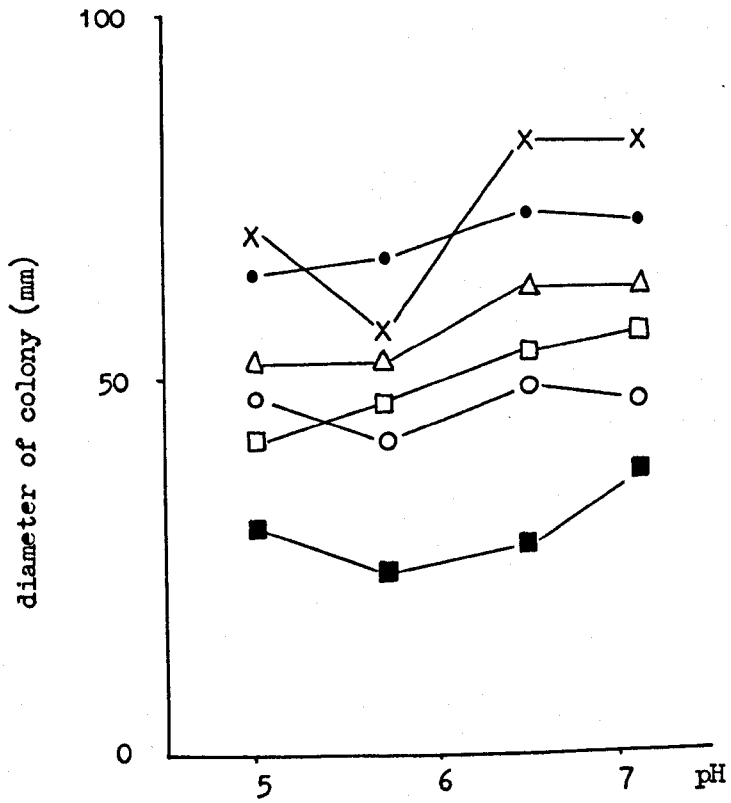
Colonisation of baits at pH 4 was extremely low; the highest recording at this value was 30% for degreased wool by M. gypseum.

Carbon and nitrogen requirements of 3 keratinophilic fungi.

The medium containing no carbon source showed that the amino acids used most readily by A. uncinatum were alanine,

Figure 66

Graph representing growth of keratinophilic fungi on solid media of different pH values



X——X M. gypseum

●——● C. keratinophilum

△——△ M. cookei

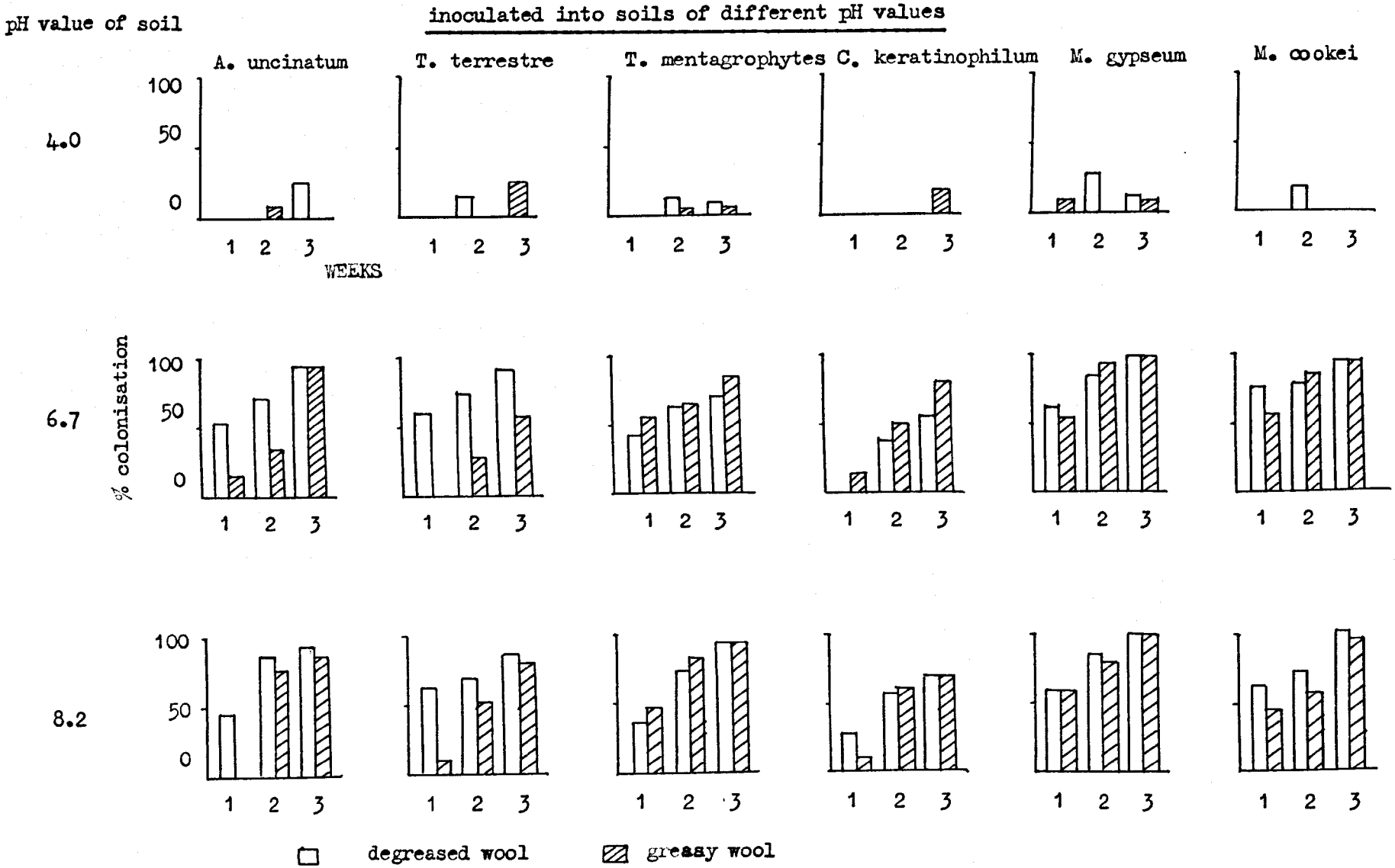
○——○ T. mentagrophytes

□——□ A. uncinatum

■——■ T. terrestre

Figure 67

Histograms representing percentage colonisation of baits by keratinophilic fungi inoculated into soils of different pH values



phenylalanine and proline. This observation also applied to media supplied with lactose, sugar or starch as carbon source. Using maltose, fructose and glucose as carbon sources the results were slightly different. Where maltose was supplied, NH_4NO_3, arginine, aspartic acid, methionine, phenylalanine and proline were used to best advantage. Where fructose and glucose were the carbon sources, arginine, asparagine, aspartic acid, proline, phenylalanine, serine and urea were utilised most. Valine, glycine and $(NH_4)_2SO_4$ were also used well in conjunction with fructose.

The amino acids used least of all, whichever carbon source was used, were leucine, hydroxyproline, cysteine, glutamic acid and tryptophan. Methionine, $(NH_4)_2SO_4$ and NH_4NO_3 were also generally utilised very little, except in combination with fructose or maltose.

A comparison of the growth on different carbon sources can be seen in Figures 68, 71 and 72.

When no carbon source was supplied to C. keratinophilum, the amino acids utilised best were cysteine, phenylalanine and proline. The last 2 agreed with those producing best growth for A. uncinatum. However, whereas cysteine promoted good growth in C. keratinophilum, it did not in A. uncinatum and vice versa for aspartic acid. The nitrogen sources used least by this fungus, with all carbon sources, were $(NH_4)_2SO_4$, asparagine, hydroxyproline, glutamic acid and methionine and also aspartic acid (except in use with starch) and valine (except with glucose). This agreed with the results for A. uncinatum, with the exception of valine, aspartic acid and asparagine. In addition to cysteine, already mentioned, C. keratinophilum used leucine, tryptophan and NH_4NO_3 more than did A. uncinatum.

The results of nitrogen sources in combination with starch, sucrose and lactose agreed well with those with no carbon source with the exception that NH_4NO_3 was used well by C. keratinophilum,

FIGURE 68

Histogram representing growth of A. uncinatum on different carbon and nitrogen sources in liquid culture.

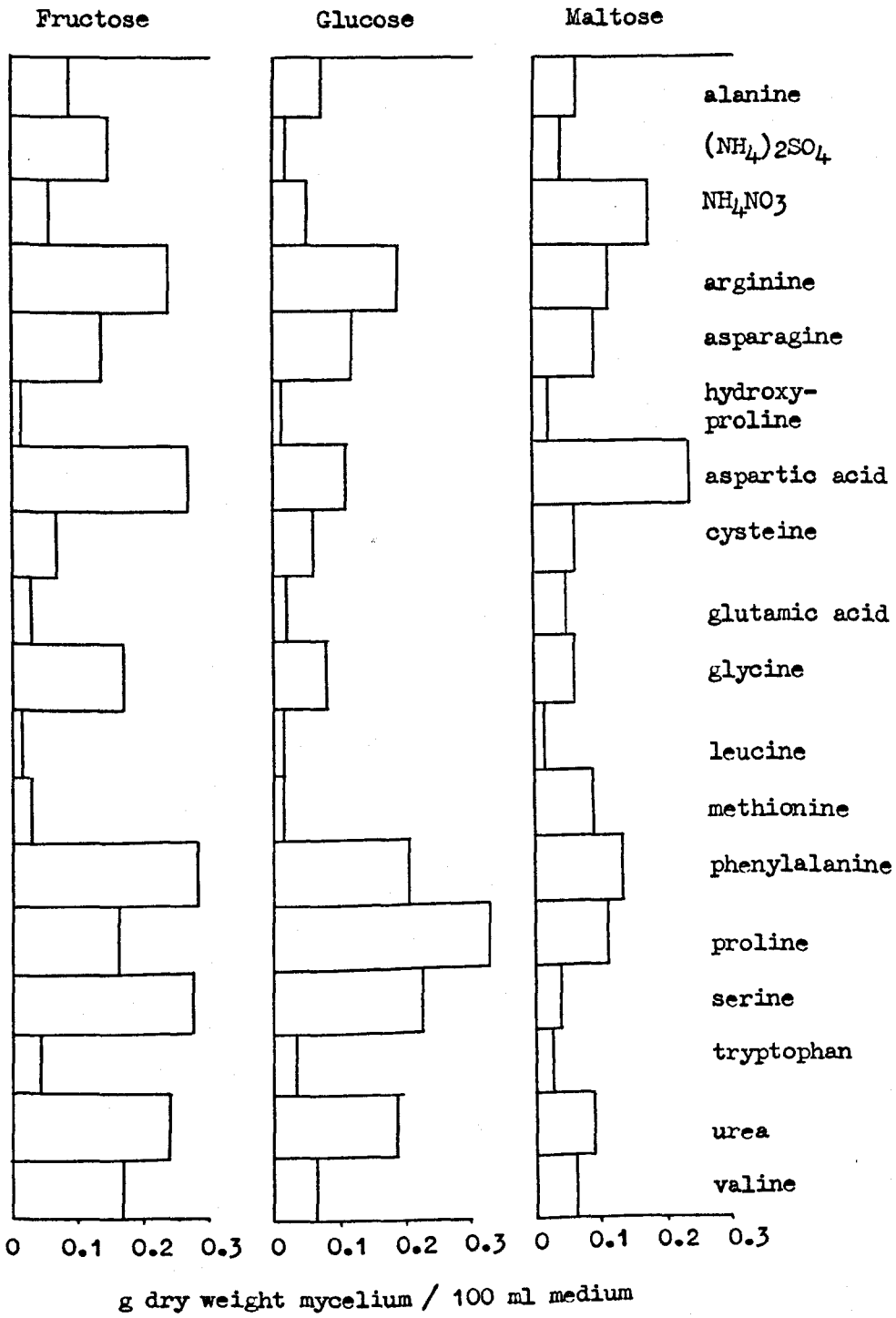


FIGURE 68 (continued)

Histogram representing growth of A. uncinatum on different carbon and nitrogen sources in liquid medium.

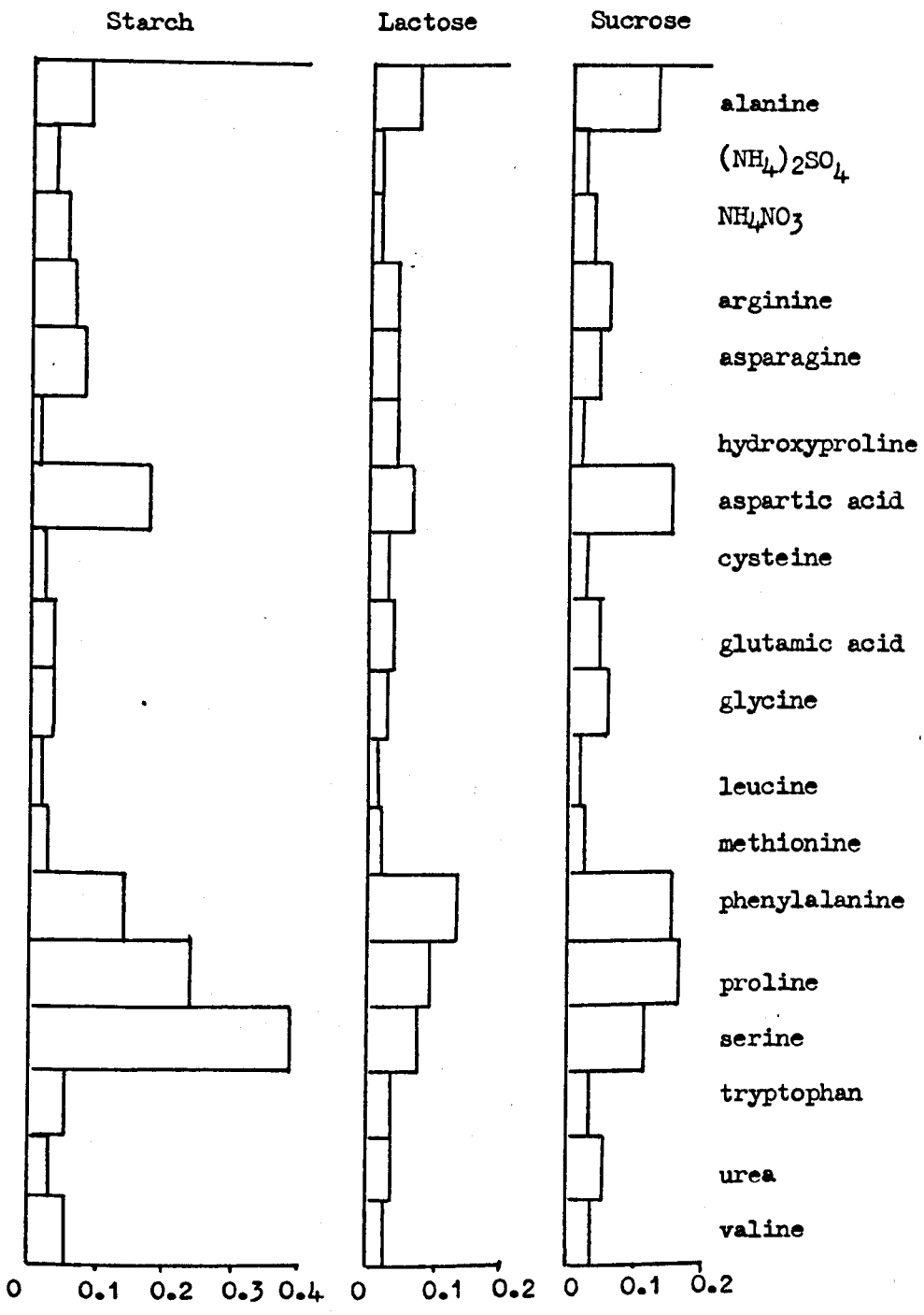


FIGURE 69

Histograms representing growth of T. terrestre on different carbon and nitrogen sources in liquid medium.

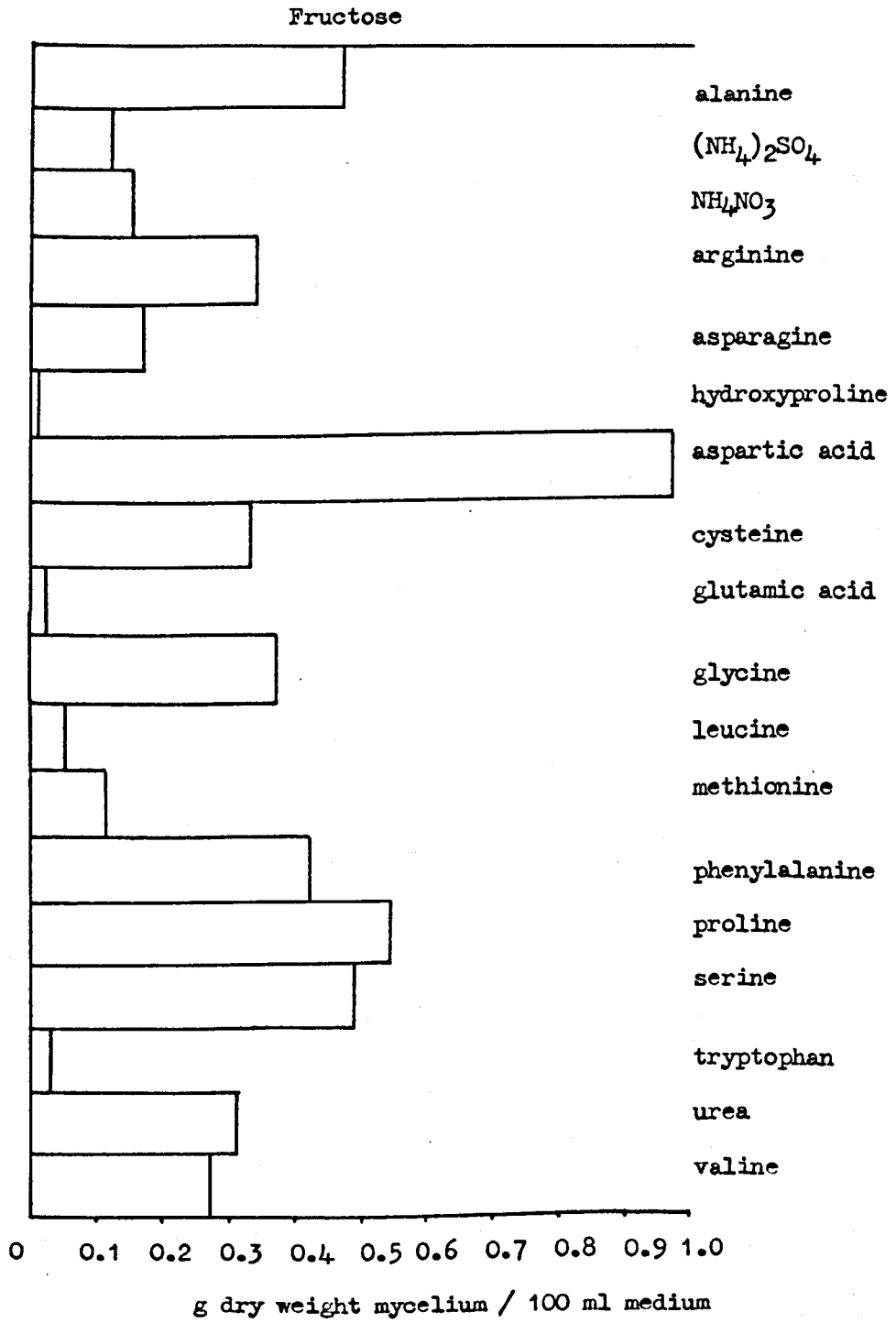


FIGURE 69 (continued)

Histograms representing growth of T. terrestre on different carbon and nitrogen sources in liquid medium.

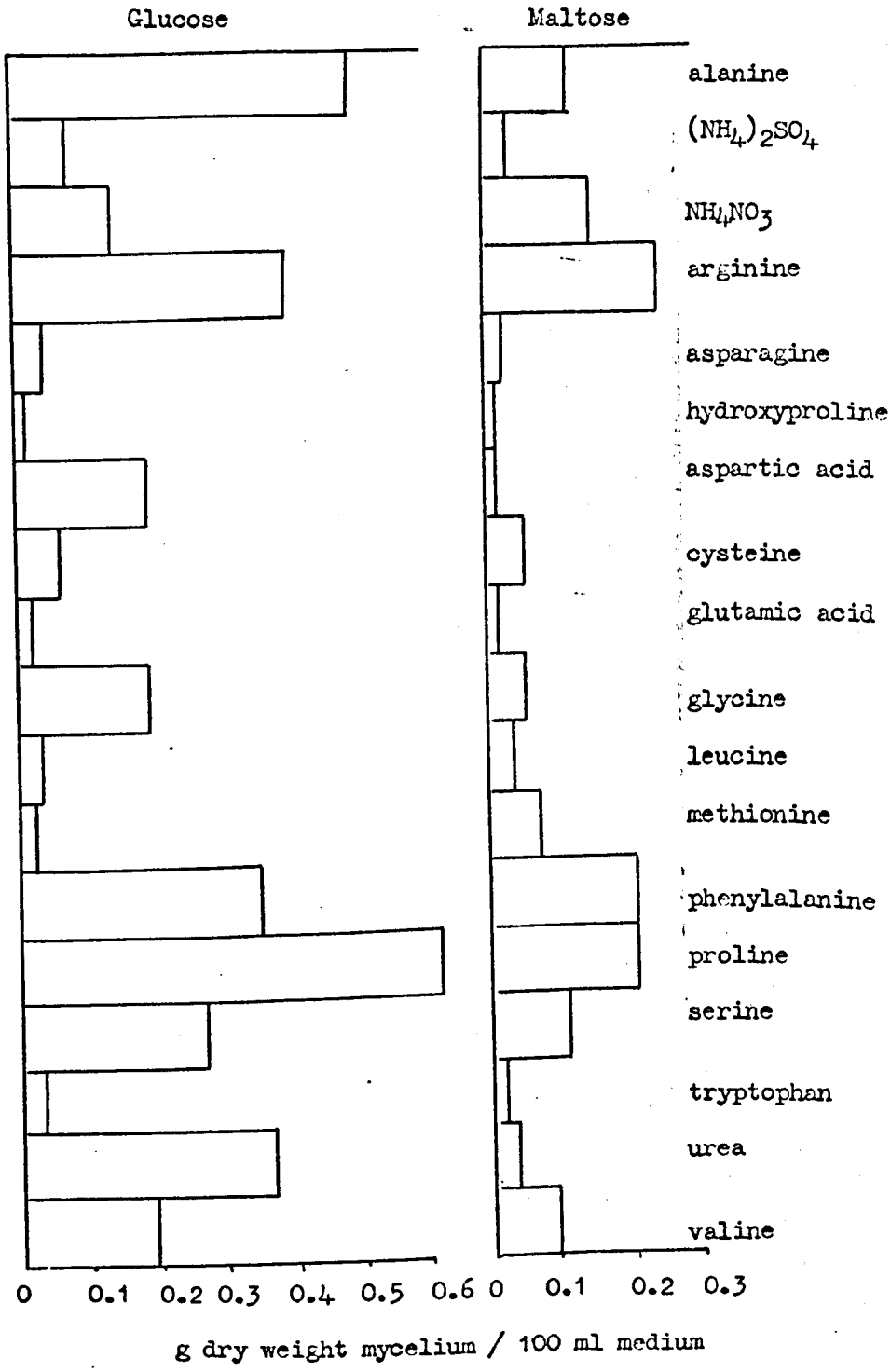


FIGURE 69 (continued)

Histograms representing growth of T. terrestre on different carbon and nitrogen sources in liquid medium.

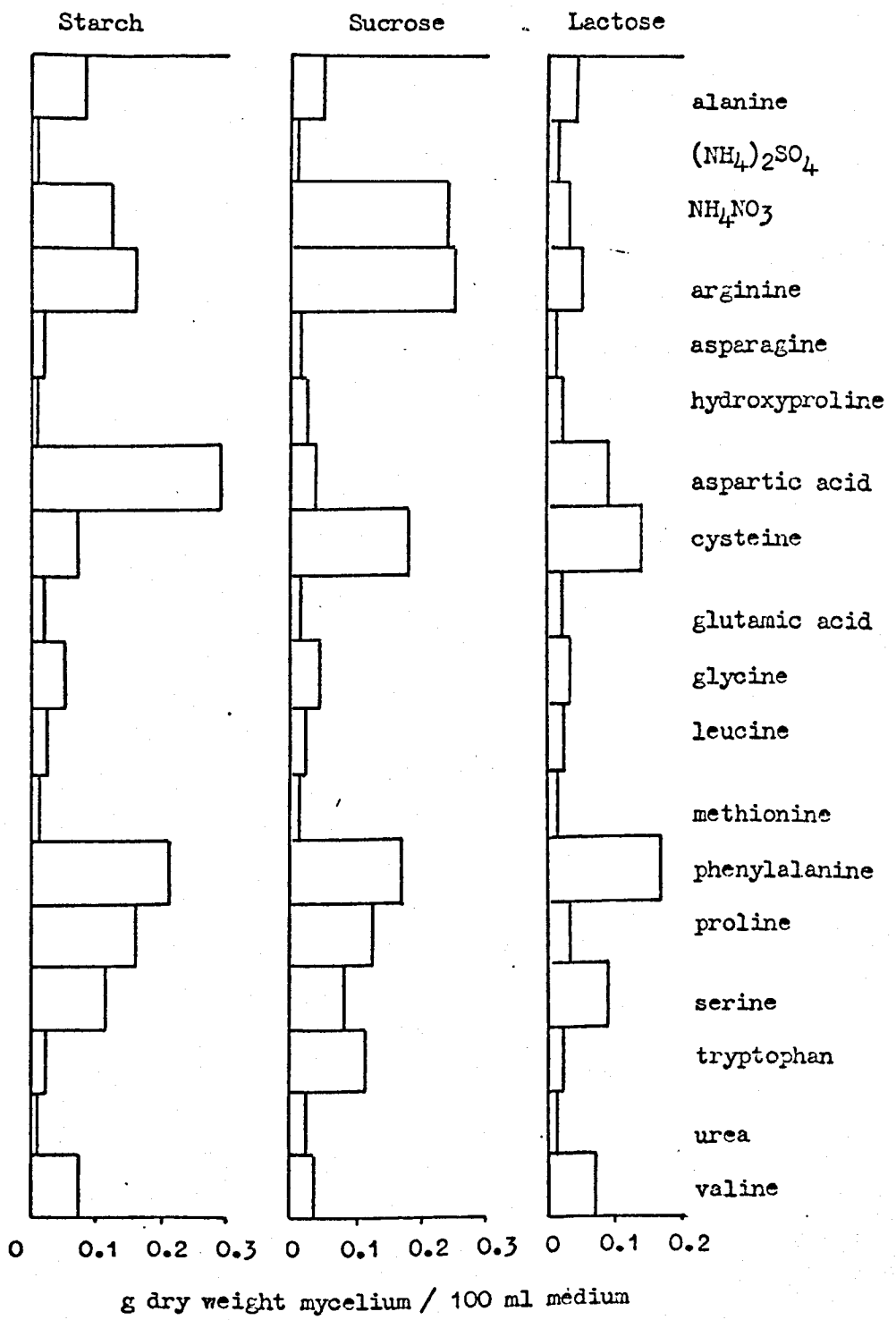


FIGURE 70

Histograms representing growth of C. keratinophilum on different carbon and nitrogen sources in liquid medium.

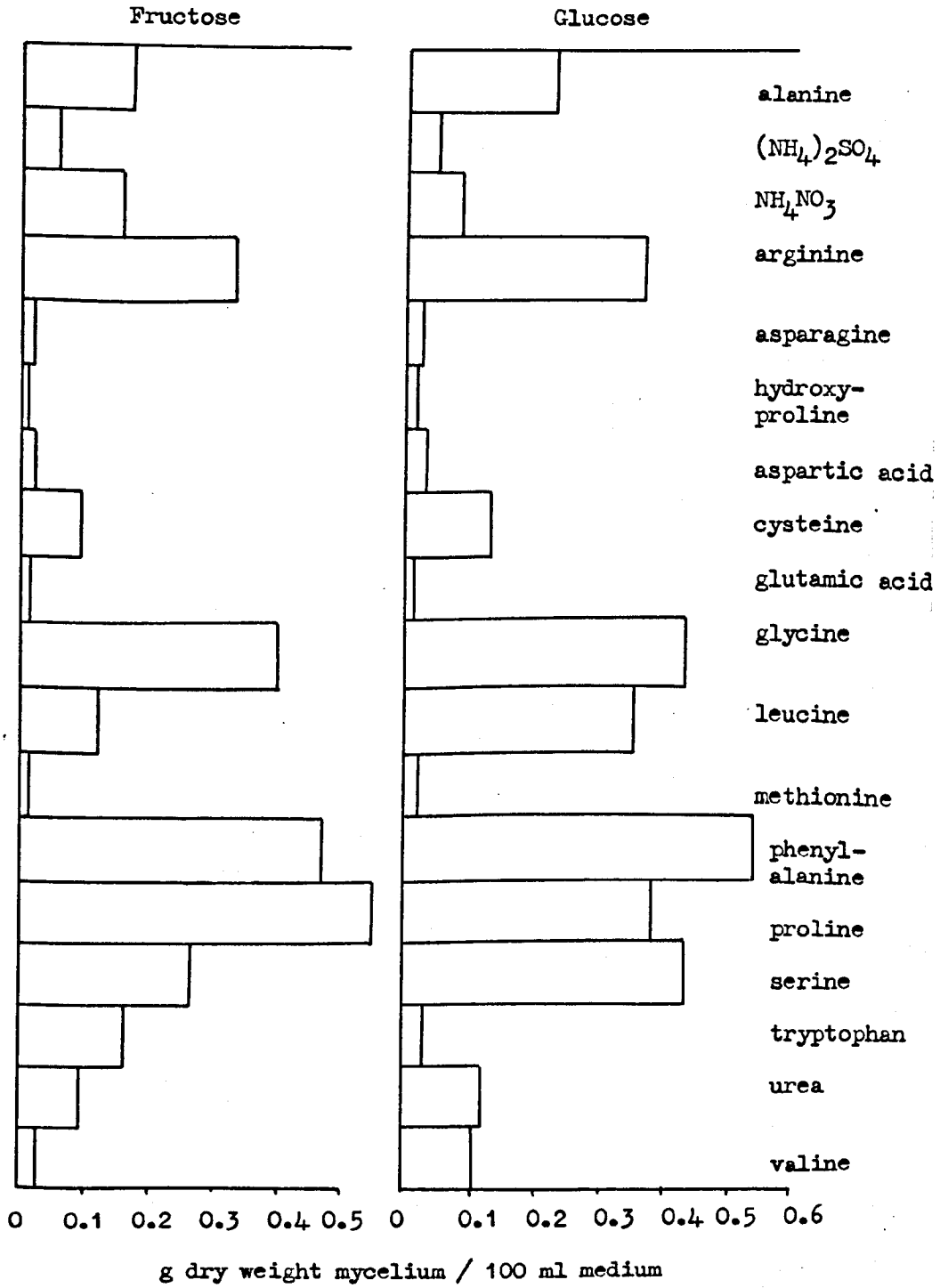


FIGURE 70 (continued)

Histograms representing growth of C. keratinophilum on different carbon and nitrogen sources in liquid medium..

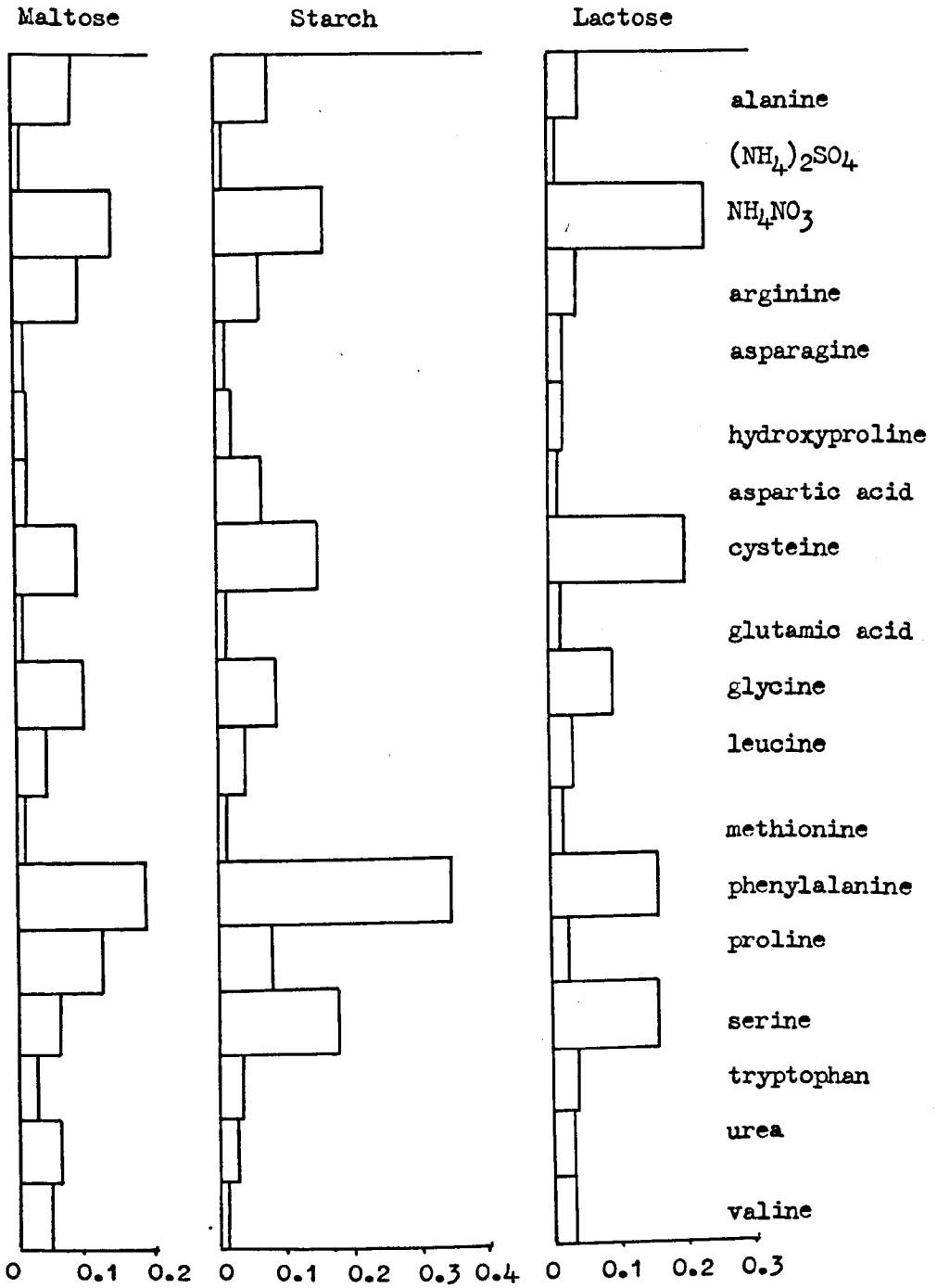


FIGURE 70 (continued)

Histograms representing growth of C. keratinophilum on different carbon and nitrogen sources in liquid medium.

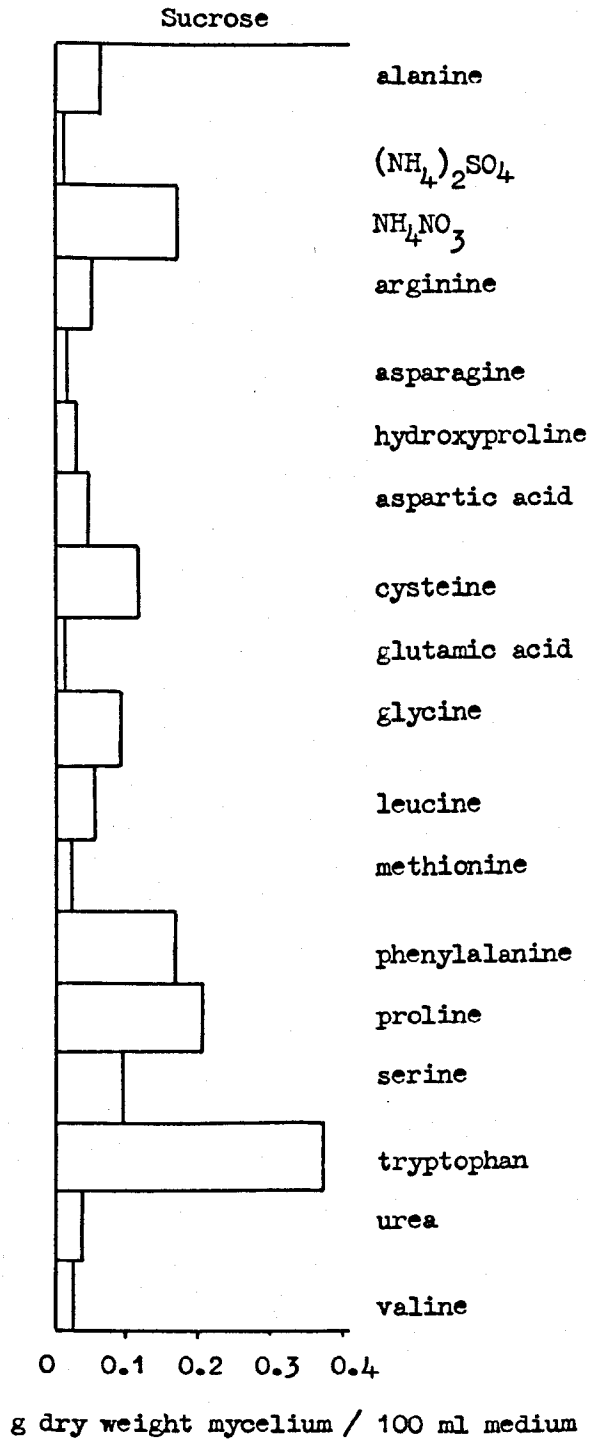


FIGURE 71

Histograms representing the growth of A. uncinatum, T. terrestre and C. keratinophilum in liquid media with no additional carbon source.

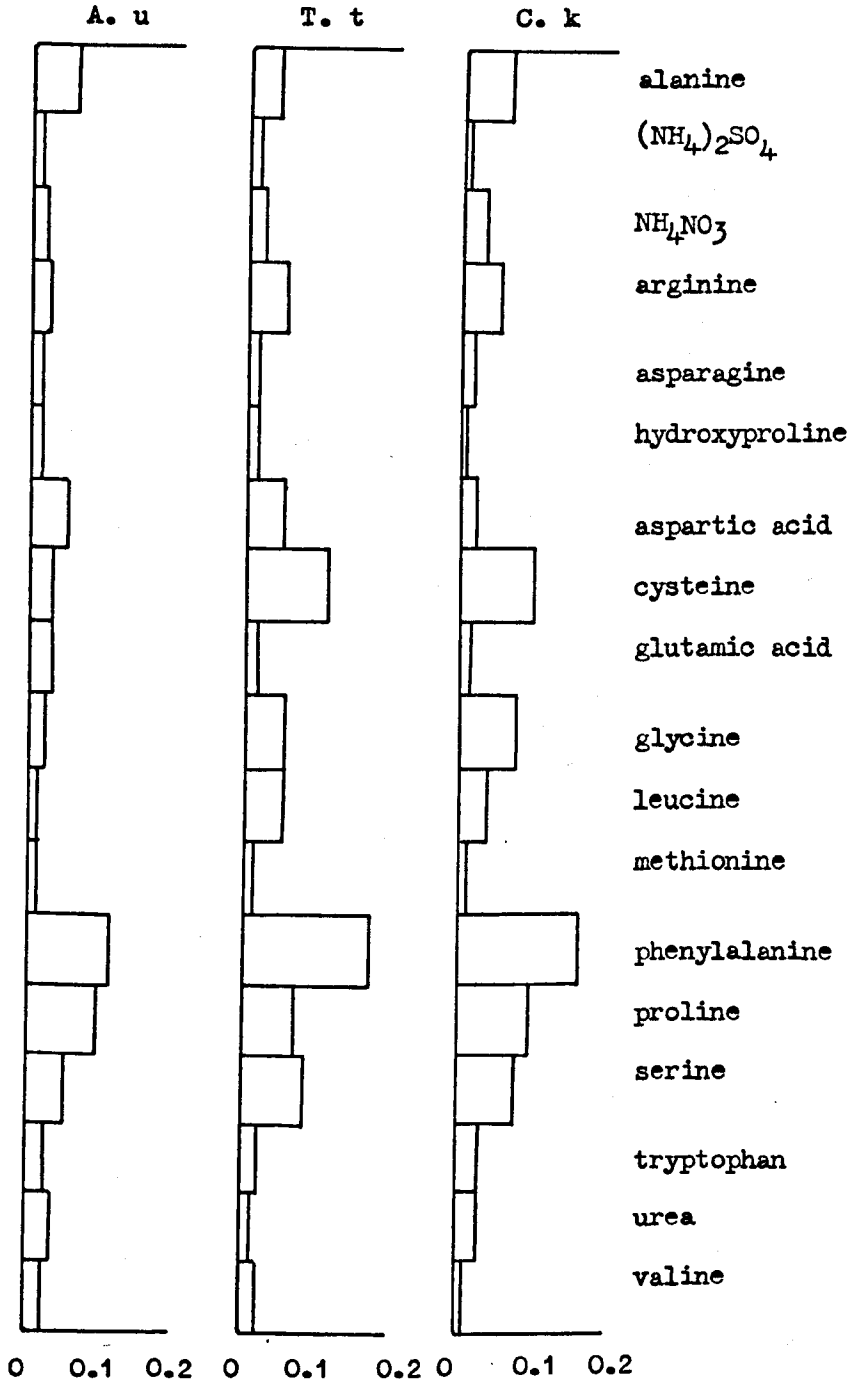
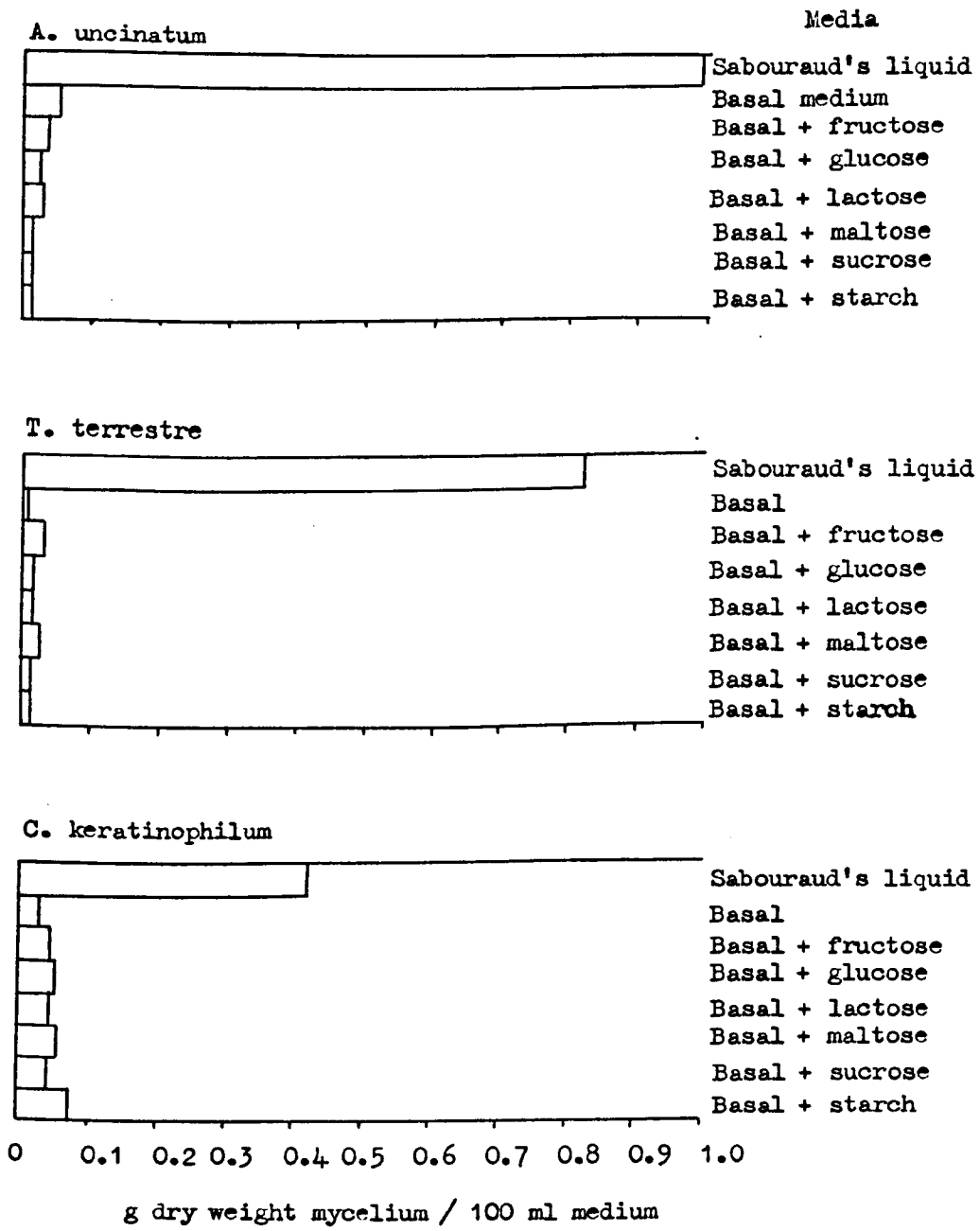


FIGURE 72

Histograms representing growth of A. uncinatum, T. terrestre and C. keratinophilum on different liquid control media.



proline was not used in conjunction with starch and lactose and tryptophan was used well in combination with sucrose. Maltose, glucose and fructose were the carbon sources which induced maximum growth. In addition to those nitrogen sources already mentioned as being promoters of good growth, NH_4NO_3 and arginine promoted good growth in combination with these 3 carbohydrates. Leucine and urea, in combination with either fructose or glucose, tryptophan plus fructose and valine and glucose also promoted good growth.

Comparisons of the growth of C. keratinophilum on different carbon and nitrogen sources can be seen in Figures 70, 71 and 72.

The nitrogen sources used most by T. terrestre, when no carbon source was provided were cysteine and phenylalanine. This means that T. terrestre resembled C. keratinophilum more than A. uncinatum in its use of nitrogen sources for best growth. The nitrogenous compounds not used well by T. terrestre in any combination of carbon source were hydroxyproline, glutamic acid and leucine, plus $(NH_4)_2SO_4$, asparagine and methionine (all except with fructose) and tryptophan (except with sucrose).

In addition to those nitrogen sources mentioned which provided good growth with no carbon source, growth was also good on starch and sucrose with NH_4NO_3 and arginine; on starch with aspartic acid; sucrose and tryptophan. Lactose and proline did not combine well to support good growth.

Besides those nitrogen sources utilised mostly when no carbon source was present, T. terrestre grew well with fructose and glucose in combination with alanine, aspartic acid, glycine, urea and valine. Using maltose, glucose and fructose, T. terrestre also produced good growth with arginine, but good growth was not seen with cysteine and maltose or glucose.

Comparisons of the growth of T. terrestre on different carbon and nitrogen sources can be seen in Figures 69, 71 and 72.

TABLE 12

Mean g dry weight mycelium/100 ml medium containing different carbon sources.

T. terrestre		*A. uncinatum*		*C. keratinophilum*	
Fructose	0.287	Fructose	0.136	Glucose	0.185
Glucose	0.195	Glucose	0.101	Fructose	0.161
Maltose	0.081	Maltose	0.085	Sucrose	0.078
Starch	0.080	Starch	0.081	Starch	0.073
Sucrose	0.078	Sucrose	0.061	Maltose	0.060
Lactose	0.046	Lactose	0.041	Lactose	0.055
No carbon	0.038	No carbon	0.031	No carbon	0.042

A. uncinatum and *T. terrestre* resembled each other in the order of utilisation of carbon sources i.e. using the monosaccharides and maltose, followed by starch. *C. keratinophilum*, on the other hand, although using glucose and fructose, did not utilise maltose to the same extent as the 2 *Trichophyton* species. *C. keratinophilum* also utilised glucose better than fructose, whereas *A. uncinatum* and *T. terrestre* used fructose better.

All 3 fungi preferred NH_4NO_3 to $(NH_4)_2SO_4$, with 1 exception, which was *A. uncinatum* with fructose.

The controls showed that Sabouraud's liquid medium, which contains peptone and glucose provided the combination which promoted the most growth for *A. uncinatum* and was the only control with high growth production for all 3 species. *C. keratinophilum* grew better on combinations of proline or phenylalanine and fructose; phenylalanine, glycine or serine and glucose, whilst *T. terrestre* grew better in a combination of aspartic acid and fructose than in Sabouraud's liquid medium.

Table 13 shows the order of utilisation of the nitrogen sources by T. terrestre, A. uncinatum and C. keratinophilum.

pH changes in liquid media containing different carbon and nitrogen sources, before and after growth.

Glutamic acid and aspartic acid always produced a low pH value before growth of below 4 and there was only one exception where glutamic acid medium was > pH 5 after harvesting mycelium of 14 days growth. Twelve media out of 21 possibilities, containing aspartic acid had a pH value of > pH 5 after growth of 14 days.

Thirteen of 21 possibilities containing $(NH_4)_2SO_4$, had media of pH < 5 after growth, nine of 21 containing NH_4NO_3 and 7 of 21 containing cysteine all showed this characteristic. Other combinations, with only a few exceptions, produced pH values of > 5 after growth.

Quantitative comparisons between the 3 species are not possible since A. uncinatum was grown in 500 ml Erlenmeyer flasks whilst T. terrestre and C. keratinophilum were grown in 250 ml flasks.

Effects of fungicides on growth of keratinophilic fungi.

a) liquid culture

Arthroderma uncinatum was inhibited by all 6 fungicides at 25 ppm and above. At 5 ppm, Triarimol had inhibited the growth of this fungus to a quarter of the control, Milcol to a third and dicloran by a half. At 10 ppm thiram and dicloran had both inhibited growth to a third of the control.

Microsporum cookei was inhibited by 4 of the fungicides at 25 ppm and higher concentrations. Only Milcol and thiram had inhibited the growth of the fungus to more than a half of the

TABLE 13

Order of preferential use of nitrogen sources by 3 keratinophilic fungi.

Arthroderma uncinatum		Chrysosporium keratinophilum		Trichophyton terrestre	
Proline	0.168	Phenylalanine	0.288	Proline	0.250
Phenylalanine	0.165	Proline	0.203	Phenylalanine	0.244
Serine	0.161	Glycine	0.180	Aspartic acid	0.234
Aspartic acid	0.149	Serine	0.179	Arginine	0.213
Arginine	0.103	Arginine	0.141	Alanine	0.199
Urea	0.093	$NH_4 NO_3$	0.134	Serine	0.173
Alanine	0.083			Cysteine	0.134
Asparagine	0.071	Cysteine	0.108	$NH_4 NO_3$	0.123
Valine	0.070	Alanine	0.102		
Glycine	0.061	Leucine	0.095	Urea	0.110
$NH_4 NO_3$	0.058	Tryptophan	0.092	Glycine	0.109
		Urea	0.053	Valine	0.104
Cysteine	0.041	Valine	0.032	Asparagine	0.040
$(NH_4)_2 SO_4$	0.040	Aspartic acid	0.021	Tryptophan	0.037
		$(NH_4)_2 SO_4$	0.015	Methionine	0.036
Glutamic acid	0.035			$(NH_4)_2 SO_4$	0.032
Tryptophan	0.032	Asparagine	0.014		
Methionine	0.026	Hydroxyproline	0.013	Leucine	0.027
Hydroxyproline	0.015	Methionine	0.008	Glutamic acid	0.017
Leucine	0.011	Glutamic acid	0.003	Hydroxyproline	0.011

(Mean g dry weight mycelium / 100 ml medium)

control at 5 ppm. Triarimol had very little effect upon the growth of M. cookei, while dicloran, although not as inhibitory as the other 4 fungicides, had inhibited the growth of this fungus to half that of the control by 25 ppm. There was stimulation of M.cookei at 5 ppm by formaldehyde and captan.

Chrysosporium keratinophilum was inhibited by thiram and Triarimol at 5 ppm and above and by Milcol at 10 ppm and above. The other 3 fungicides only inhibited this fungus to half the control at 100 ppm.

Trichophyton terrestre was inhibited by 4 fungicides at 50 ppm and above. The exceptions were Milcol and dicloran which only inhibited growth to half the control at 100 ppm. Triarimol had the greatest and soonest inhibitory effect upon T. terrestre at 5 ppm and above. Thiram and captan were the fungicides which inhibited growth to half the control at 25 ppm and above.

Microsporum gypseum was inhibited by 4 fungicides at 50 ppm and above. At 10 ppm 3 of the 4 inhibited the growth to half that of the control and at 25 ppm all 4, plus dicloran, inhibited the growth to a quarter of the control. Triarimol was the fungicide with least effect. Even at 200 ppm M. gypseum had not been inhibited to half the growth of the control. This fungicide had a stimulatory effect at 5, 10, 25 and 50 ppm upon M. gypseum.

Figures 73 - 77 are graphs showing the effect of the 5 fungicides upon the 5 fungi in liquid media.

b) solid media

Figures 78 - 82 are graphs showing the results of growth of the 5 fungi on media containing fungicides.

All fungicides inhibited the growth of A. uncinatum to half or less of that of the control at concentrations of 100 ppm. Only dicloran, Milcol and Triarimol had any effect upon inhibiting the growth of this fungus at concentrations as low as 10 ppm to half that of the control. A similar result was observed for C. keratinophilum but in this case captan and formaldehyde did

Figures 73 - 77

Graphs representing growth of keratinophilic fungi in liquid media containing fungicides.

Key

△ —— △ Captan

▲ —— ▲ Dicloran

● —— ● Formaldehyde

○ —— ○ Milcol

□ —— □ Thiram

× —— × Triarimol

Figure 73 M. cookei

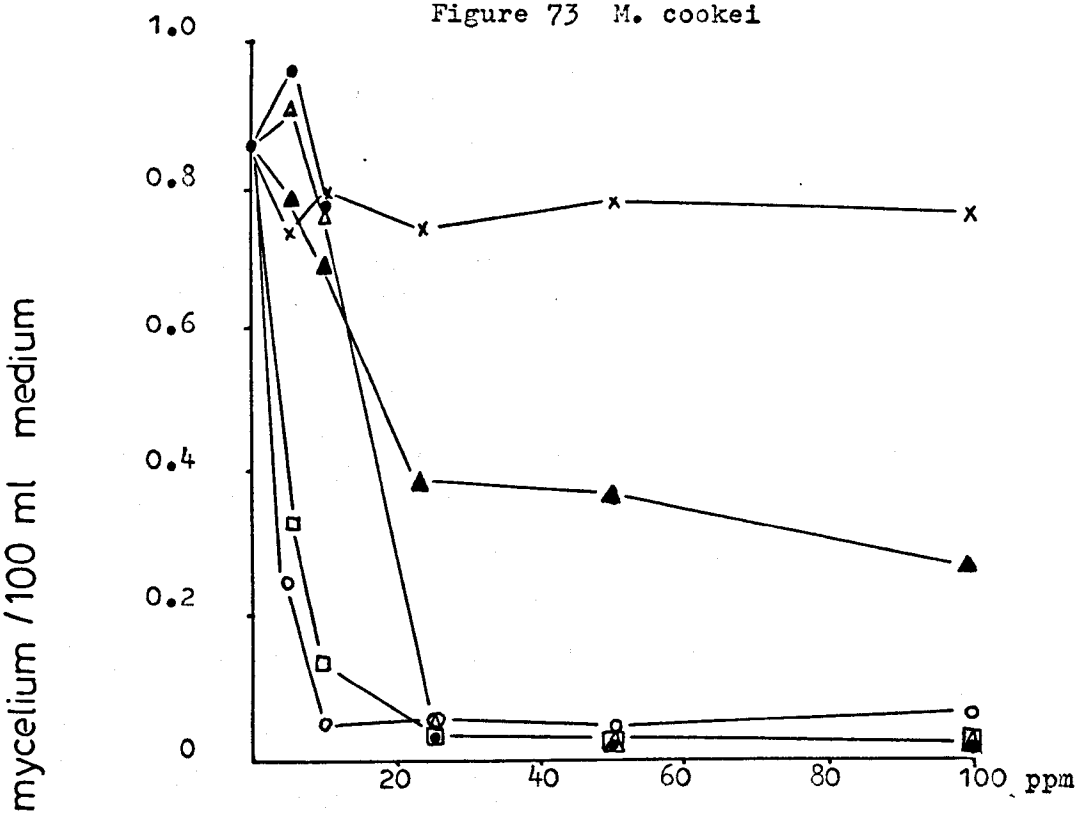


Figure 74 A. uncinatum

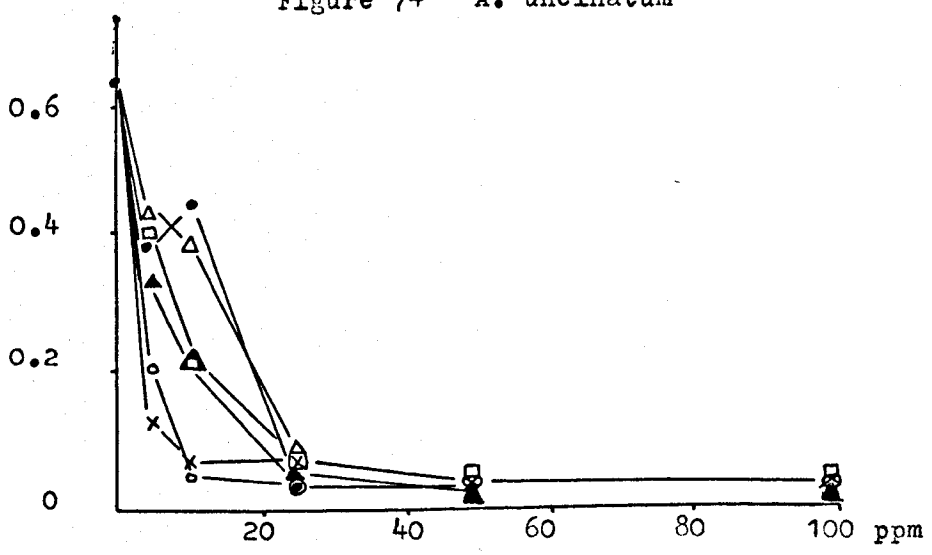
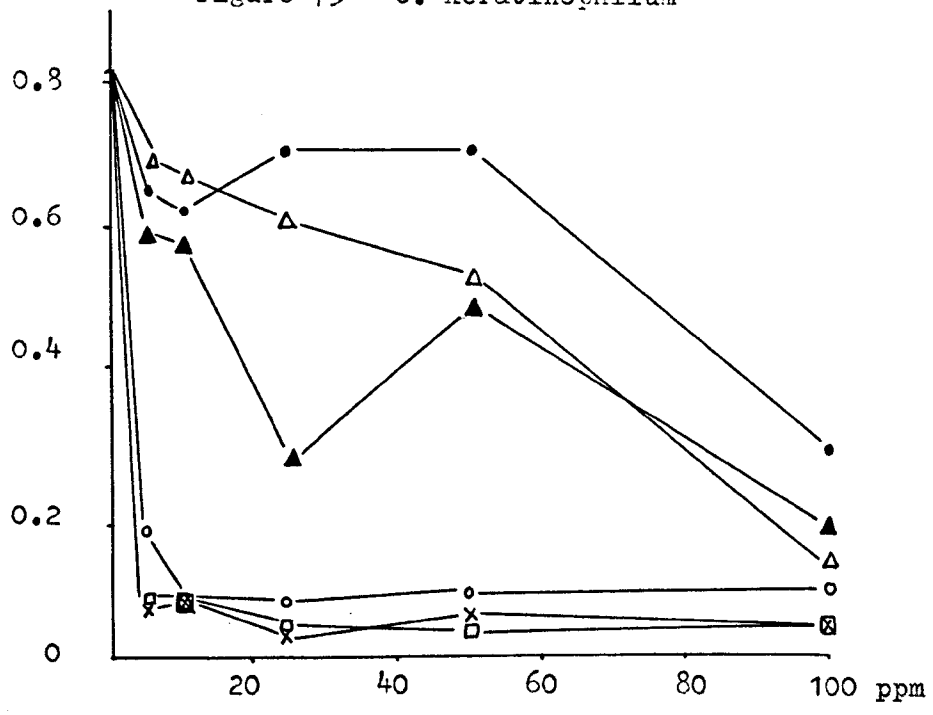


Figure 75 C. keratinophilum



g dry weight mycelium / 100 ml medium

Figure 76 T. terrestre

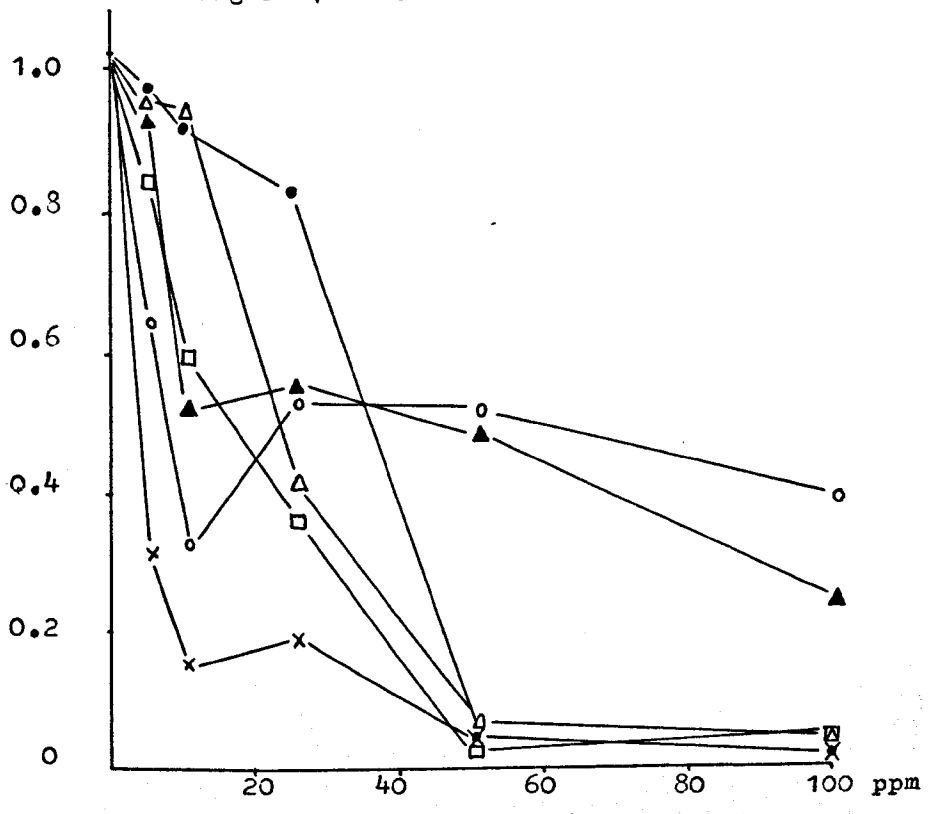
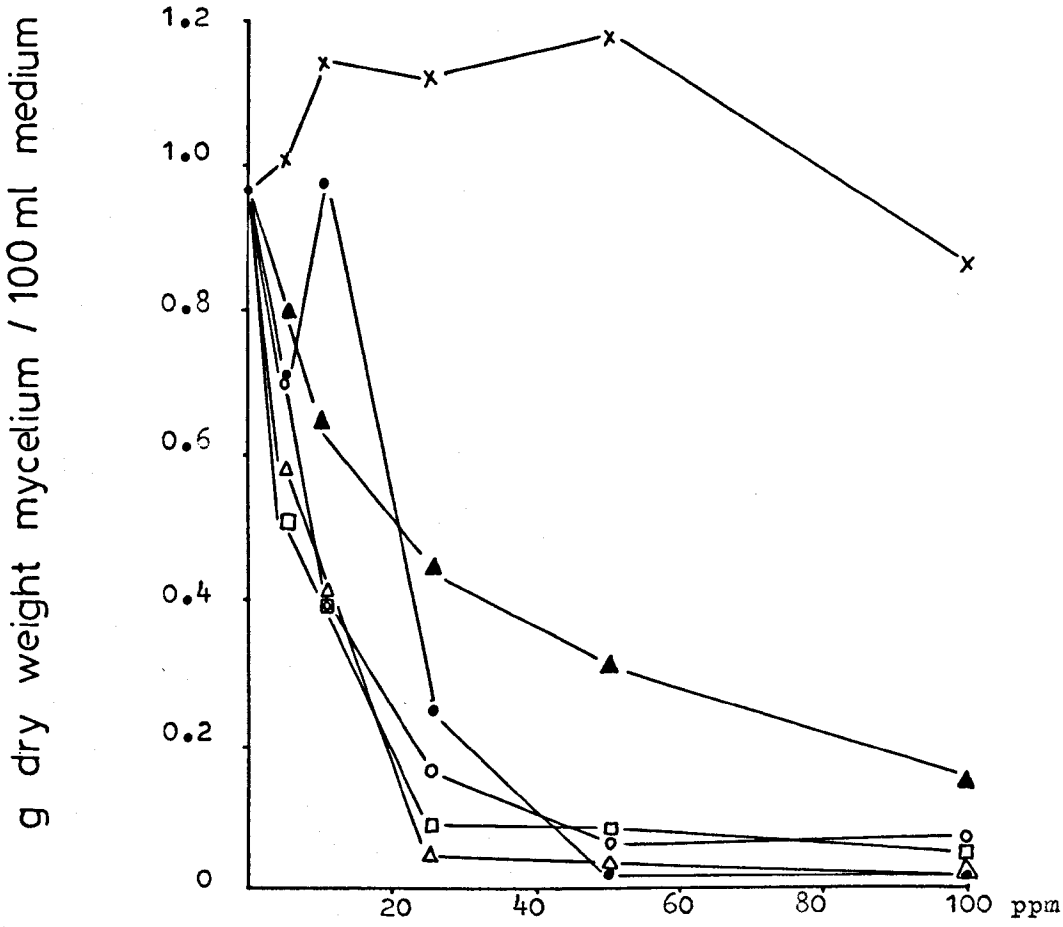


Figure 77 M. gypseum



Figures 79 - 82

Graphs representing growth of keratinophilic fungi on solid media containing fungicides

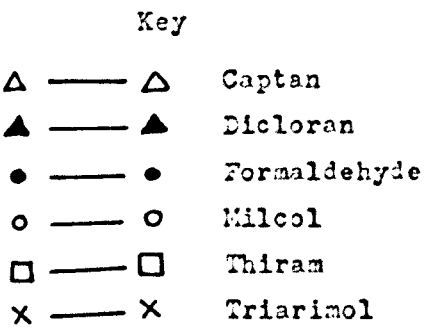


Figure 78 A. uncinatum

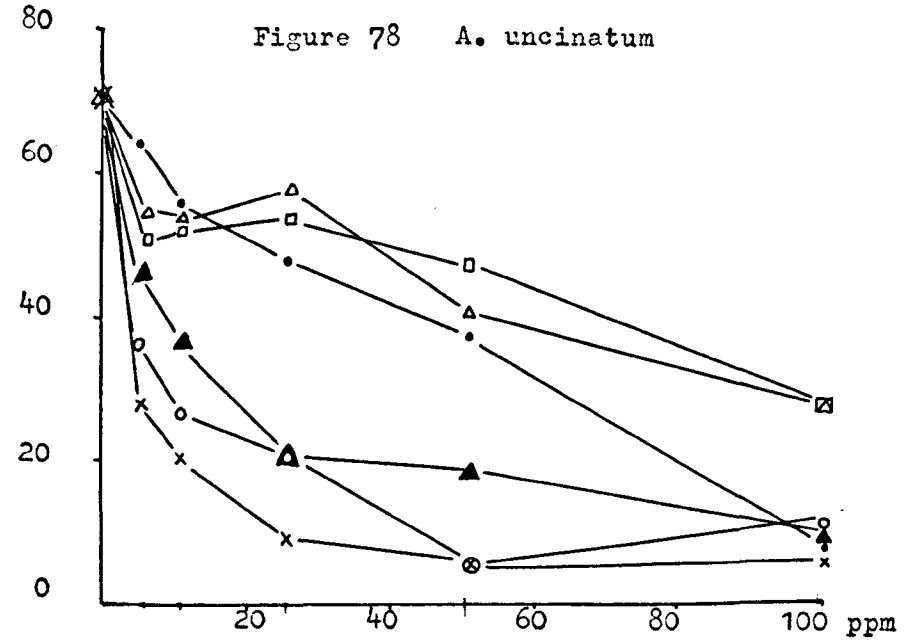


Figure 79 C. keratinophilum

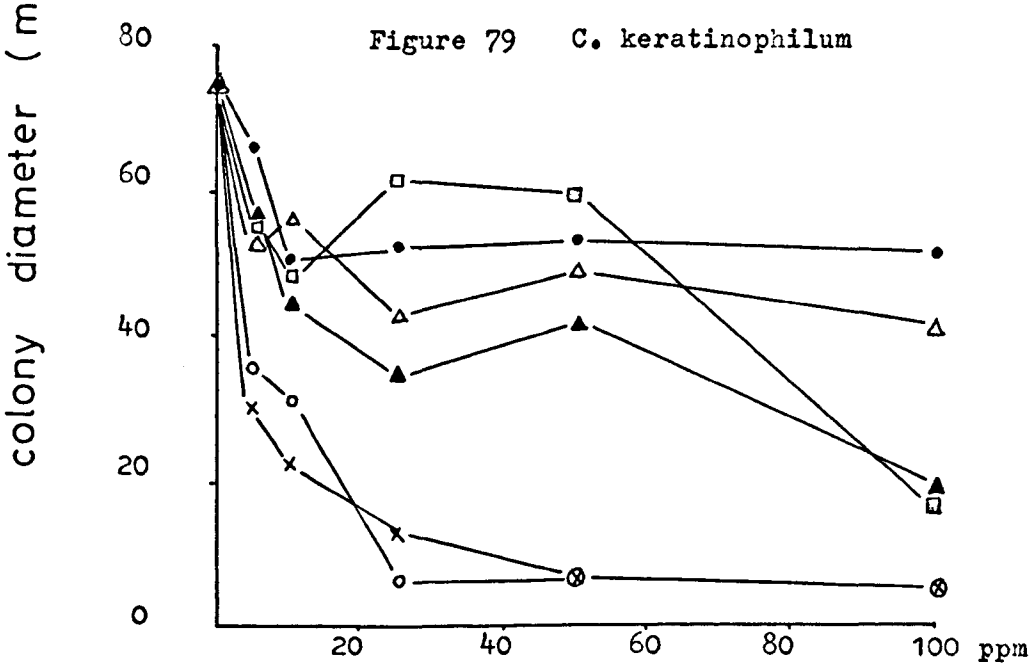


Figure 80 T. terrestre

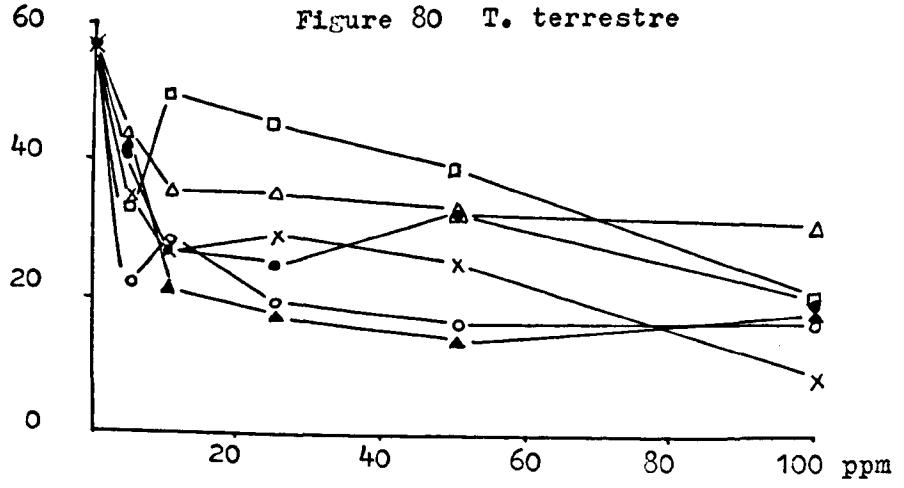


Figure 81 M. cookei

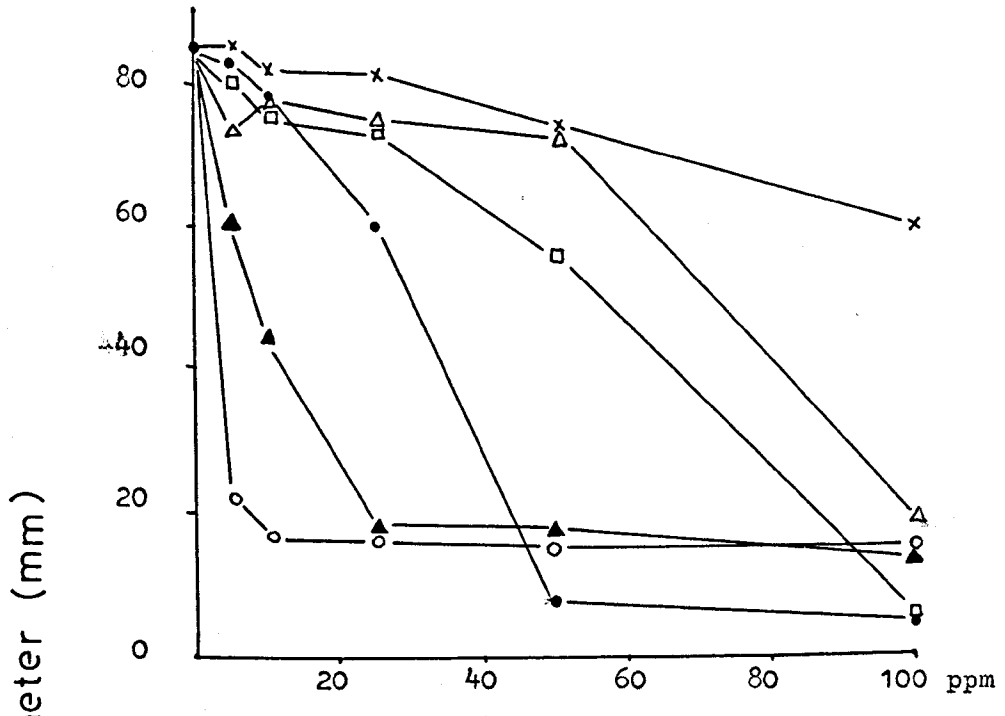
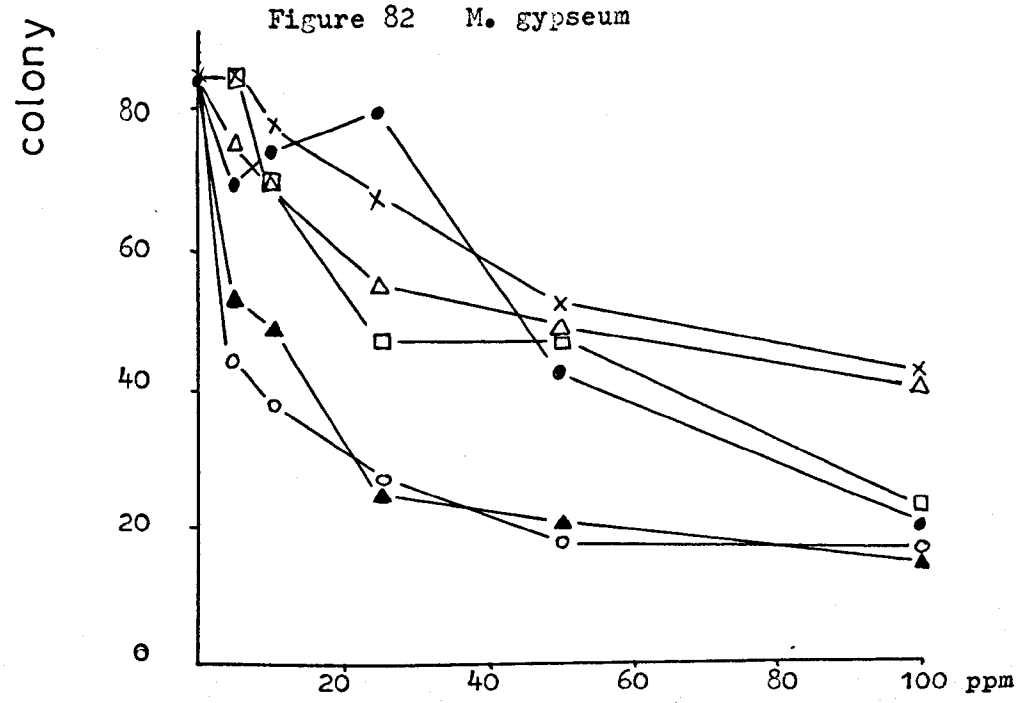


Figure 82 M. gypseum



not inhibit growth to half the control, even at 100 ppm. There was an anomaly with dicloran at 50 ppm and only at a concentration of 25 ppm was growth inhibited to half that of the control. All but captan inhibited growth of T. terrestre by half at 100 ppm. At 10 ppm and above, all but captan and thiram had inhibited growth of this fungus to at least half that of the control, although growth in media containing 50 ppm formaldehyde was slightly stimulated, but not above that of the control.

Microsporum cookei was inhibited to half the growth of the control at 100 ppm by all the fungicides except Triarimol. Only Milcol and dicloran had any effect at concentrations of as low as 5 ppm and 10 ppm respectively. Formaldehyde inhibited at 50 ppm, whereas thiram and captan only took effect at 100 ppm.

All 6 fungicides inhibited M. gypseum at 100 ppm to half the growth of the control. Milcol had the most effect : growth was inhibited at 5 ppm ; dicloran was inhibitory at 10 ppm, formaldehyde at 50 ppm and the other 3 at 100 ppm.

Figures 83 - 112 are photographs of colonies of the 5 fungi after 14 days growth on media containing fungicides.

Effect of sea water on the growth of keratinophilic fungi.

a) isolations from 3 soils watered with different concentrations of sea water.

Percentage colonisation by A. uncinatum on greasy wool decreased with increasing concentrations of sea water on the farm hedge and University soils, but increased on the farm field soils. Isolations on degreased wool varied very little within each sample site, but the University and farm field soils had less percentage colonisation when watered with sea water than with sterile distilled water. Lowest percentage colonisation was, with the exception of the farm field soils, on greasy wool watered with sea water.

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

a) A. uncinatum

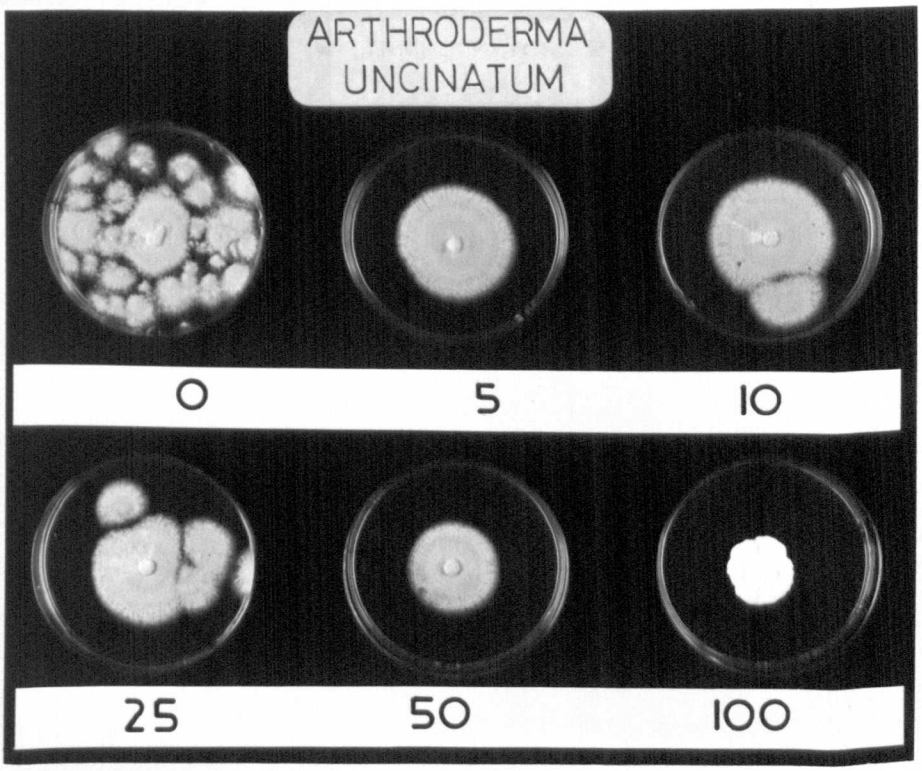
Figure 83

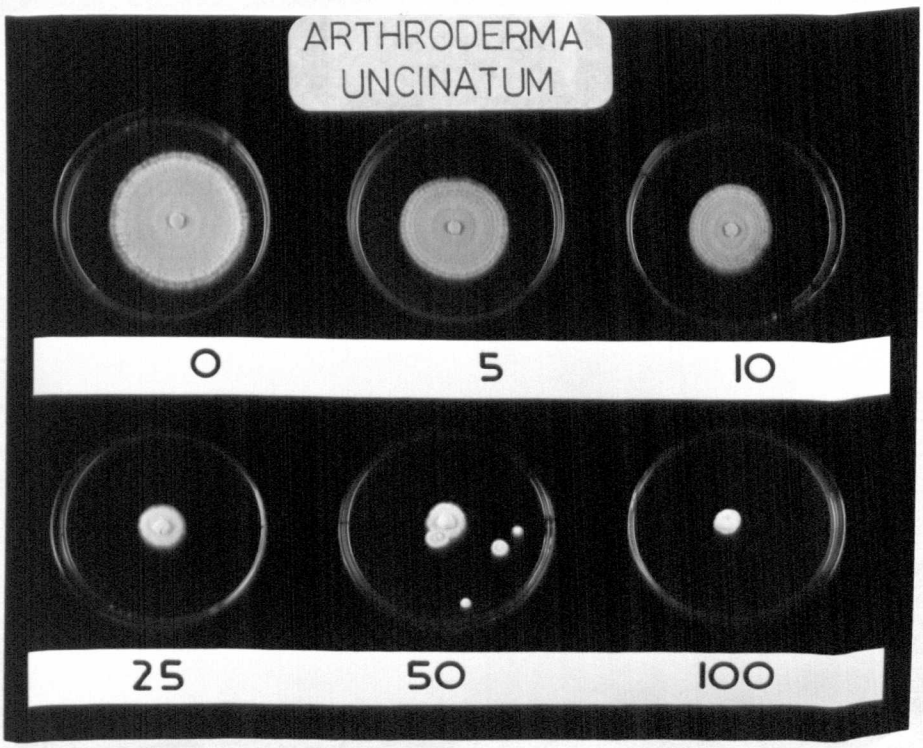
Captan

Figure 84

Dicloran

83


ARTHRODERMA
UNCINATUM
0
5
10
25
50
100


ARTHRODERMA
UNCINATUM
0
5
10
25
50
100

84

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

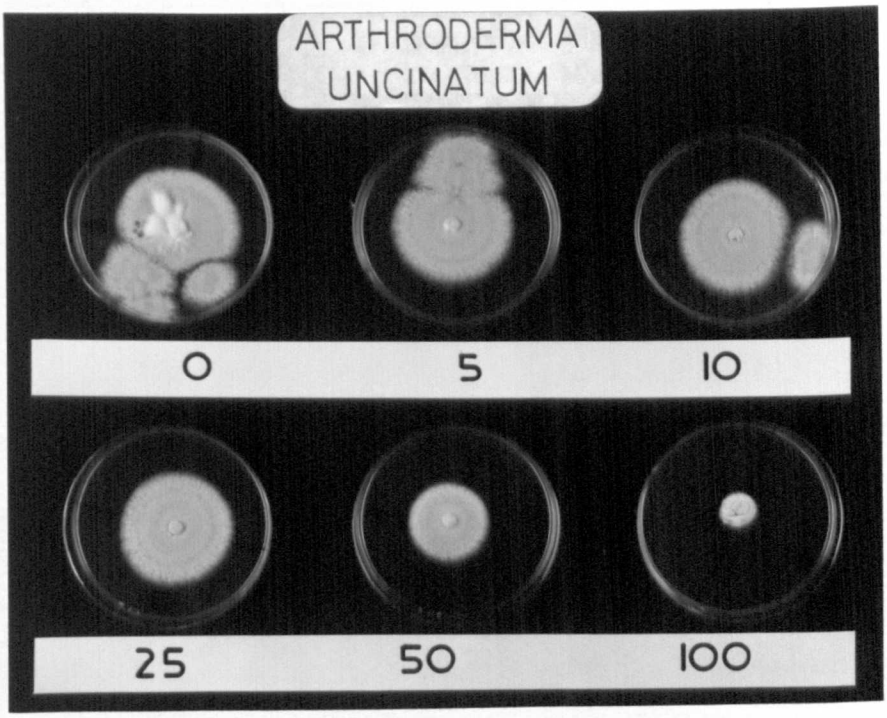
Figure 85

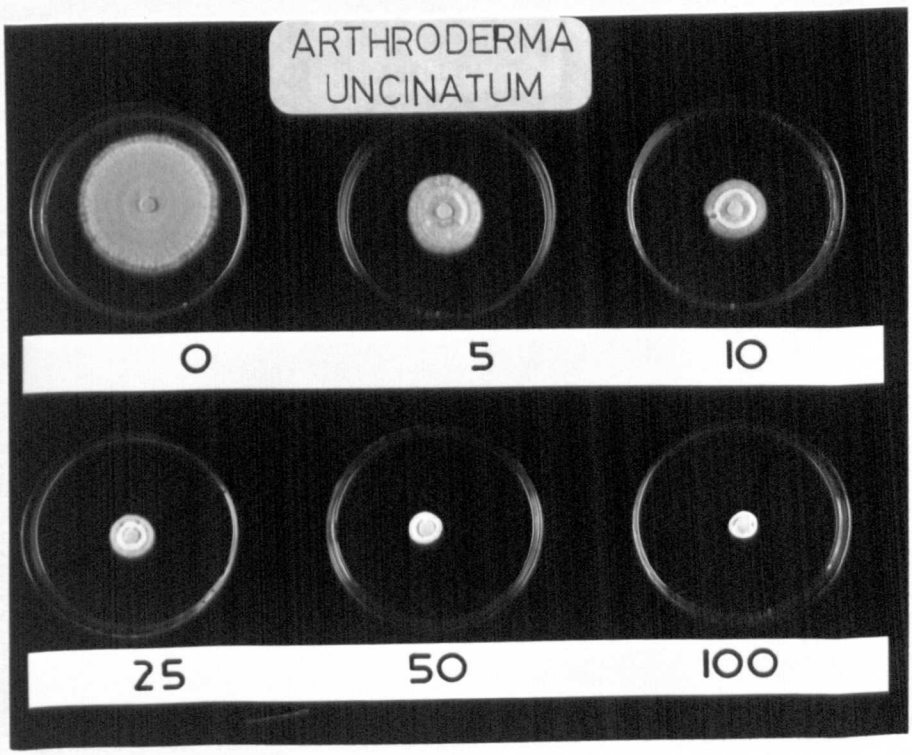
Formaldehyde

Figure 86

Milcol

85


ARTHRODERMA
UNCINATUM
0
5
10
25
50
100


ARTHRODERMA
UNCINATUM
0
5
10
25
50
100

86

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

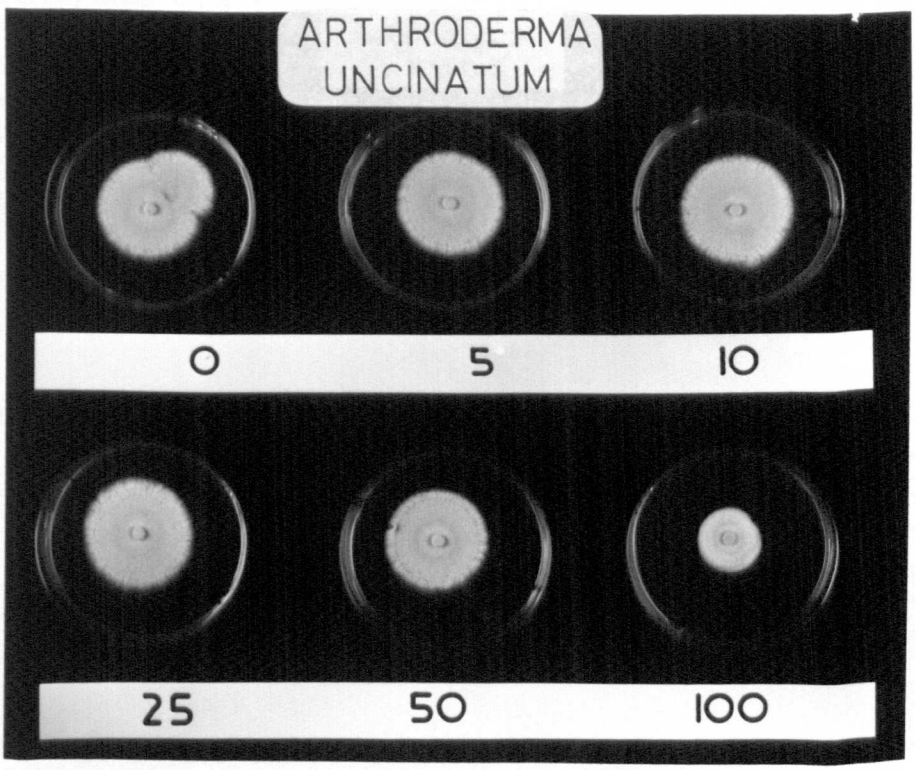
Figure 87

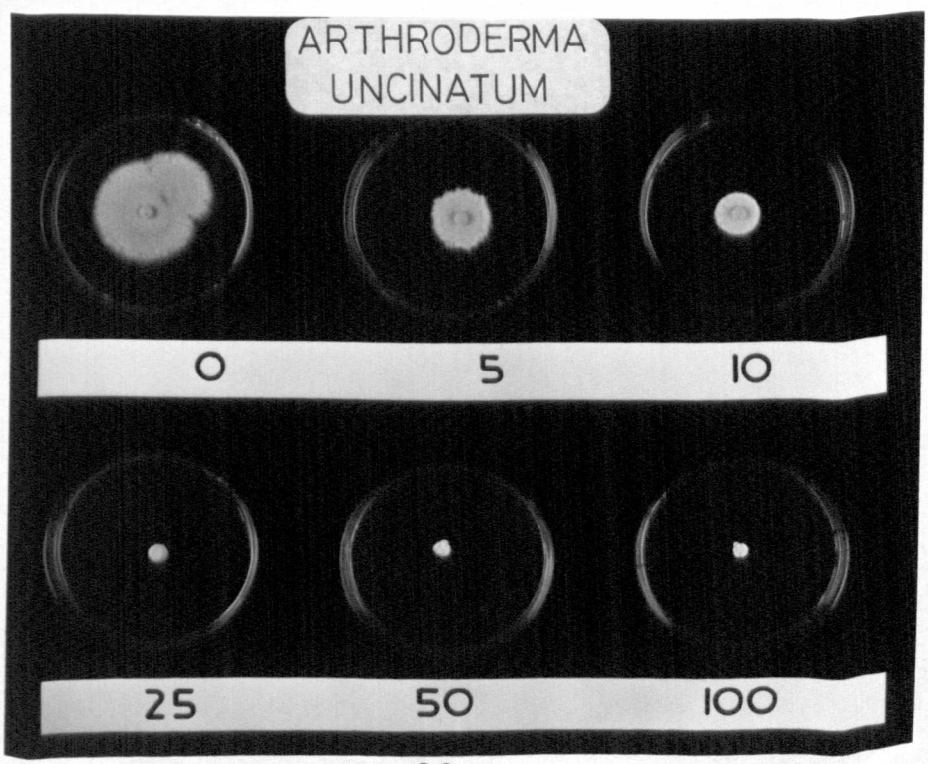
Thiram

Figure 88

Triarimol

87


ARTHRODERMA
UNCINATUM
0
5
10
25
50
100


ARTHRODERMA
UNCINATUM
0
5
10
25
50
100

88

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

b) C. keratinophilum

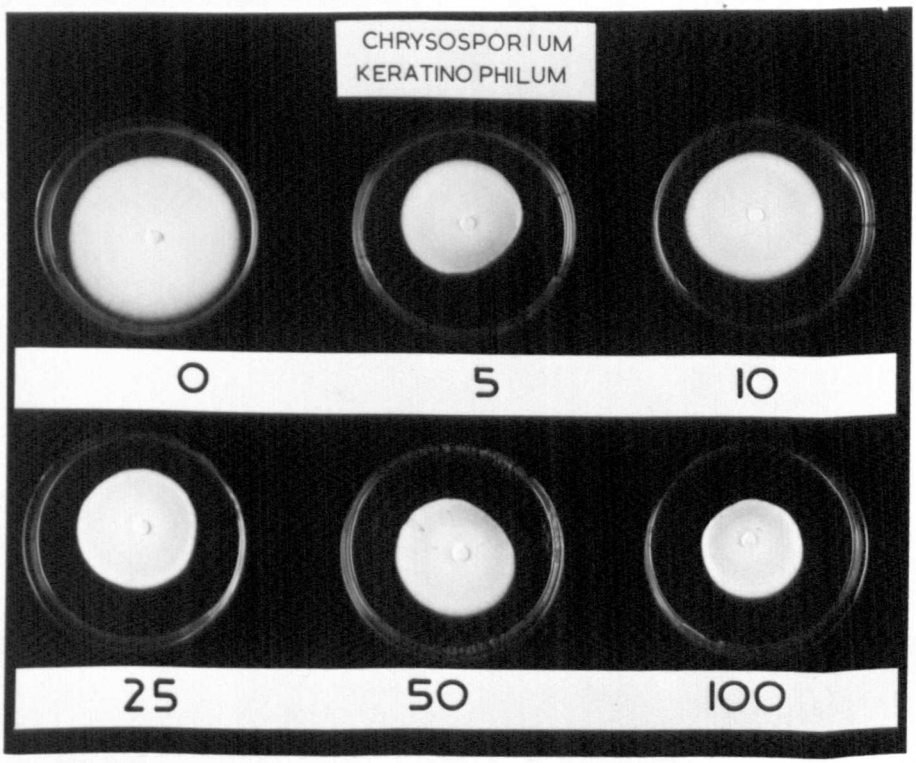
Figure 89

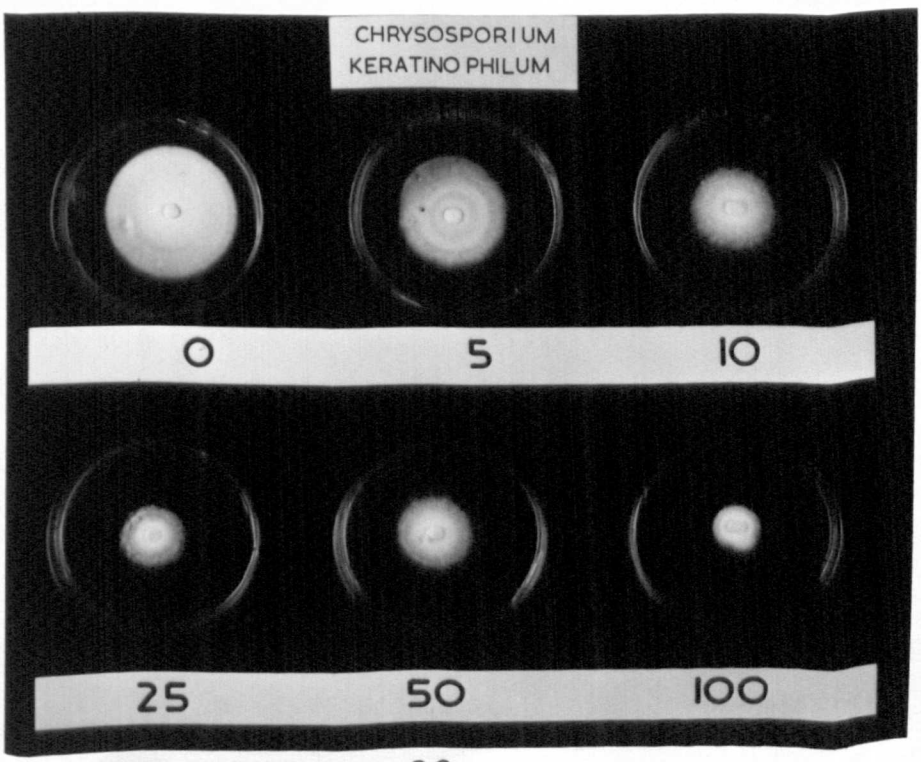
Captan

Figure 90

Dicloran

89


CHRYSOSPORIUM
KERATINO PHILUM
0
5
10
25
50
100


CHRYSOSPORIUM
KERATINO PHILUM
0
5
10
25
50
100

90

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

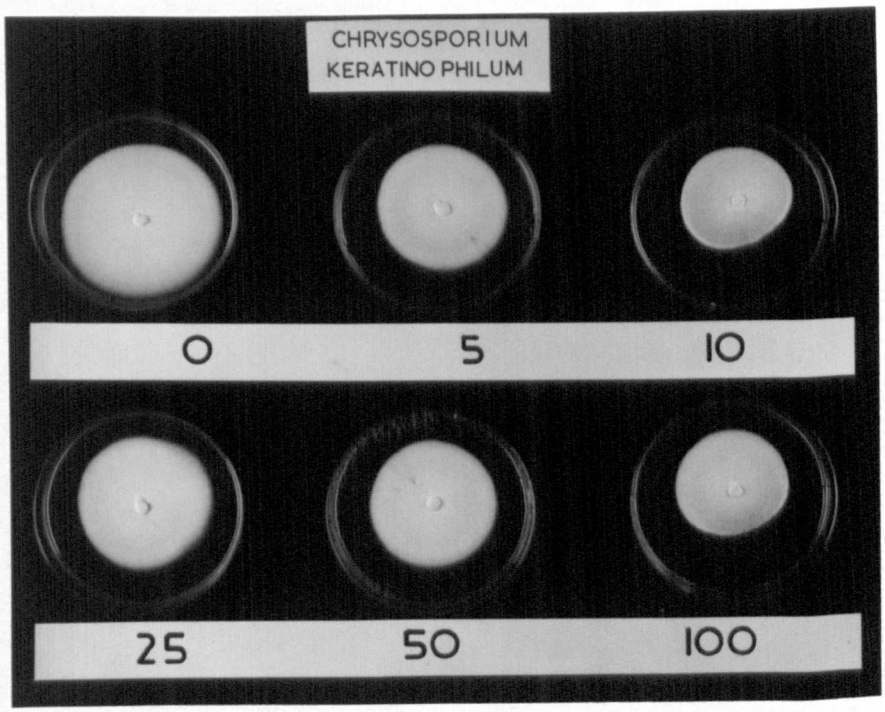
Figure 91

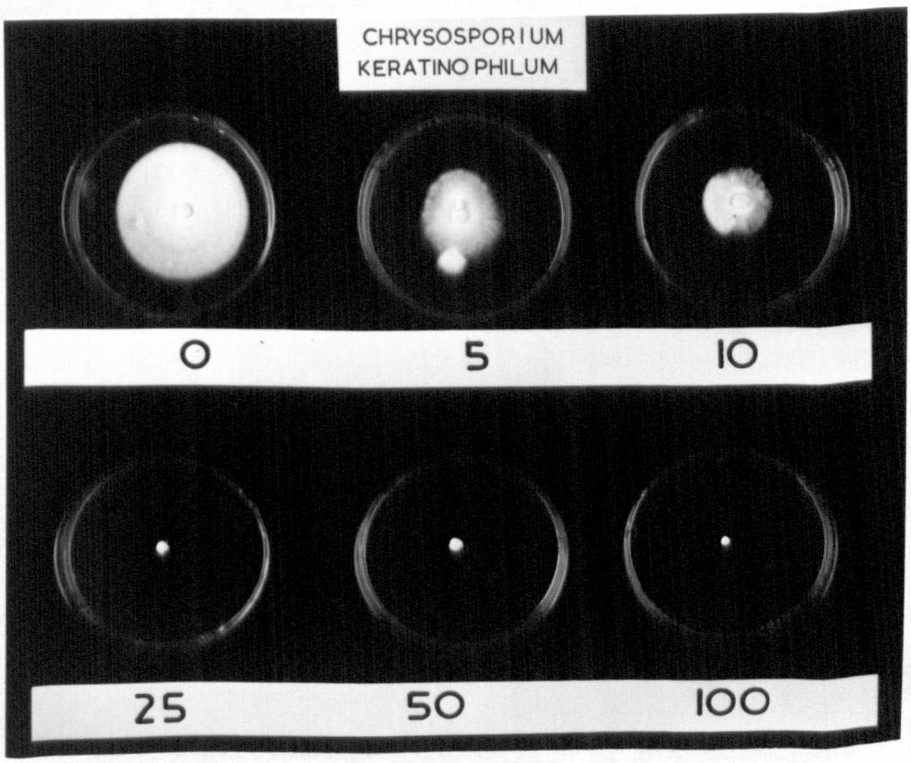
Formaldehyde

Figure 92

Milcol

91


CHRYSOSPORIUM
KERATINO PHILUM
0
5
10
25
50
100


CHRYSOSPORIUM
KERATINO PHILUM
0
5
10
25
50
100

92

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

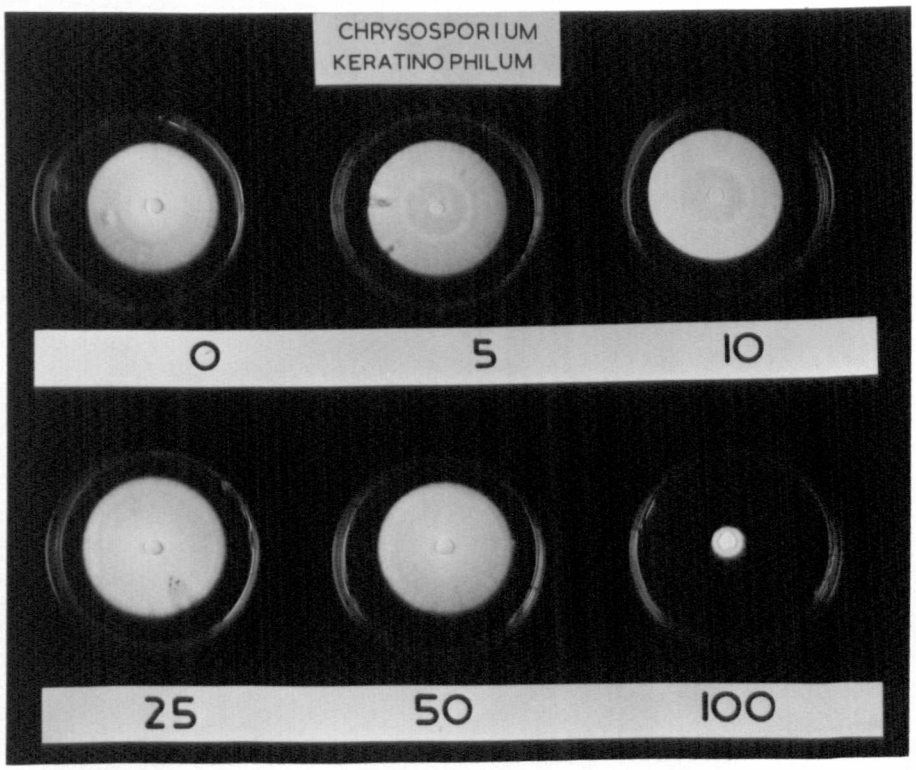
Figure 93

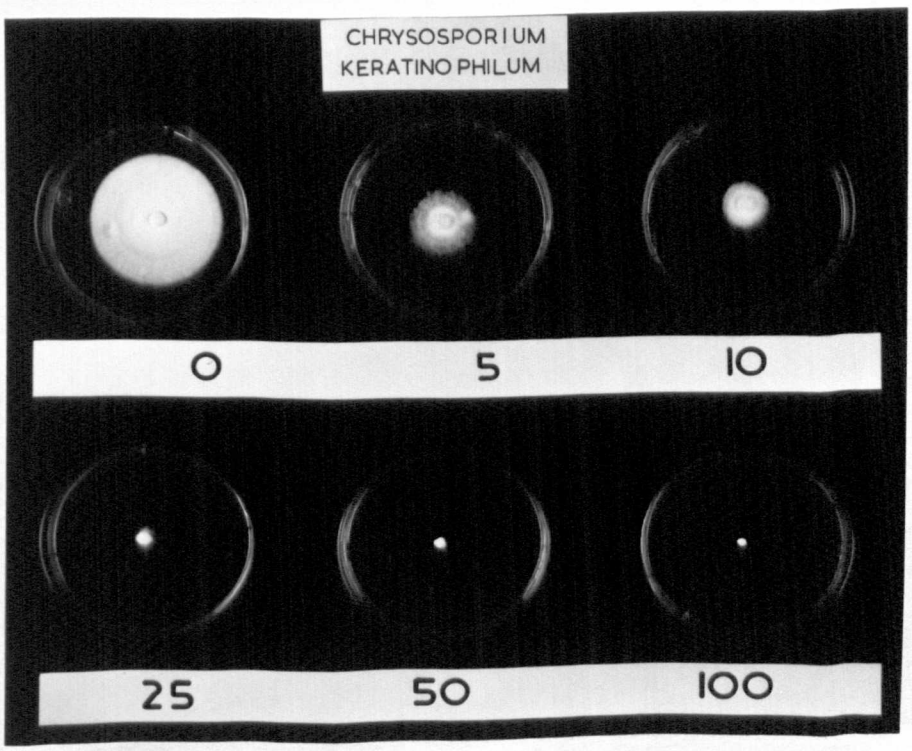
Thiram

Figure 94

Triarimol

93


CHRYSOSPORIUM
KERATINOPHILUM
0
5
10
25
50
100


CHRYSOSPORIUM
KERATINOPHILUM
0
5
10
25
50
100

94

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

c) M. cookei

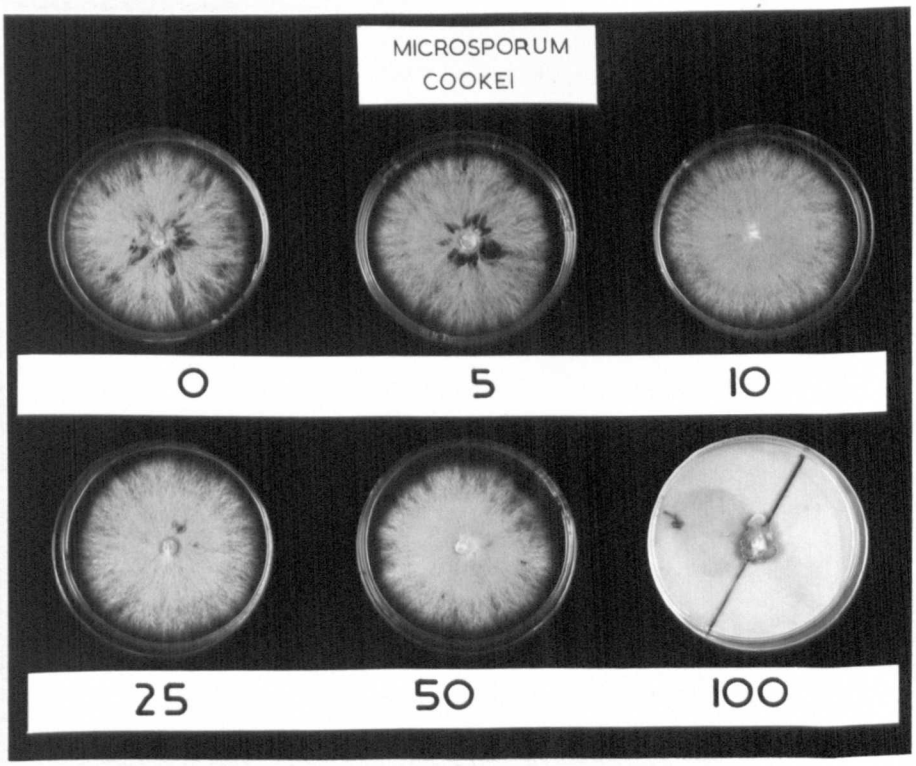
Figure 95

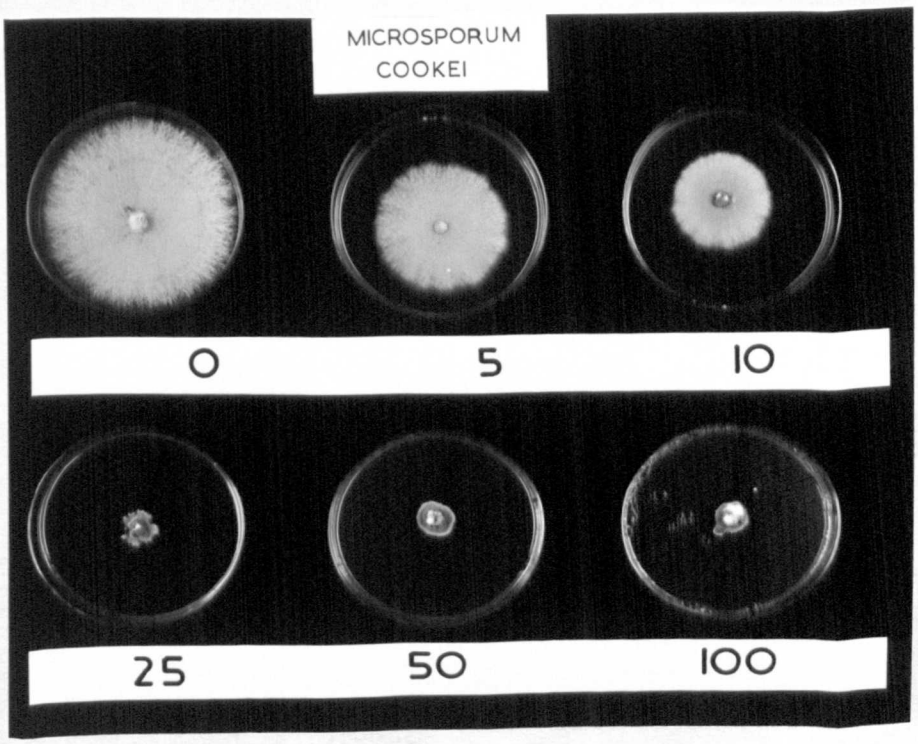
Captan

Figure 96

Dicloran

95


MICROSPORUM
COOKEI
0
5
10
25
50
100


MICROSPORUM
COOKEI
0
5
10
25
50
100

96

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

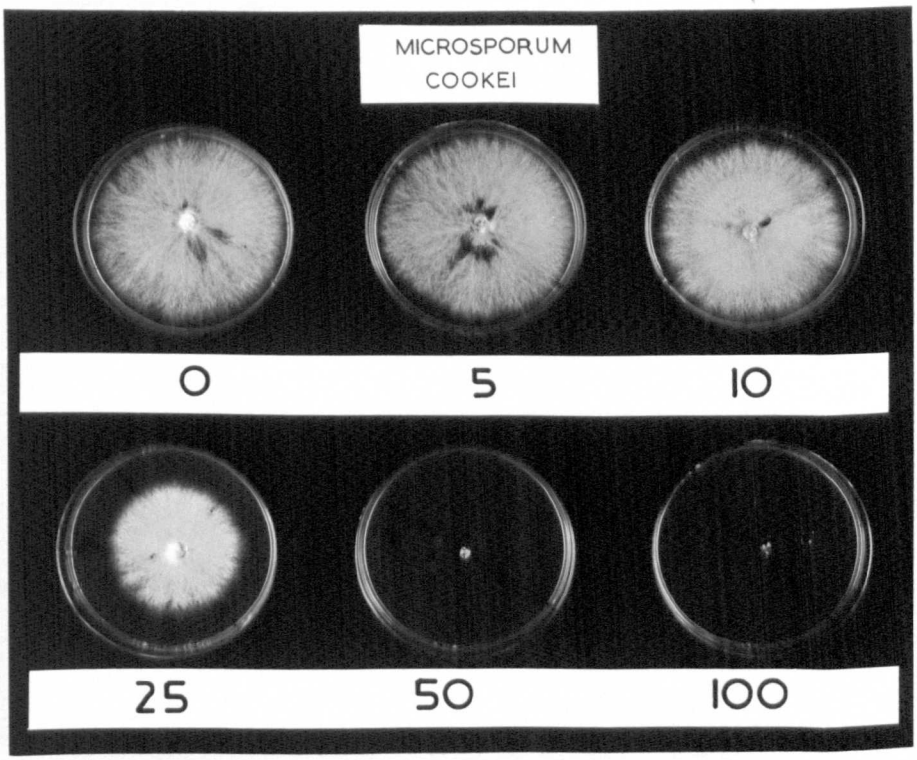
Figure 97

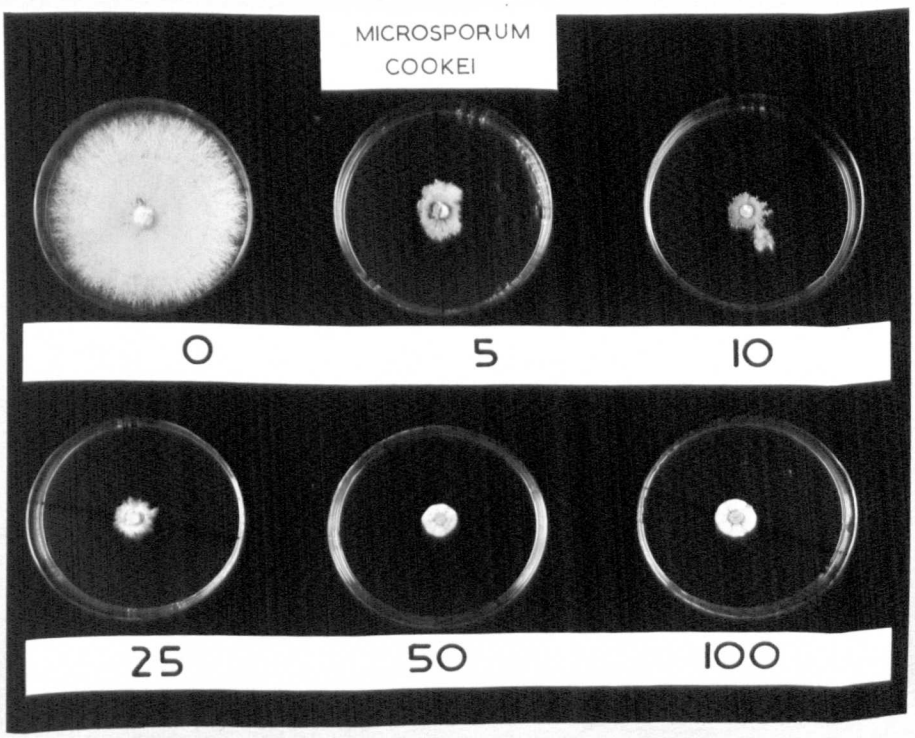
Formaldehyde

Figure 98

Kilcol

97


MICROSPORUM
COOKEI
0
5
10
25
50
100


MICROSPORUM
COOKEI
0
5
10
25
50
100

98

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

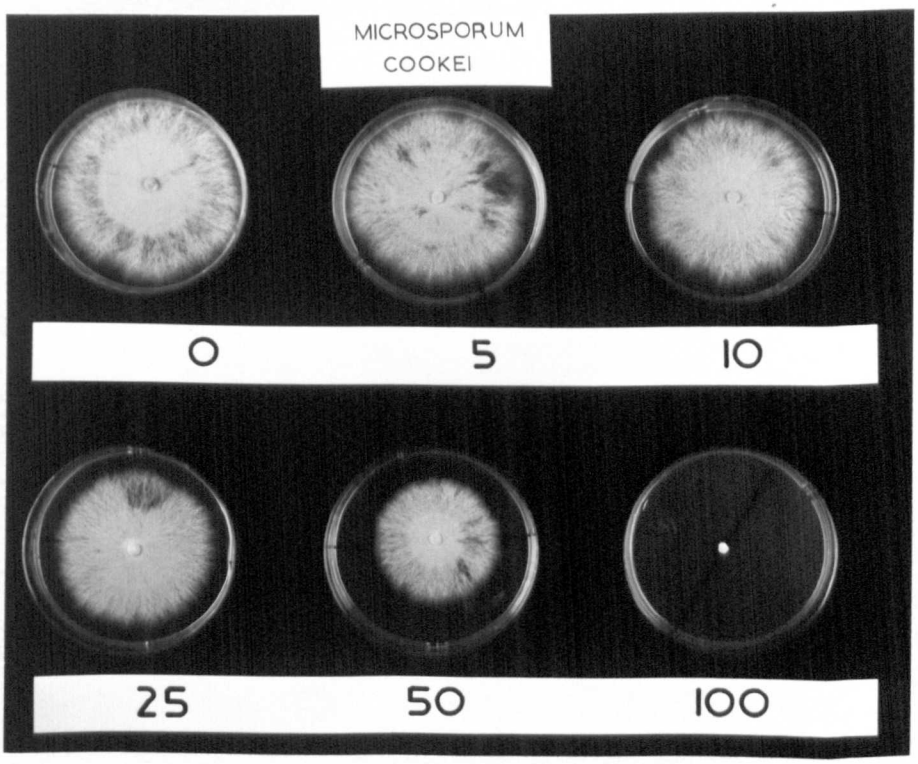
Figure 99

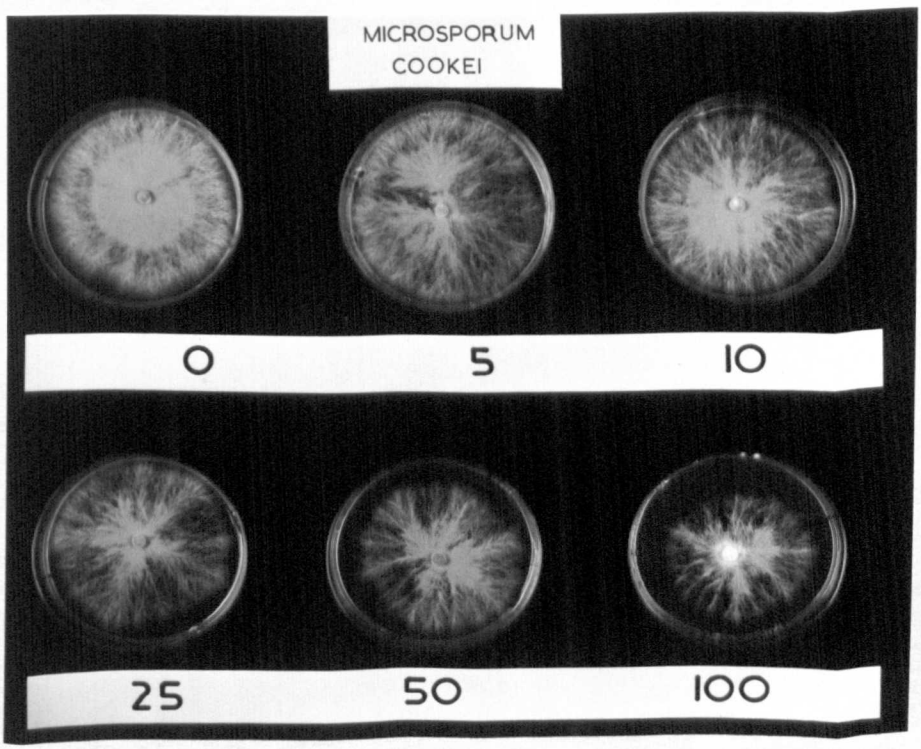
Thiram

Figure 100

Triarimol

99


MICROSPORUM
COOKEI
0
5
10
25
50
100


MICROSPORUM
COOKEI
0
5
10
25
50
100

100

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

d) M. gypseum

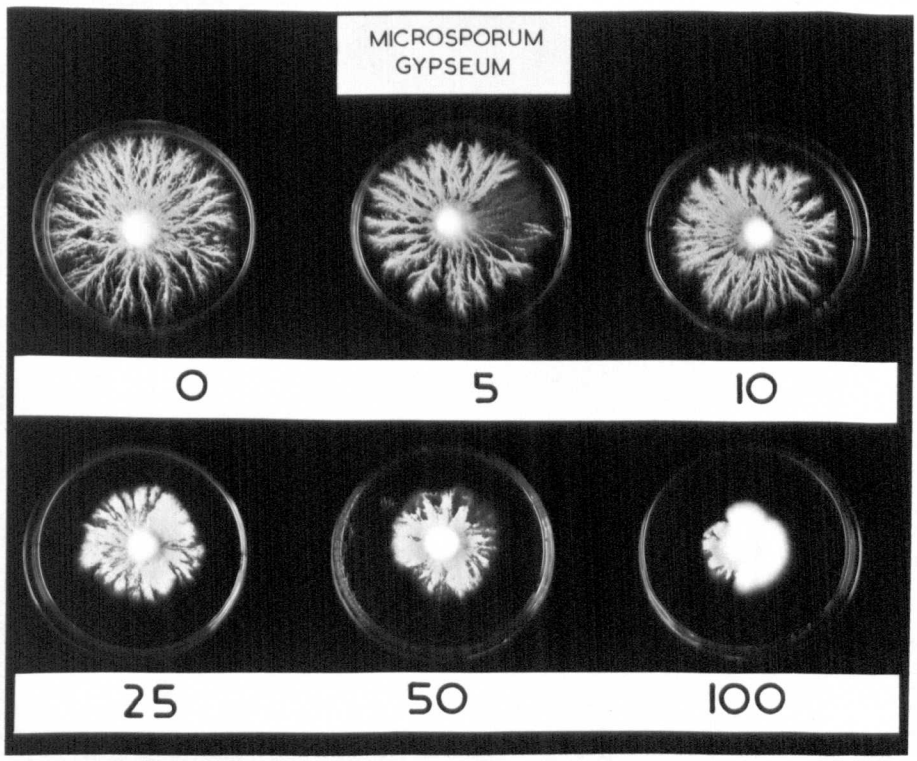
Figure 101

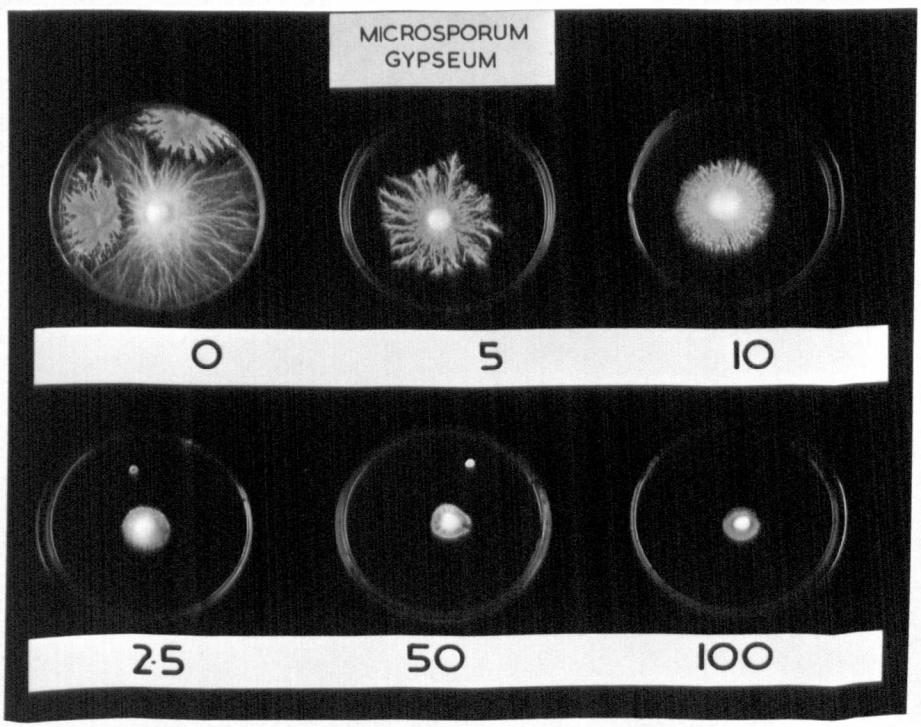
Captan

Figure 102

Dicloran

101


MICROSPORUM
GYPSEUM
0
5
10
25
50
100


MICROSPORUM
GYPSEUM
0
5
10
25
50
100

102

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

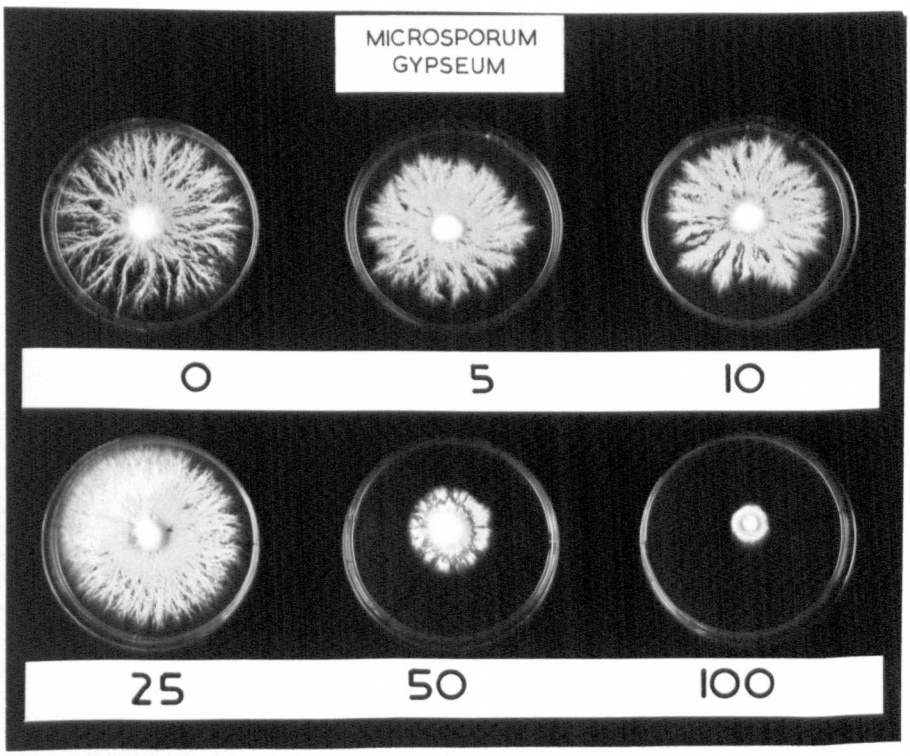
Figure 103

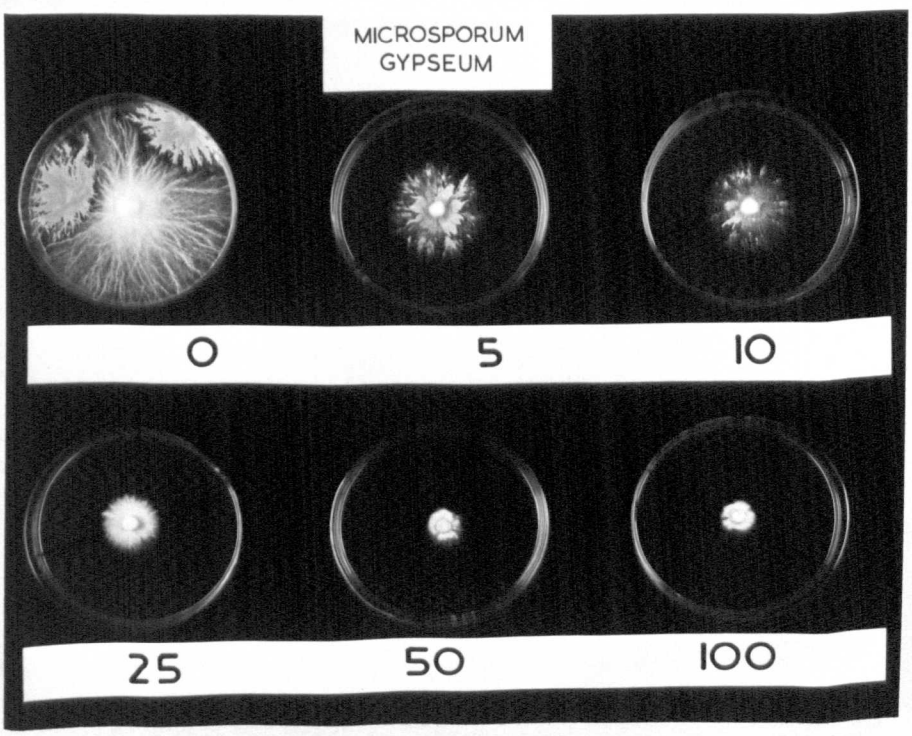
Formaldehyde

Figure 104

Milcol

103


MICROSPORUM
GYPSEUM
0
5
10
25
50
100


MICROSPORUM
GYPSEUM
0
5
10
25
50
100

104

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

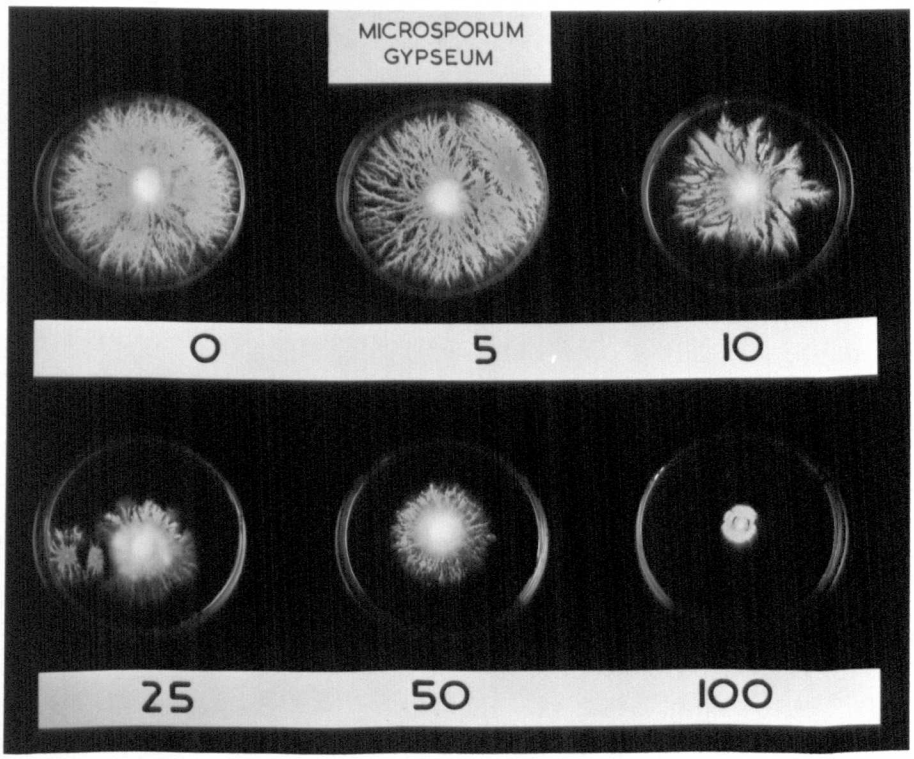
Figure 105

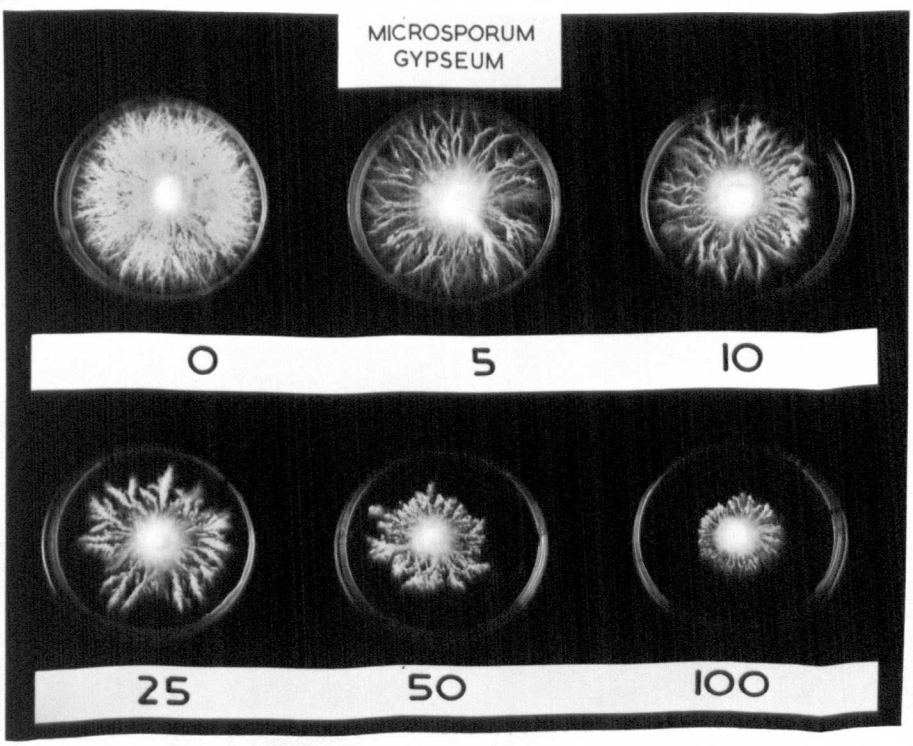
Thiram

Figure 106

Triarimol

105


MICROSPORUM
GYPSEUM
0
5
10
25
50
100


MICROSPORUM
GYPSEUM
0
5
10
25
50
100

106

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

e) T. terrestre

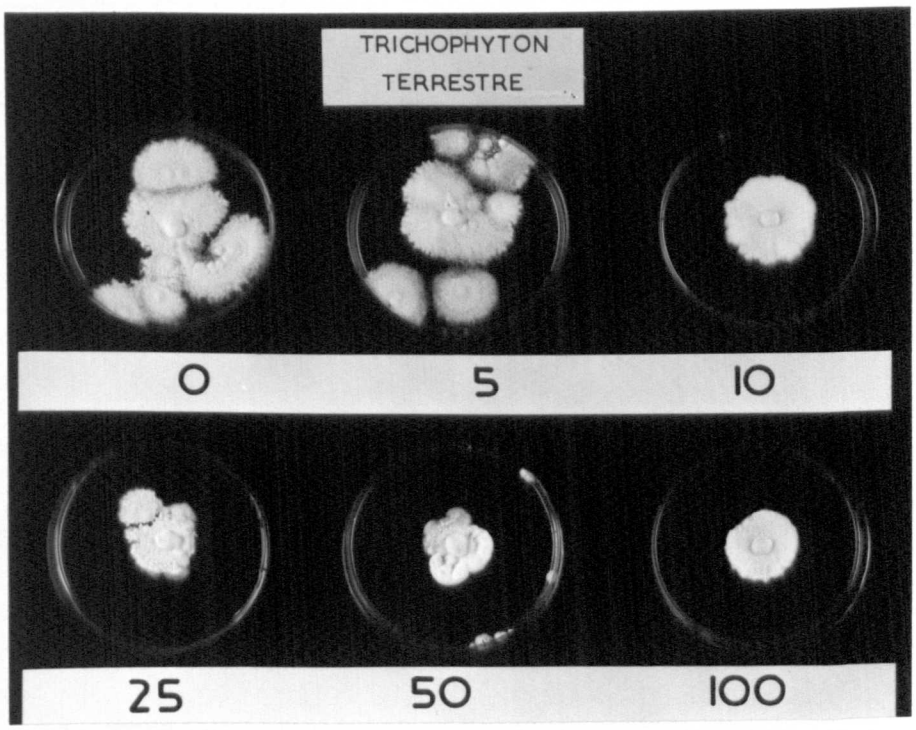
Figure 107

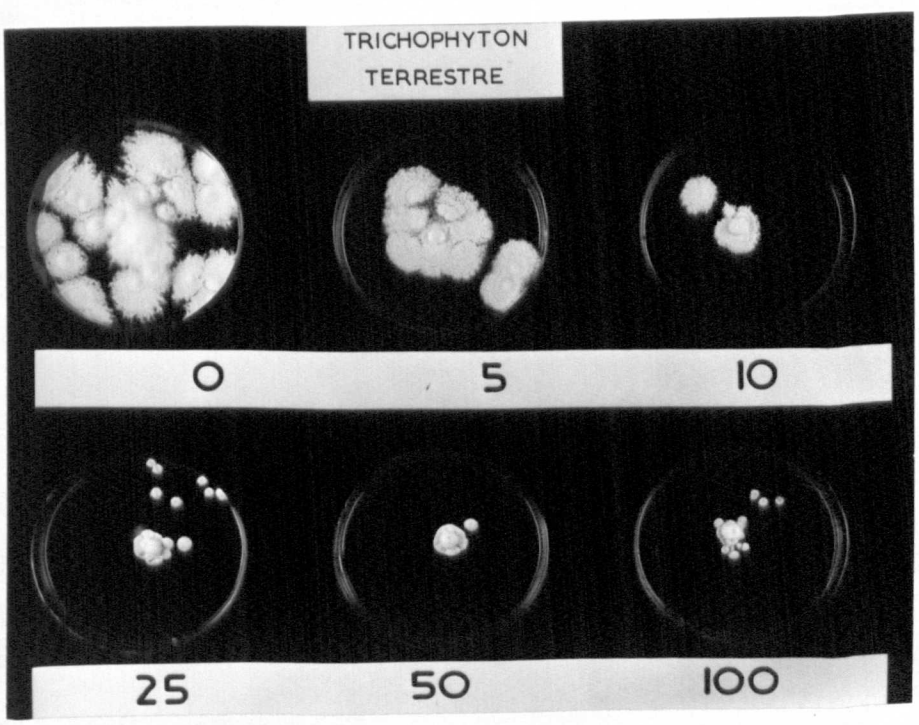
Captan

Figure 108

Dicloran

107


TRICHOPHYTON
TERRESTRE
0
5
10
25
50
100


TRICHOPHYTON
TERRESTRE
0
5
10
25
50
100

108

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

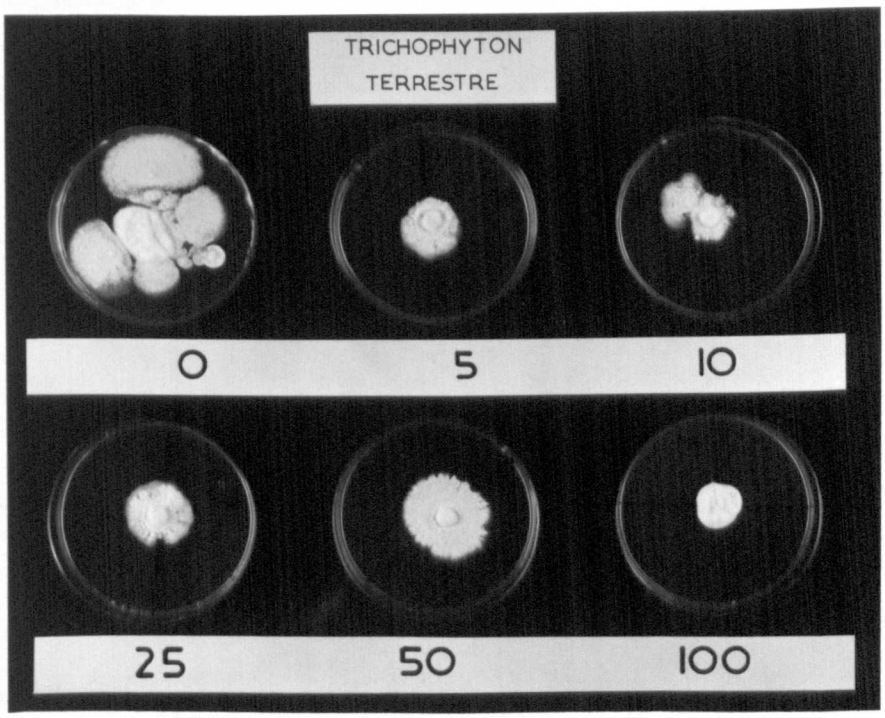
Figure 109

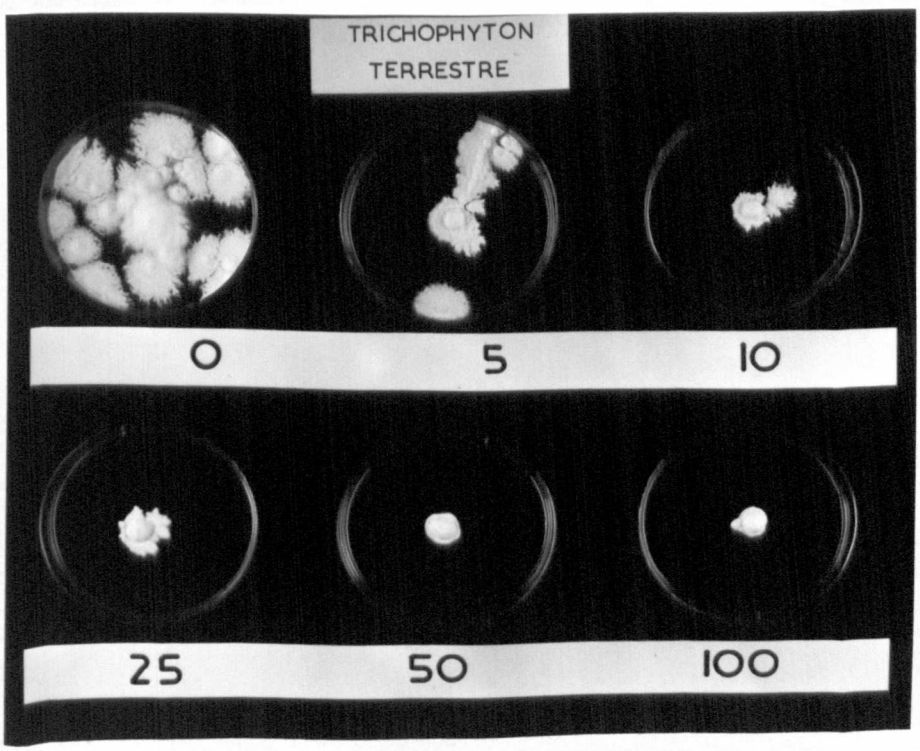
Formaldehyde

Figure 110

Milcol

109


TRICHOPHYTON
TERRESTRE
0
5
10
25
50
100


TRICHOPHYTON
TERRESTRE
0
5
10
25
50
100

110

Growth of keratinophilic fungi on media containing different concentrations of fungicides.

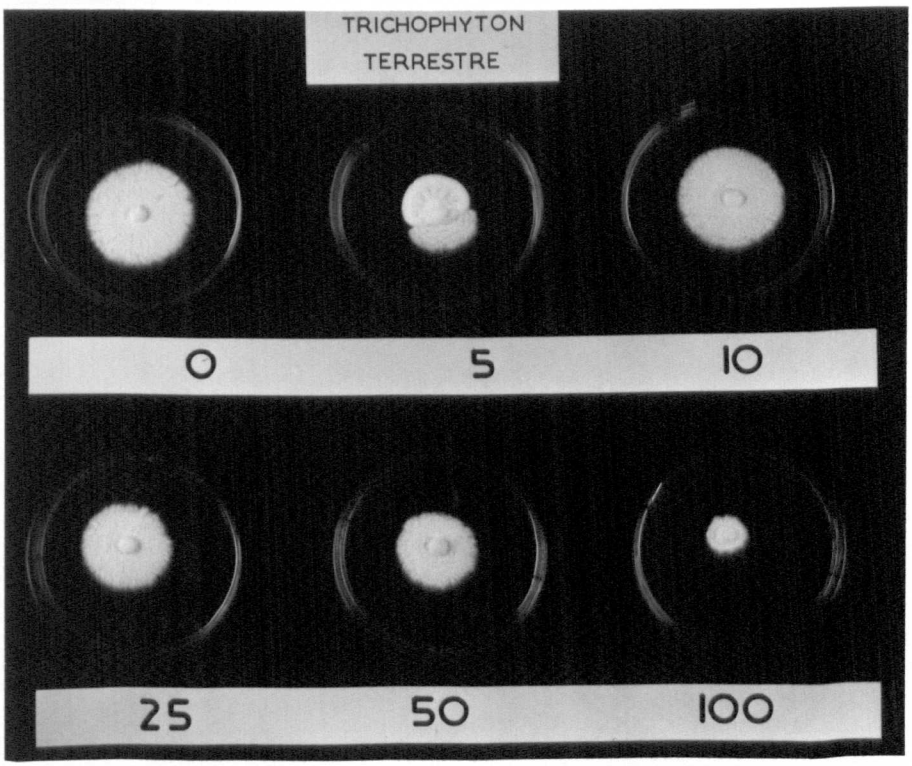
Figure 111

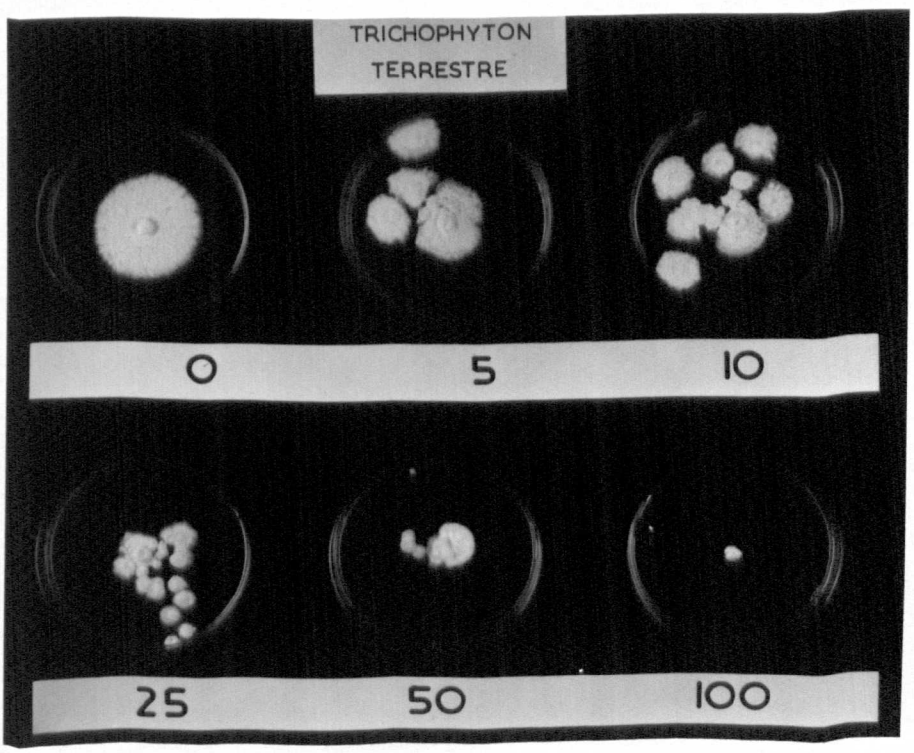
Thiram

Figure 112

Triarimol

111


TRICHOPHYTON
TERRESTRE
0
5
10
25
50
100


TRICHOPHYTON
TERRESTRE
0
5
10
25
50
100

112

The highest percentage colonisation by C. keratinophilum was on greasy wool, both on University and farm hedge soils, watered with sterile distilled water. However, greasy wool was not colonised at all by this fungus when University soils were watered with sea water. Degreased wool was more highly colonised than greasy wool, by C. keratinophilum on the soil from the treated field, and isolations were the same on both control and sea water-treated soils. Chrysosporium keratinophilum was inhibited by sea water from farm hedge and University soils. Arthroderma uncinatum and C. keratinophilum were isolated more from control soils of the University, on greasy wool, than from either the field or hedge soil. Isolation of these 2 fungi was only greater in the treated field soils than those of the hedge when baited with degreased wool.

Trichophyton terrestre showed a gradual increase in percentage colonisation when the University soils were treated with increasing concentrations of sea water. No colonisation of either greasy or degreased wool by T. terrestre was observed in these particular control soil samples, although this species had been recorded in these soils on other occasions. Trichophyton terrestre colonised about 35% of the baits, only from farm field soil watered with half strength sea water. The colonisation of baits on soils from the farm hedge was seen only in control soils kept moist with sterile distilled water. (See Figures 113-115).

b) reisolation of keratinophilic fungi inoculated into sterile soil and watered with different concentrations of sea water.

Chrysosporium keratinophilum and M. cookei were the fungi most affected by sea water when reisolation of them was attempted by baiting soils which had already been inoculated with one of the 5 fungi under investigation.

In sandy soil, all the fungi were difficult to reisolate

Histograms representing the effect of sea water upon percentage colonisation of degreased and greasy wool by keratinophilic fungi (after 4 weeks baiting) from 3 different soils.

Figure 113 - A. uncinatum

Figure 114 - C. keratinophilum

Figure 115 - T. terrestre

SDW sterile distilled water

SW sea water

DG degreased wool

GW greasy wool

Figure 113

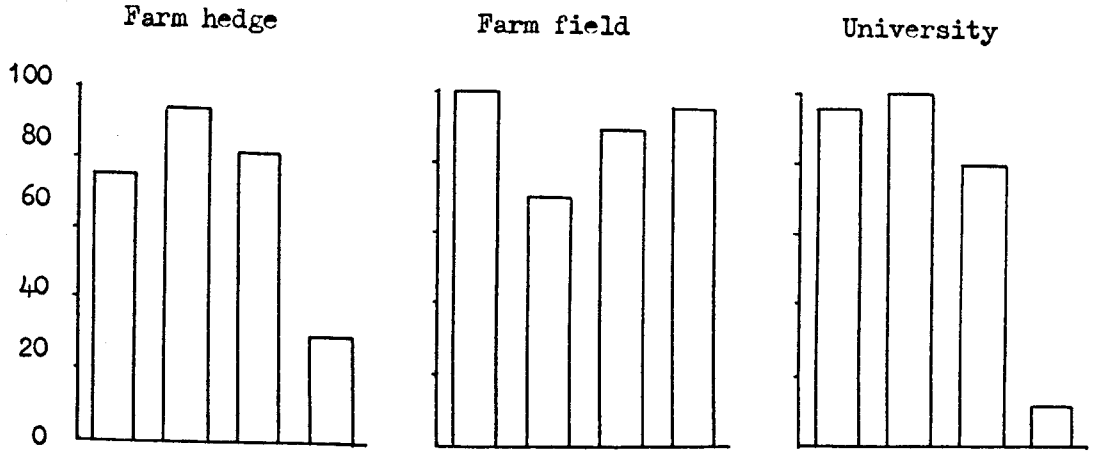


Figure 114

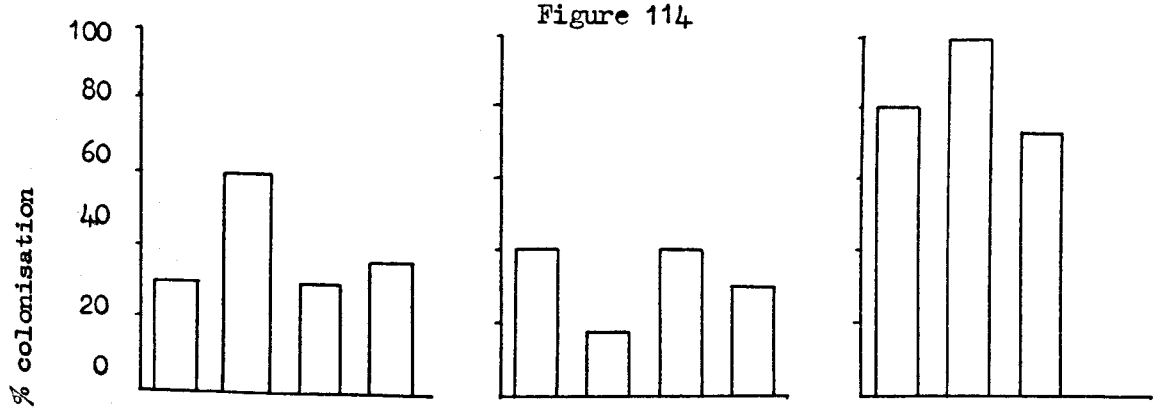
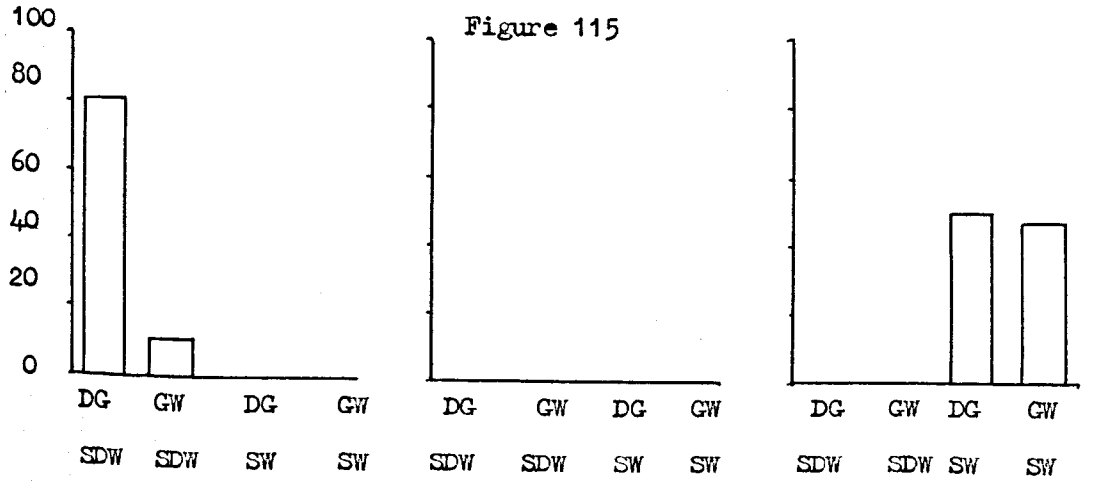


Figure 115



when the soil was watered with different sea concentrations. This time C. keratinophilum was not reisolated at all from these soils. All fungi were isolated more on degreased wool than greasy wool, and all were isolated more from soils watered with sterile distilled water rather than sea water. The combination of salt water and greasy wool produced fewest reisolations with the exception of M. gypseum in the loamy soils. (See Figures 131-135)

Figures 116 - 120 are histograms representing the effect of sea water on the reisolation of keratinophilic fungi from sterile soils and sands.

c) growth of keratinophilic fungi on solid media containing different concentrations of sea water.

The picture of growth was more or less the same as in liquid media results (see below), but the difference between the 2 lower concentrations of sea water and controls varied very little from one another.

d) growth of keratinophilic fungi in liquid media containing different concentrations of sea water.

A. uncinatum, M. cookei and M. gypseum showed an inverse correlation between concentration of sea water and growth. Trichophyton terrestre and C. keratinophilum in general showed this, but growth of T. terrestre was slightly stimulated on media containing full strength sea water and C. keratinophilum was stimulated by $\frac{1}{4}$ strength sea water. These latter 2 species seemed to be the least inhibited by sea water in liquid media.

Figures 121 - 125 are colour photographs showing the growth of the 5 fungi studied, upon media containing various concentrations of sea water.

Figures 126 - 130 are histograms and graphs representing growth of the 5 fungi on solid and liquid media, containing different concentrations of sea water.

FIGURE 116

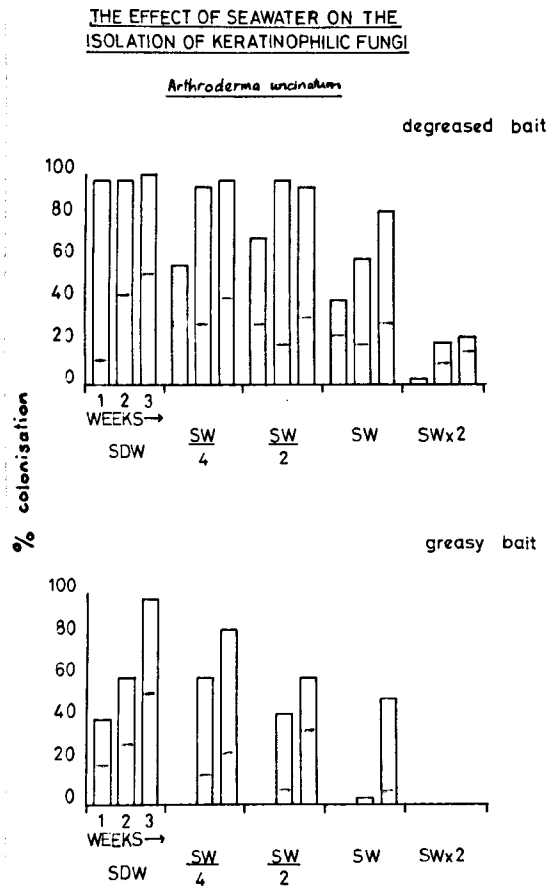
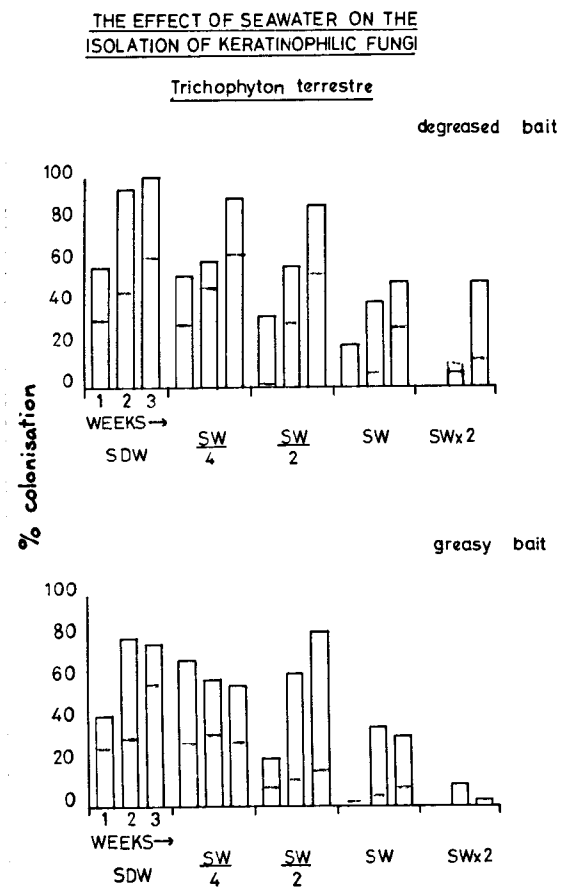


FIGURE 117



— sand
— soil

FIGURE 118

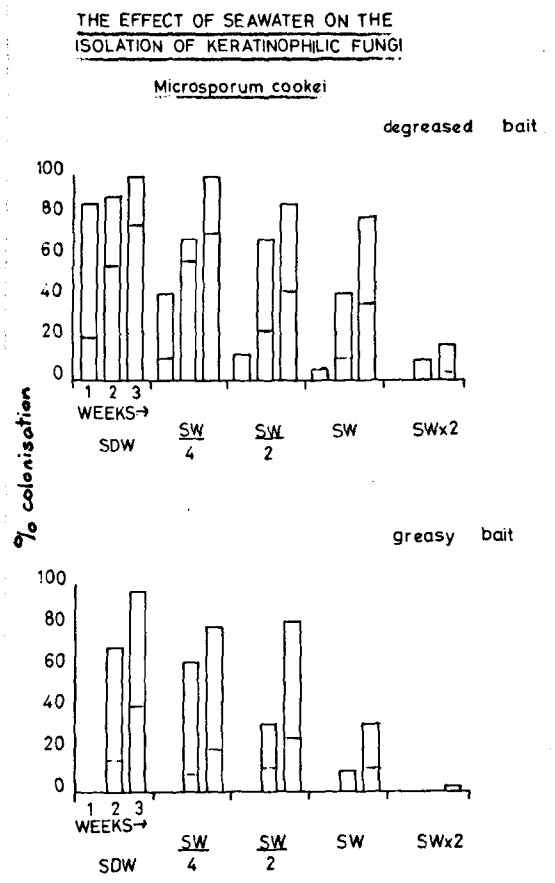
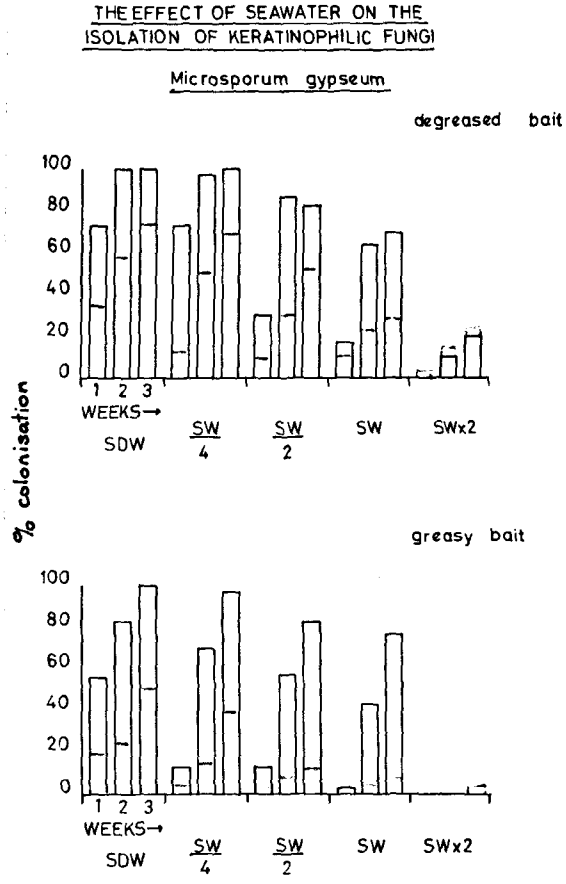
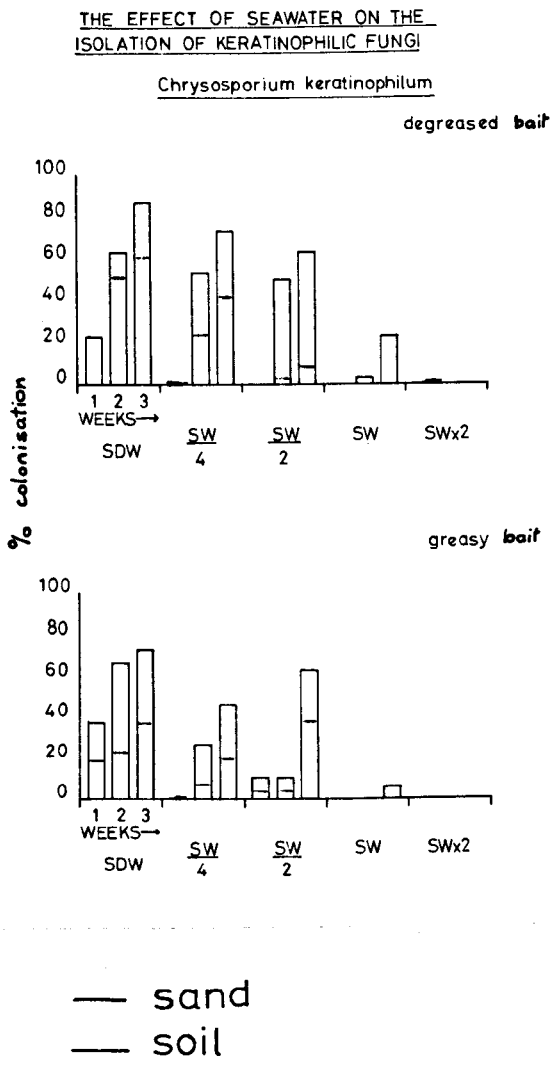


FIGURE 119



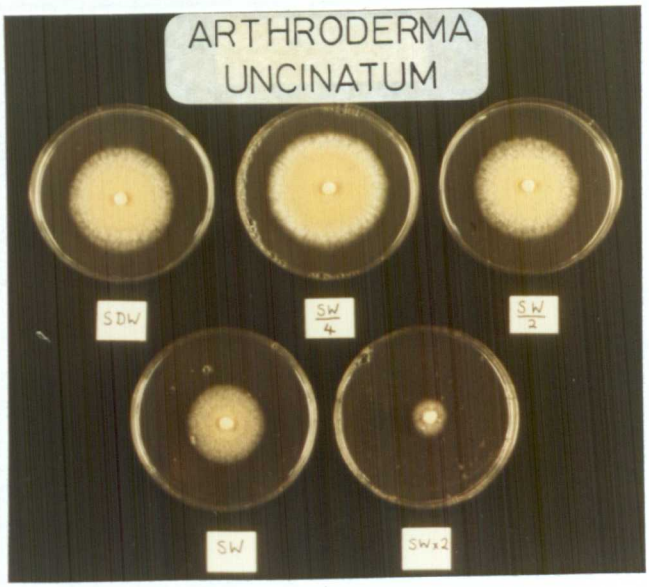
— sand
— soil

FIGURE 120


THE EFFECT OF SEAWATER ON THE ISOLATION OF KERATINOPHILIC FUNGI
Chrysosporium keratinophilum
degreased bait
% colonisation
100
80
60
40
20
0
1 2 3
WEEKS→
SDW
SW/4
SW/2
SW
SWx2
greasy bait
100
80
60
40
20
0
1 2 3
WEEKS→
SDW
SW/4
SW/2
SW
SWx2
— sand
— soil

Growth of keratinophilic fungi on media containing different concentrations of sea water

FIGURE 121



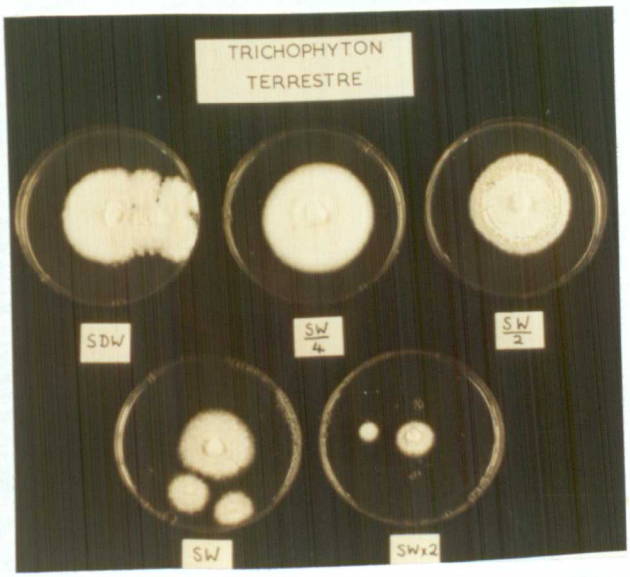
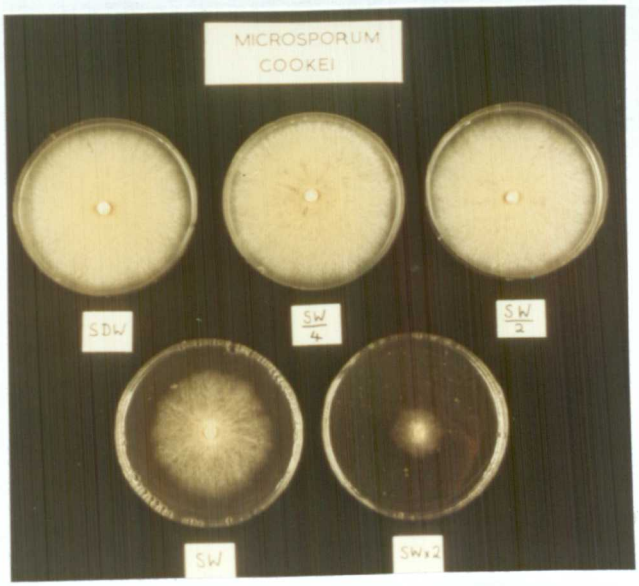


FIGURE 122

Growth of keratinophilic fungi on media containing different concentrations of sea water

FIGURE 123



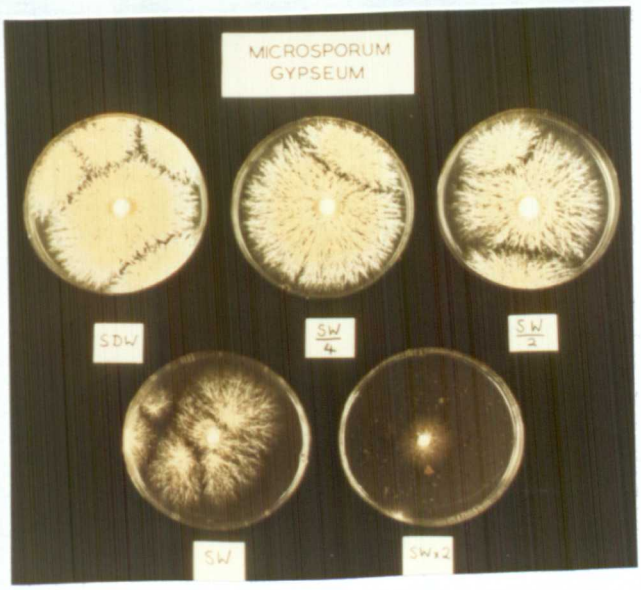
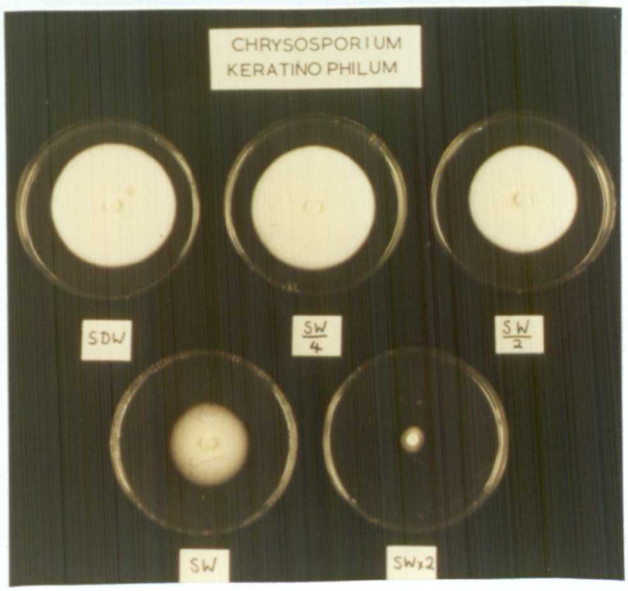


FIGURE 124

Growth of keratinophilic fungi on media containing different concentrations of sea water

FIGURE 125



Graphs and histograms representing growth of keratinophilic fungi on solid and in liquid media

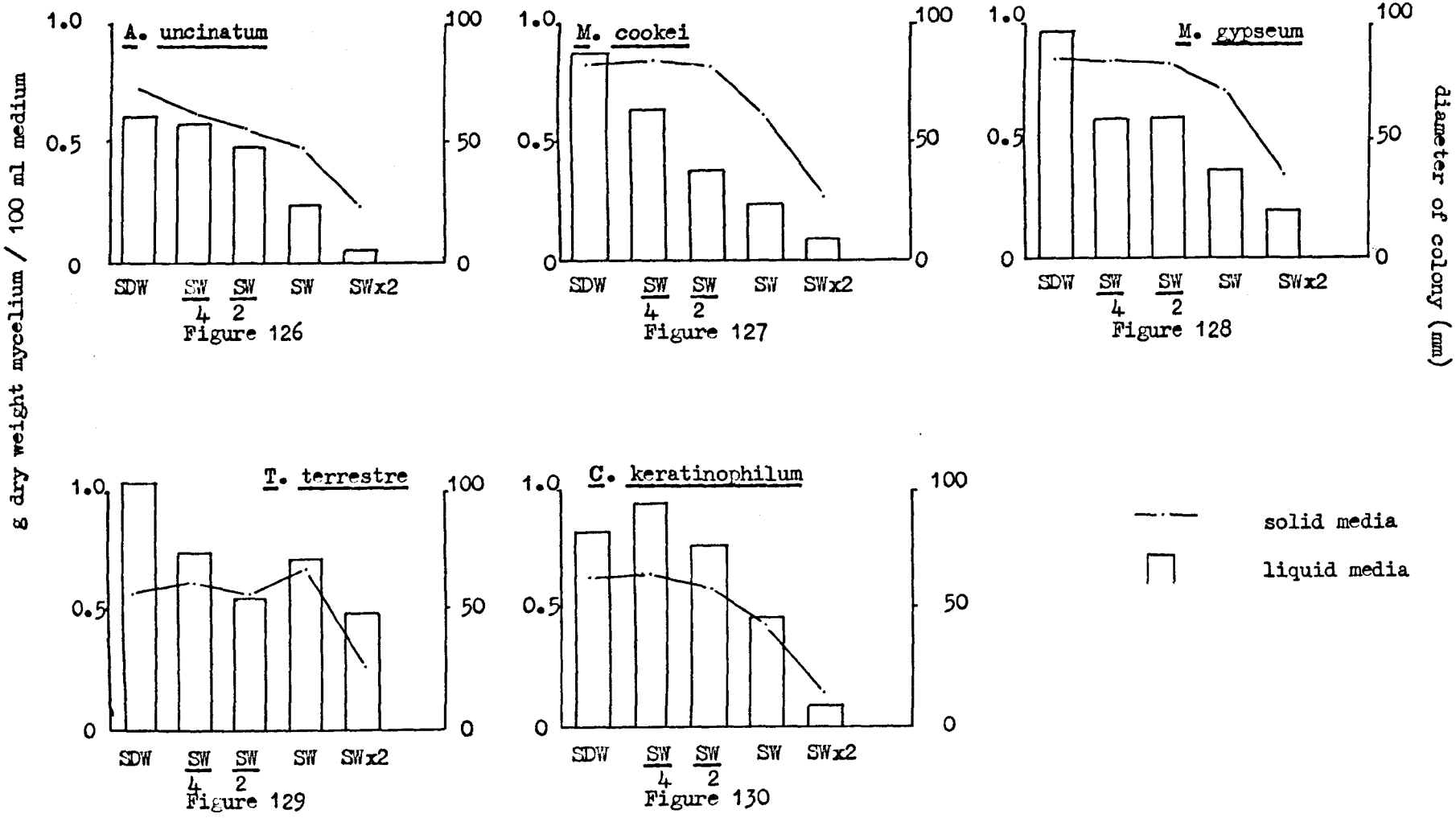


Figure 126

Figure 127

Figure 128

Figure 129

Figure 130

Figures 131-135 are histograms comparing reisolations of 5 keratinophilic fungi with degreased and greasy wool, sea water and sterile distilled water.

Results of the application of the fluorescent antibody technique to a study of A. uncinatum and T. mentagrophytes.

All slides of control cultures other than A. uncinatum were negative for fluorescence.

Fluorescence of pure cultures of A. uncinatum can be seen at 4 and 9 days in Figures 136 and 137, respectively. The older mycelia have not fluoresced to such an extent as those of the 4-day-old culture, but the multiseptate nature of the macroconidia is clear and the thick walls of the cells are evident. Figures 138, 139 and 140 show spores and mycelium of A. uncinatum which have grown between 2 wool baits, 3.5 cm apart. (Although slides were studied weekly, no growth was detected until 5 weeks, but this does not mean that growth had not taken place. The hyphae may just not have come into contact with the surface of the particular slide being studied.). Arthroderma uncinatum conidia and hyphae could be seen on the inoculated bait in non-sterile soil. The soil smears showed that, after 7 weeks burial, some spores and many cleistocarps were present in the soil surrounding the baits, but no hyphae were visible. The time of cleistocarp formation was unknown, but the fact that no hyphae were detected, suggests that they were formed earlier than 7 weeks. Figure 141 shows non-lysed spores inoculated into non-sterile soil.

There were no problems with autofluorescence, but there was some non-specific adsorption of the fluorescein-labelled antirabbit globulin by soil particles still adhering to slides. However, this was not sufficient to interfere with detection of A. uncinatum. No other fungi were detected in the soil by this

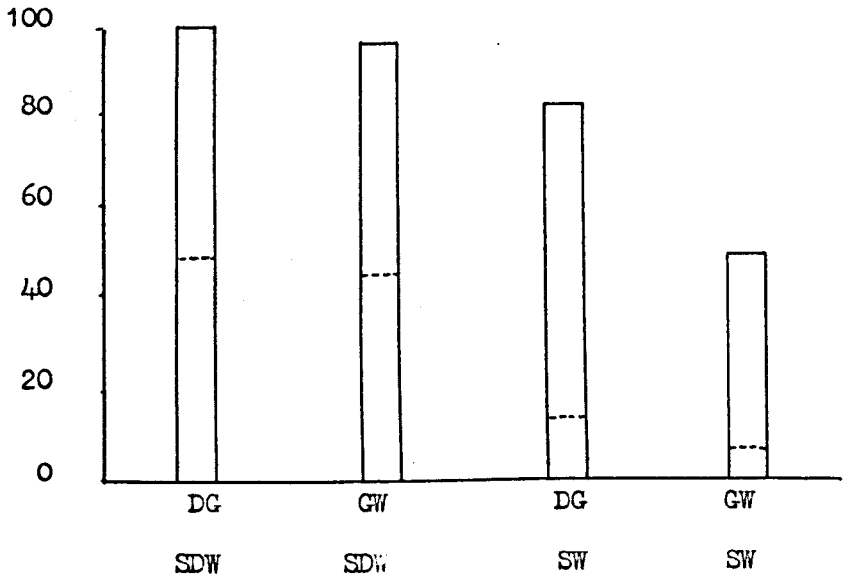
Figures 131 - 135

Histograms representing reisolation of keratinophilic fungi on greasy and degreased wools on sands and soils watered with sea water or distilled water

SW	sea water
SDW	sterile distilled water
DG	degreased wool
GW	greasy wool
——	soils
----	sands

Figure 131

A. uncinatum



% colonisation

Figure 132

T. terrestre

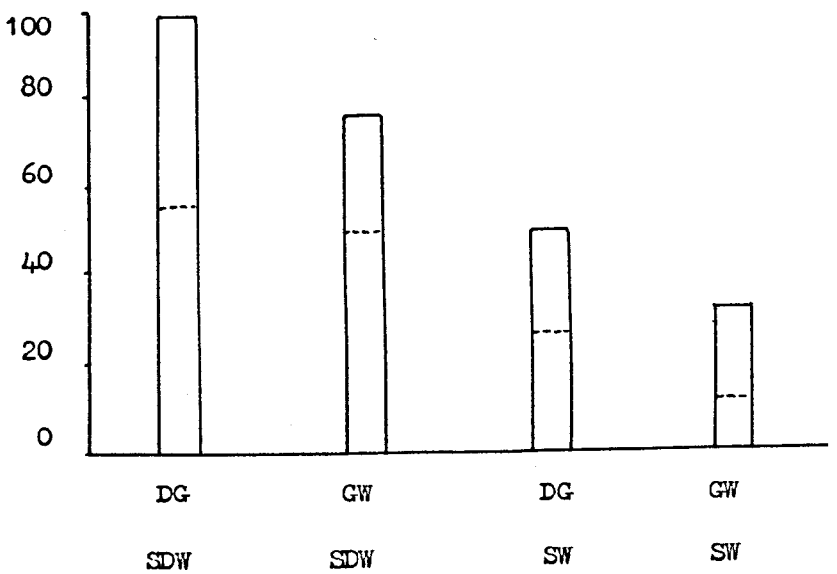


Figure 133

M. gypseum

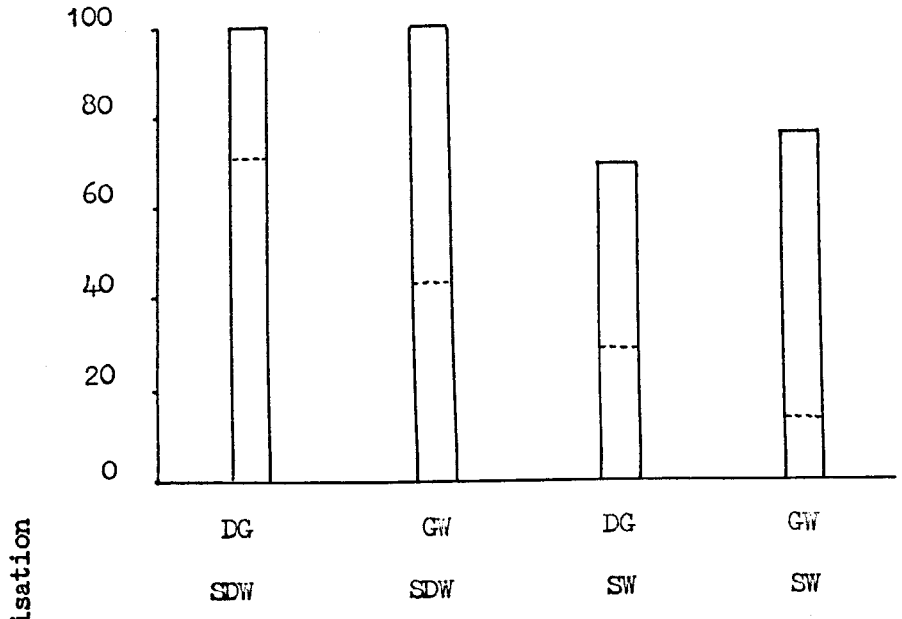


Figure 134

M. cookei

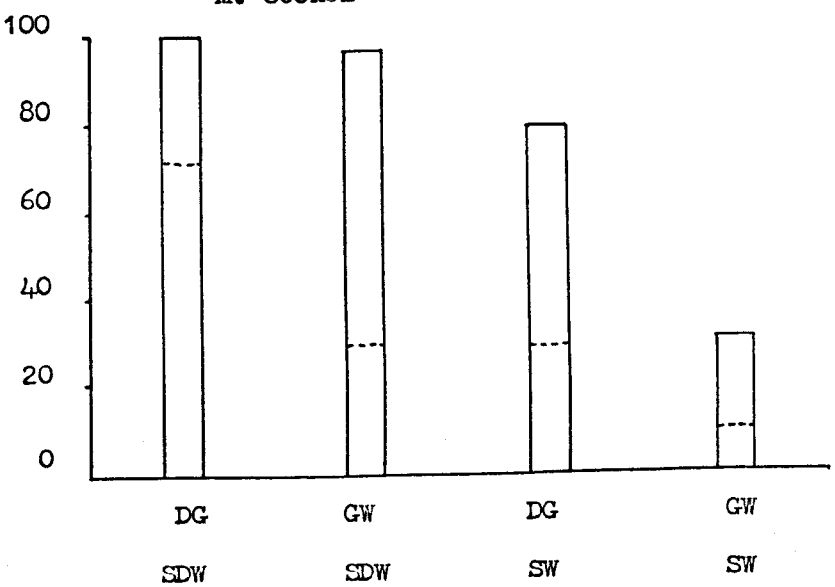
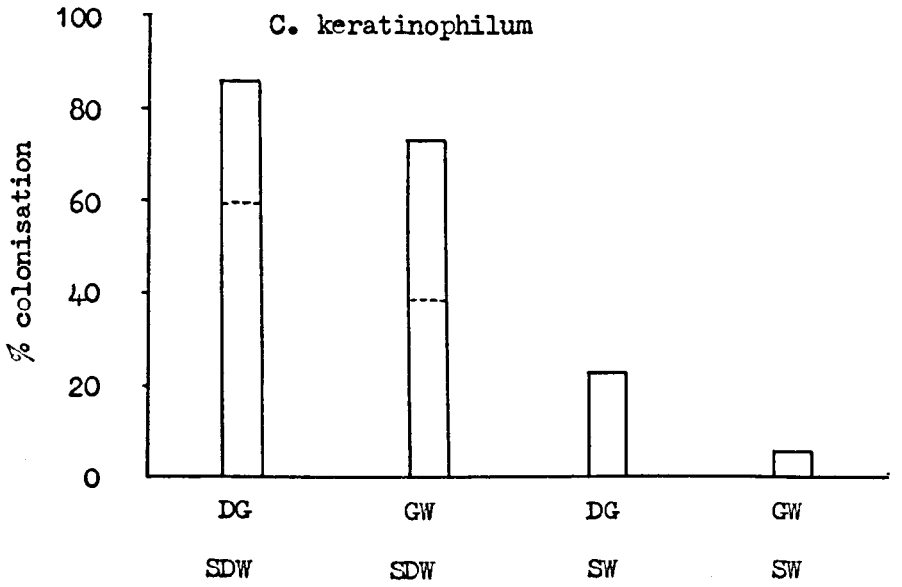


Figure 135


C. keratinophilum
% colonisation
100
80
60
40
20
0
DG
SDW
GW
SDW
DG
SW
GW
SW

Arthroderma uncinatum

Pure culture 4 days x 120

FIGURE 136

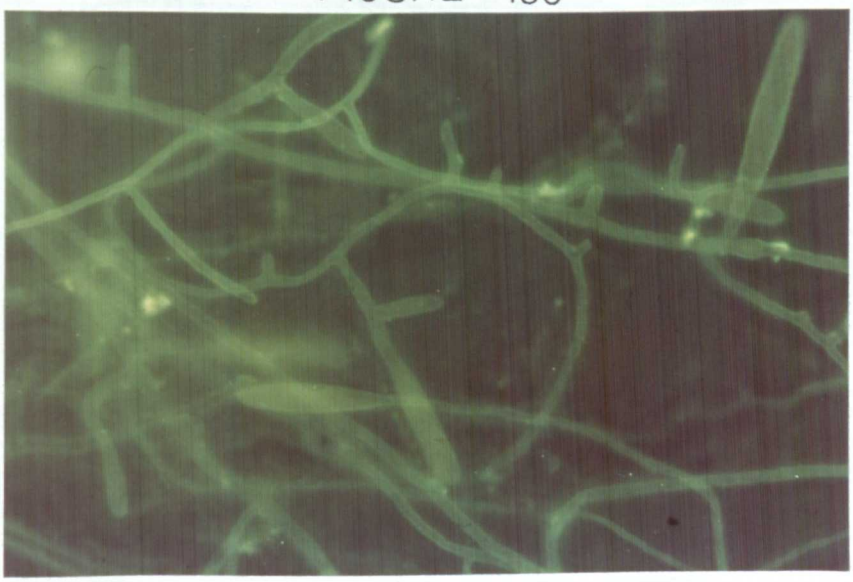


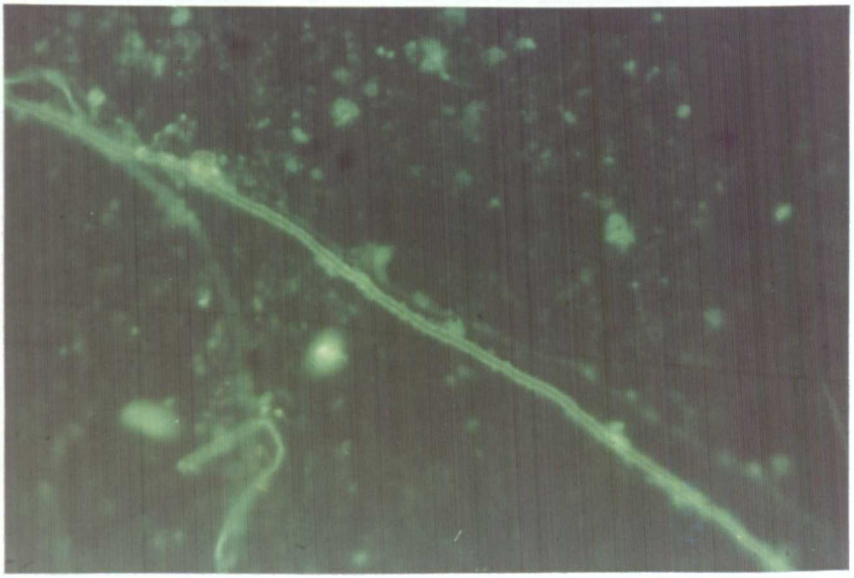
FIGURE 137

Pure culture 9 days x 90

Arthroderma uncinatum

Hypha between 2 baits in non-sterile soil x 120

FIGURE 138



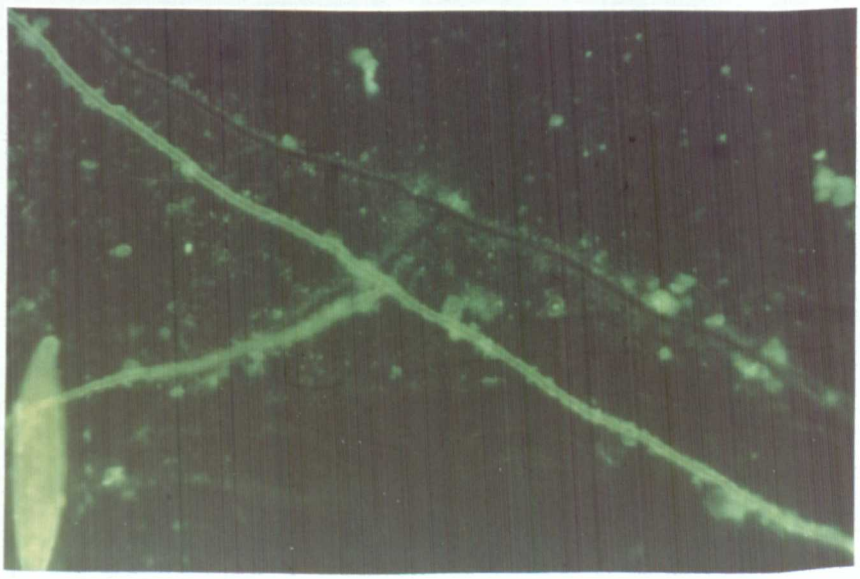


FIGURE 139

Hypha and detached spore between 2 baits in non-sterile soil

x 120

Arthroderma uncinatum

Spore and hypha on non-inoculated bait x 120

FIGURE 140

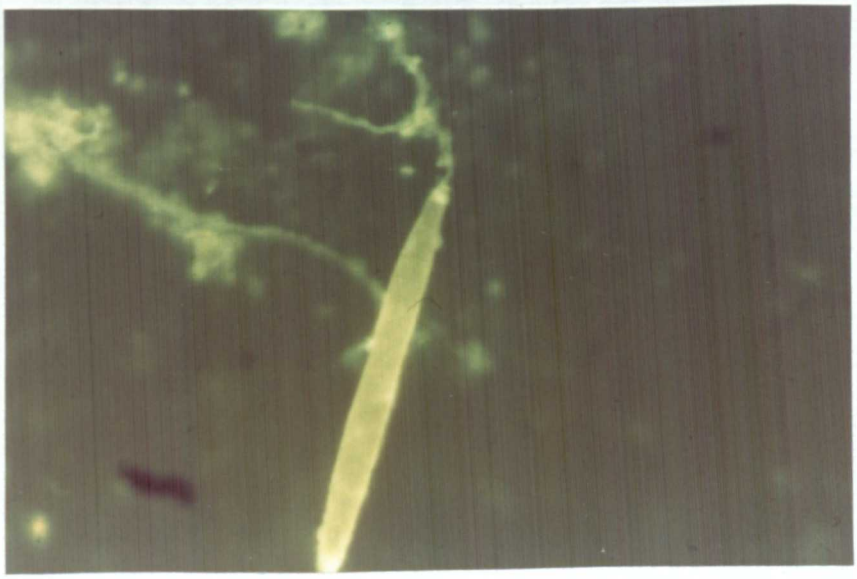


FIGURE 141

Spores inoculated into non-sterile soil. No growth or germination after 7 weeks. No keratin bait provided x 90

method, although it was known that C. keratinophilum, M. cookei and T. terrestre were present, because they had previously been isolated by the hairbaiting technique using greasy and degreased wool, from the same soil sample.

The pure cultures of T. mentagrophytes (Figures 142, 143 and 144) show that the spores fluoresce more than the hyphae. In addition, the cleistocarps also are stained by the fluorescein -labelled antiglobulin. However, fluorescence was also seen with Cephalosporium (Figure 145), A. uncinatum (Figure 146), Fusarium (Figure 147), an unidentified species (Figure 148), T. terrestre (Figure 149), M. gypseum (Figure 150) and M. cookei.

Germinating spores of T. mentagrophytes were seen in non-sterile soil (Figure 152), but in general, because the microconidia of T. mentagrophytes are so small and the soil particles adsorb some fluorescein dye, the spores are difficult to distinguish.

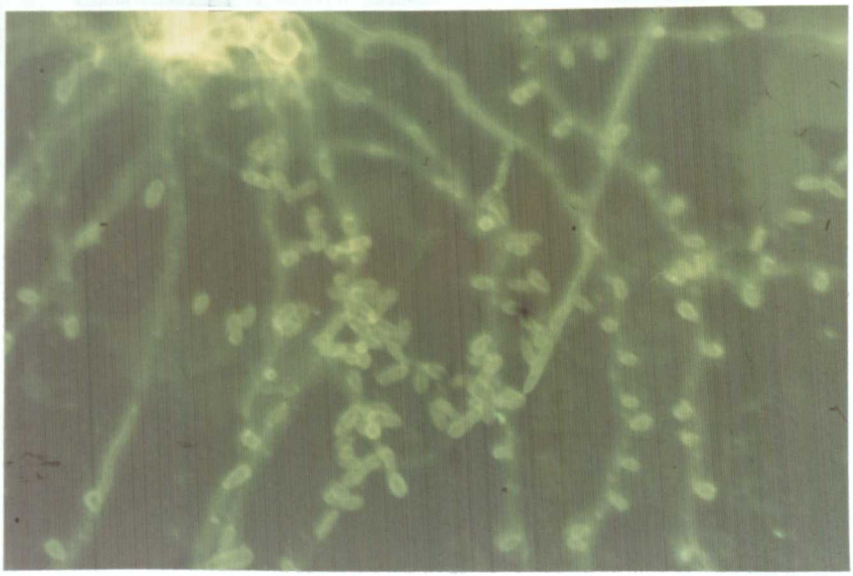
Slides were examined weekly for 10 weeks. Fluorescing hyphae could be seen in the soil slides (Figure 153), but since non-specific staining had taken place, it was impossible to determine whether they were hyphae of T. mentagrophytes or not. Hyphae and spores of T. mentagrophytes were seen on the inoculated bait in non-sterile soil and in 9 weeks no lysis was observed.

Cleistocarps were seen on the inoculated bait after 4 weeks. It is possible that these were A. benhamiae (the perfect state of T. mentagrophytes), but since fluorescence appeared to be non-specific, there is the possibility that these could be A. uncinatum. Peridial hyphae were not clearly seen, thereby preventing identification.

T. mentagrophytes

FIGURE 142

Pure culture 8 days x 75



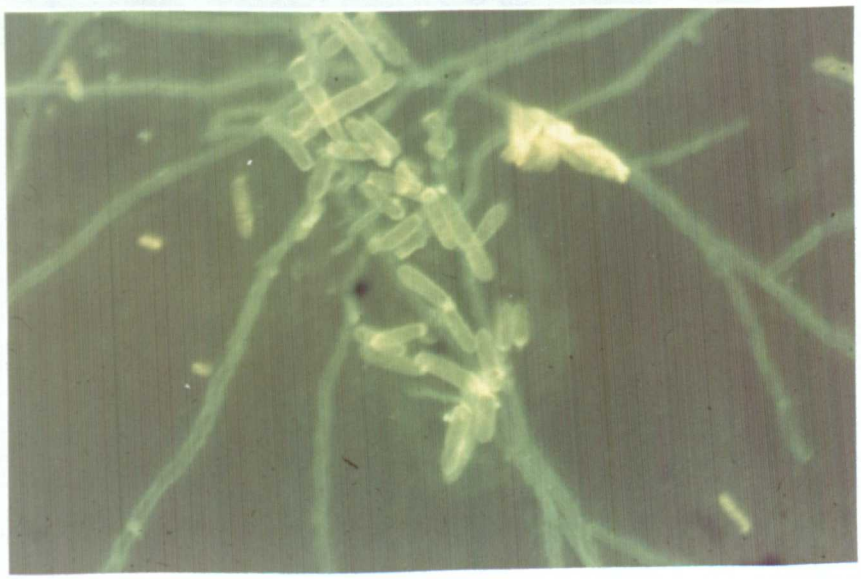


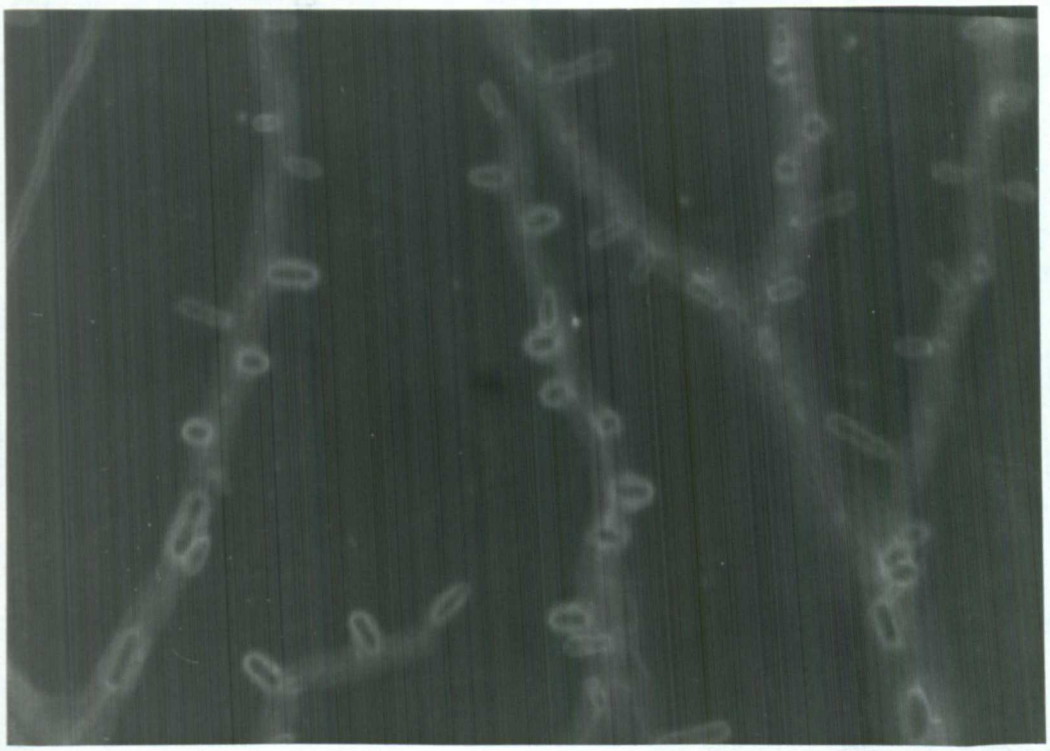
FIGURE 143

Pure culture 8 days x 120

T. mentagrophytes

FIGURE 144

Pure culture 8 days x 140



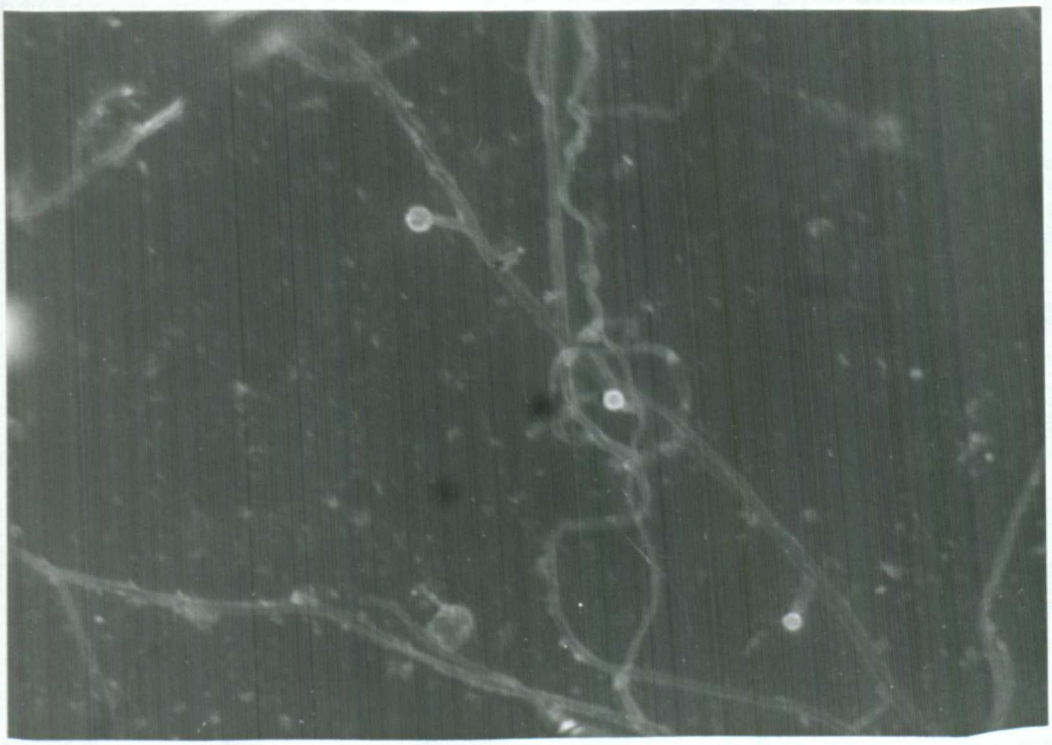
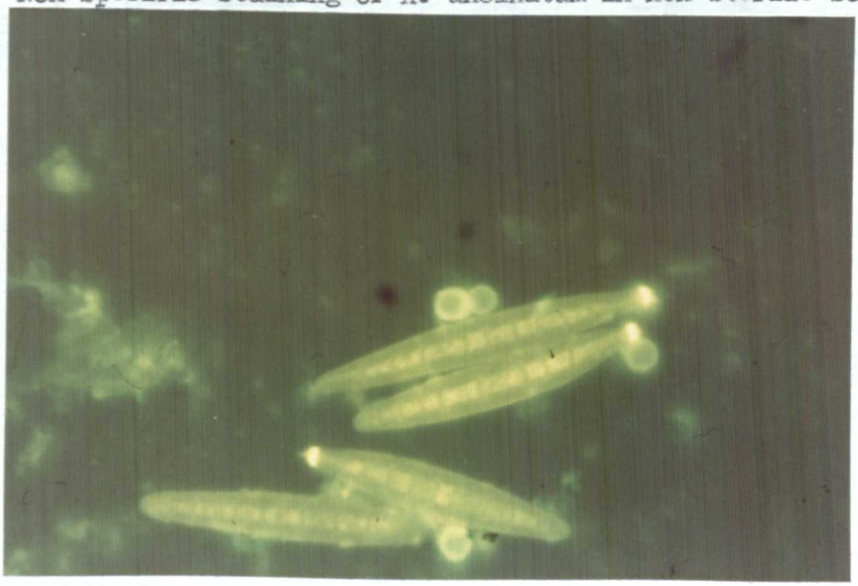


FIGURE 145

Non-specific staining of ?Cephalosporium in non-sterile soil x 90

FIGURE 146

Non-specific staining of A. uncinatum in non-sterile soil x 120



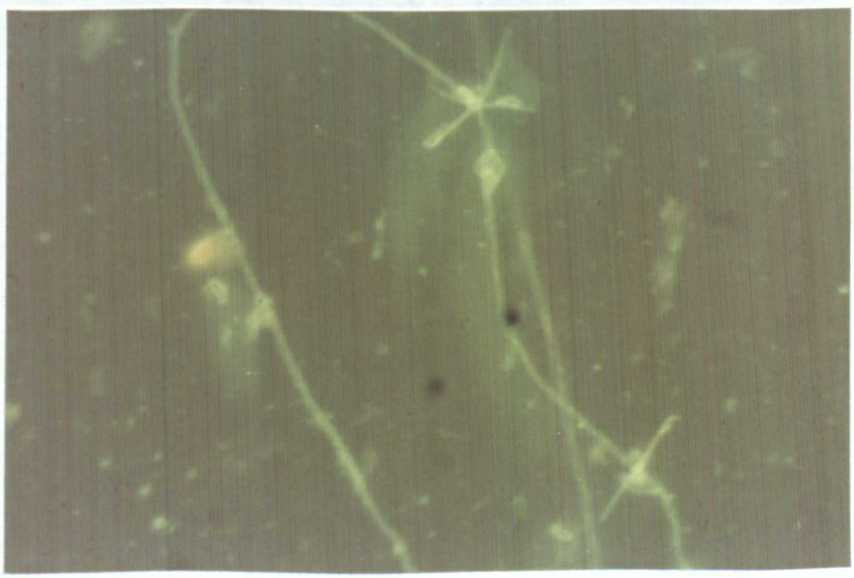


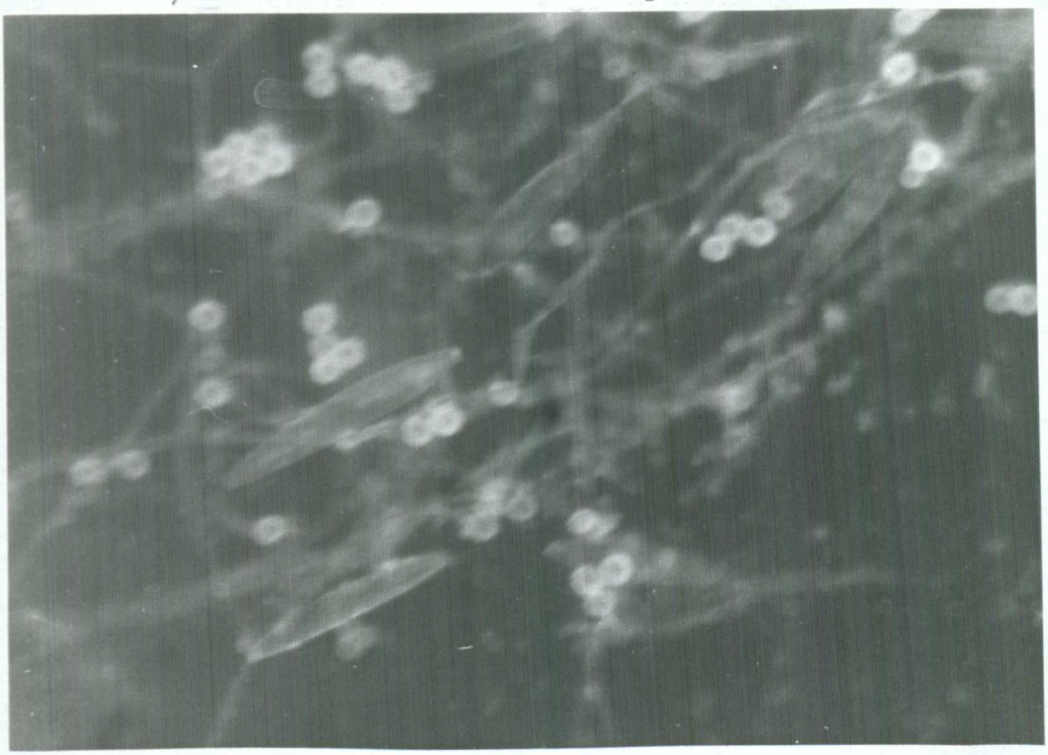
FIGURE 147

Non-specific staining of Fusarium in non-sterile soil x 120

Non-specific staining of species in non-sterile soil

FIGURE 148

a) A. uncinatum and an unknown species x 90



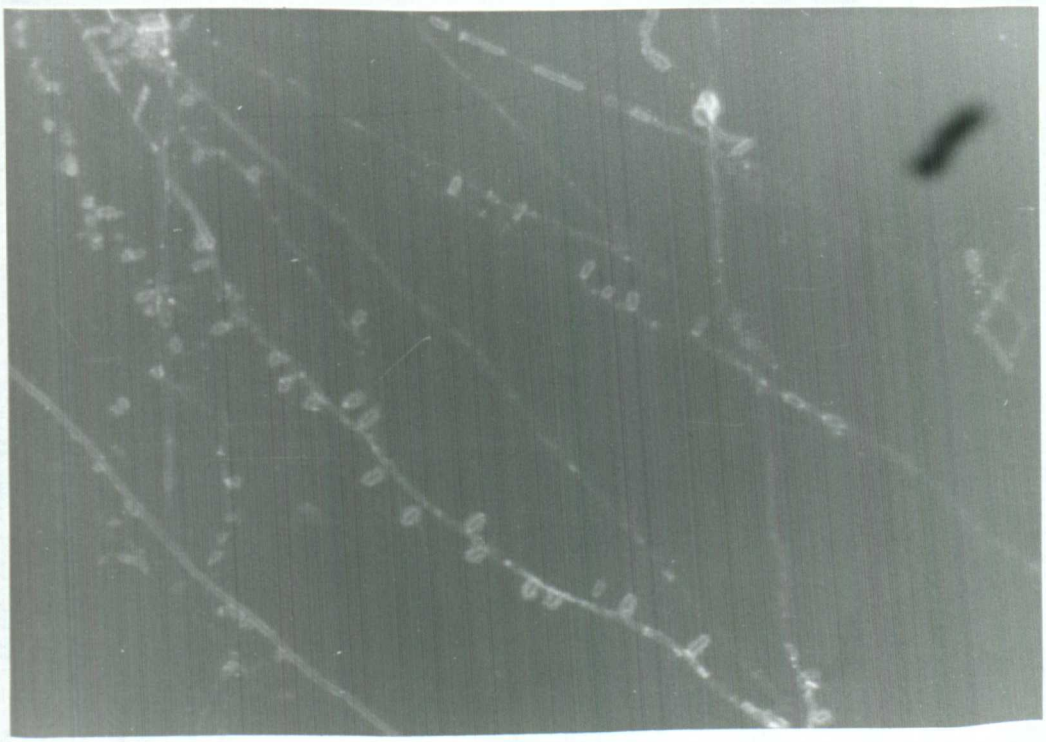


FIGURE 149

b) T. terrestre x 90

Non-specific staining of species in non-sterile soil

FIGURE 150
a) M. gypseum x 140

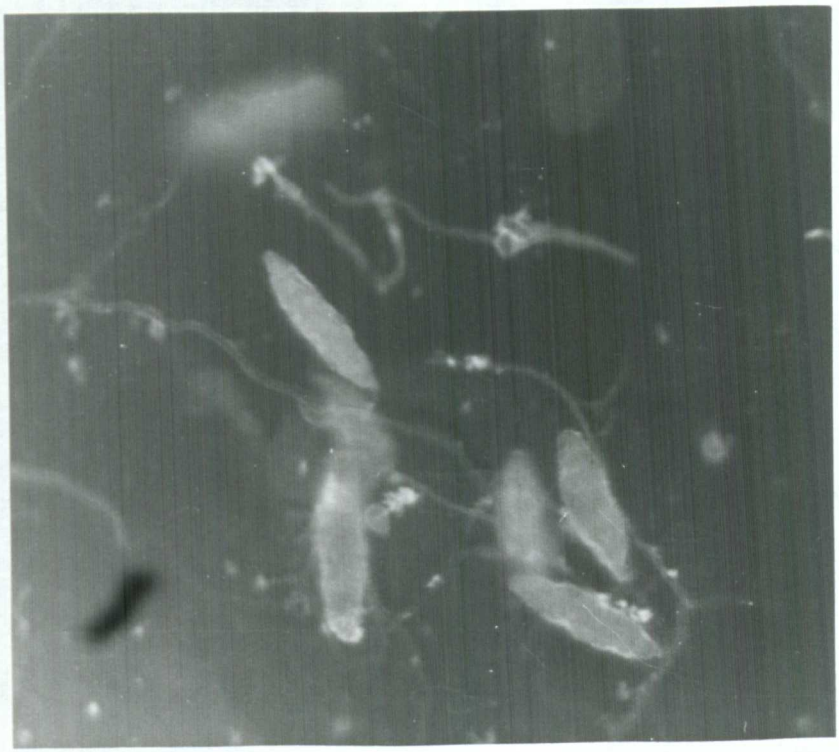
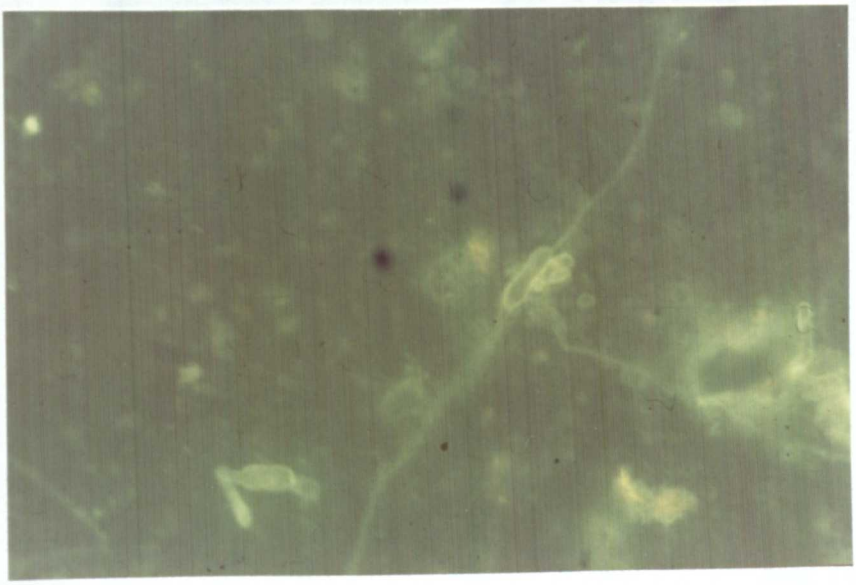


FIGURE 151
b) M. cookei x 140

T. mentagrophytes

FIGURE 152

Germinating spore in non-sterile soil x 120



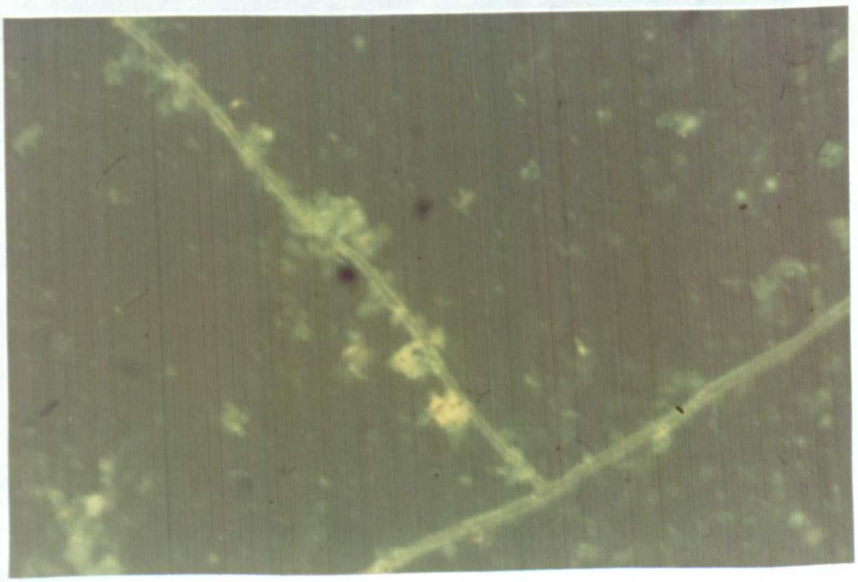


FIGURE 153

Hypha near 2nd bait in non-sterile soil. ?T. mentagrophytes

x 120

Results of growth of the 3 keratinophilic fungi on natural substrates in soil.

The number of colonies turning the medium from yellow to red was counted and was used to represent the number of propagules, i.e. 1 propagule = 1 colony.

Figures 156 - 161 show the numbers of propagules isolated from sterile and non-sterile soil, with or without hair, over a period of 31 days. The number of isolations at 0 days was, with one exception, always higher in non-sterile soils than in sterile soils. The exception (conidial A. uncinatum) was where only minimal differences were seen.

Trichophyton terrestre, inoculated into non-sterile soils with no hair, remained at more or less the same level throughout the sampling period. In sterile soil, despite an initial drop in the number of propagules isolated, numbers increased steadily and reached a plateau at 24 days. In the soils containing hair, the numbers in both sterile and non-sterile soil increased steadily throughout the sampling despite an initial fall in isolations at 3 and 7 days, respectively. The numbers in sterile soil were always more than in non-sterile soil after 7 days.

Conidial A. uncinatum inoculated into non-sterile and sterile soil with no hair remained in more or less the same numbers throughout the sampling period, with an initial drop in both soils at 3 and 7 days. In contrast to T. terrestre, conidial A. uncinatum did not increase in numbers in sterile soil with no hair. The numbers isolated in sterile soil with hair, after an initial drop between 3 and 10 days, rose steadily until the 24 th day, when a slight decrease was seen. In non-sterile soil with hair, the numbers of propagules increased slightly, but in general, remained more or less at the same level during the sampling time. This was also in contrast to T. terrestre, where numbers in sterile and non-sterile soil, with hair, both increased,

Key to Figures 156 - 161

● —— ●	Non-sterile soil with no hair bait
o —— o	Sterile soil with no hair bait
x —— x	Non-sterile soil with hair bait
△ —— △	Sterile soil with hair bait

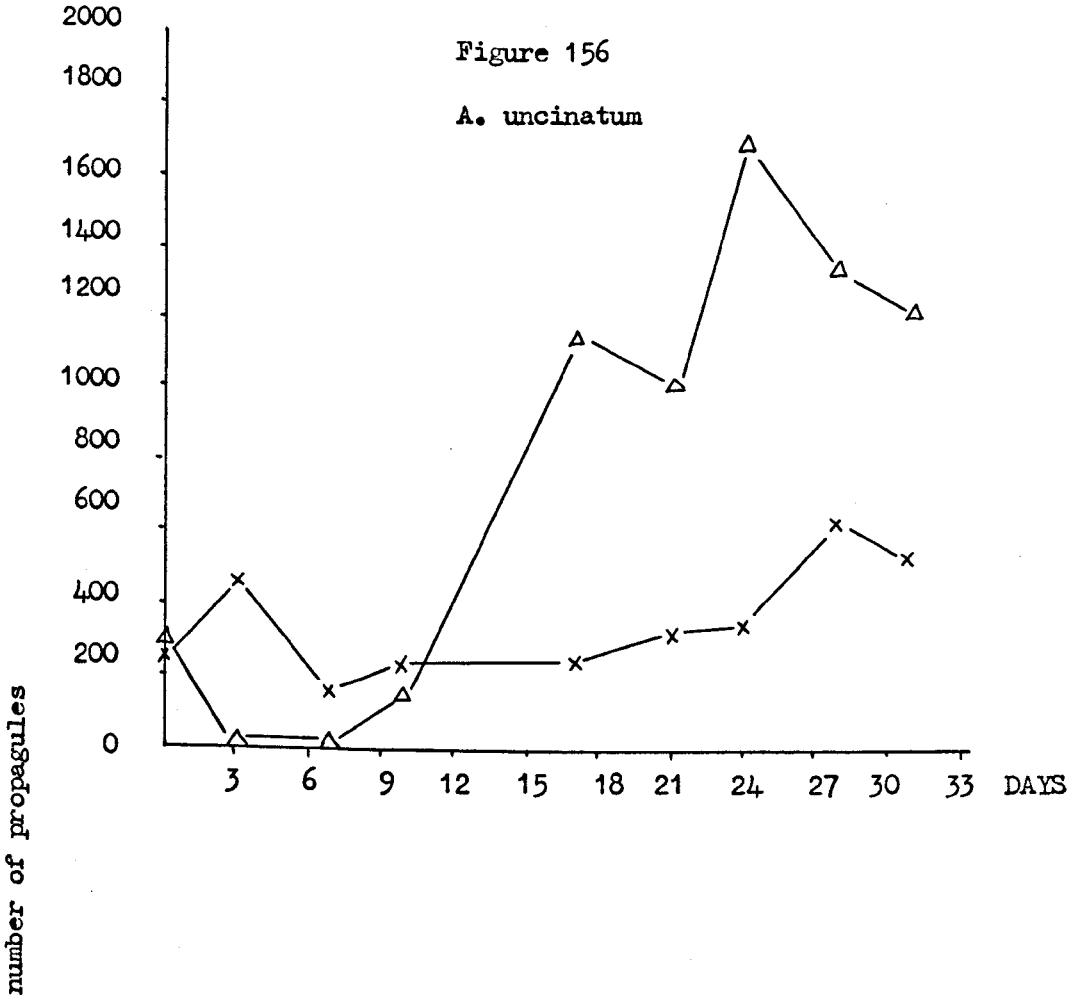


Figure 156
A. uncinatum
number of propagules
2000
1800
1600
1400
1200
1000
800
600
400
200
0
3
6
9
12
15
18
21
24
27
30
33
DAYS

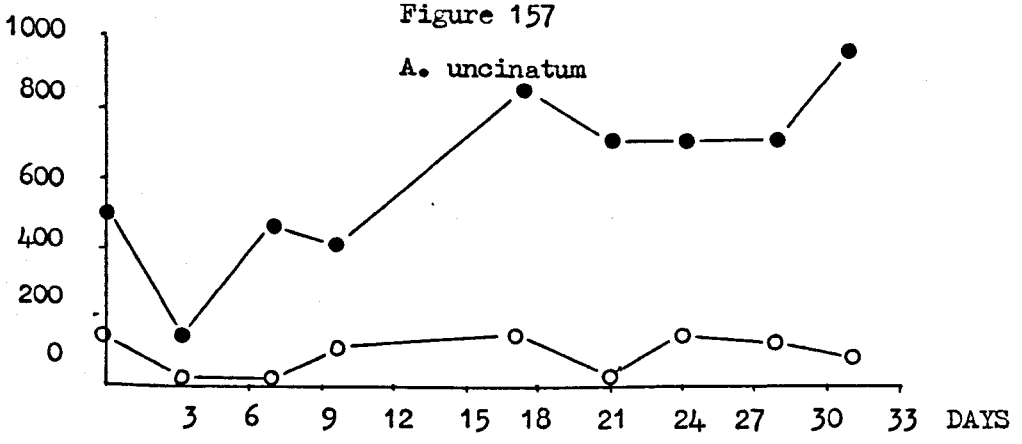


Figure 157
A. uncinatum
1000
800
600
400
200
0
3
6
9
12
15
18
21
24
27
30
33
DAYS

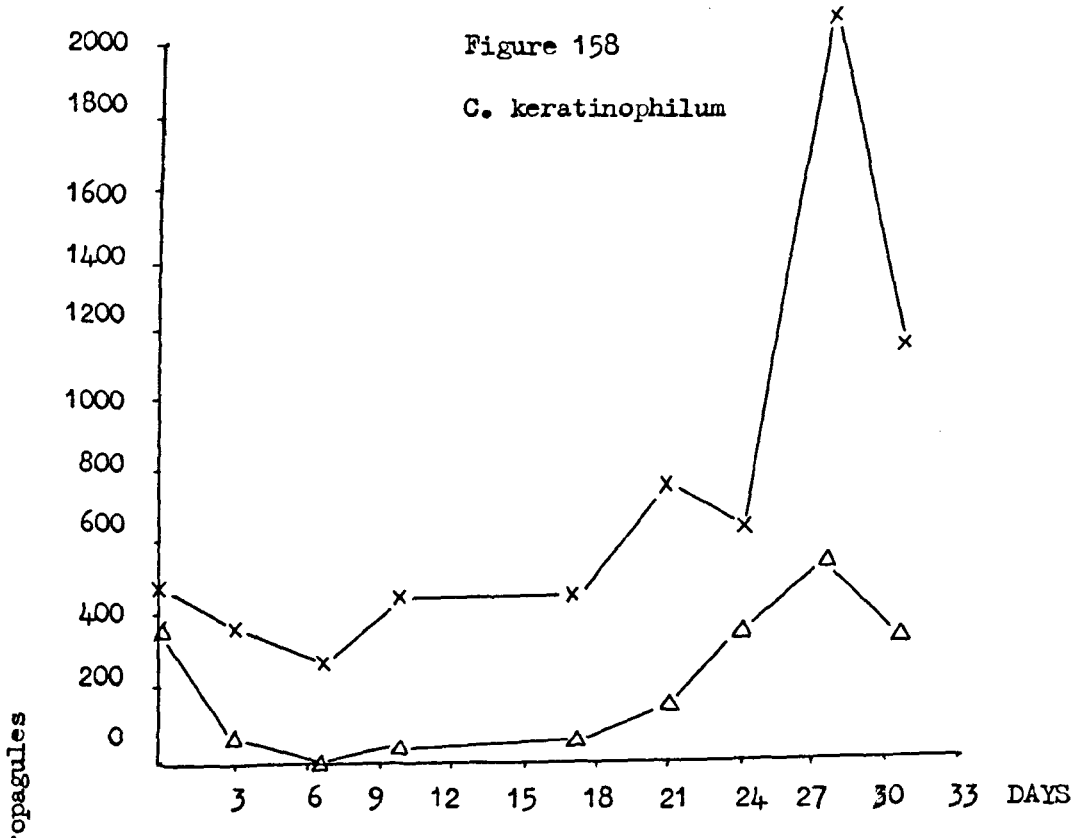


Figure 158
C. keratinophilum
2000
1800
1600
1400
1200
1000
800
600
400
200
0
3
6
9
12
15
18
21
24
27
30
33
DAYS

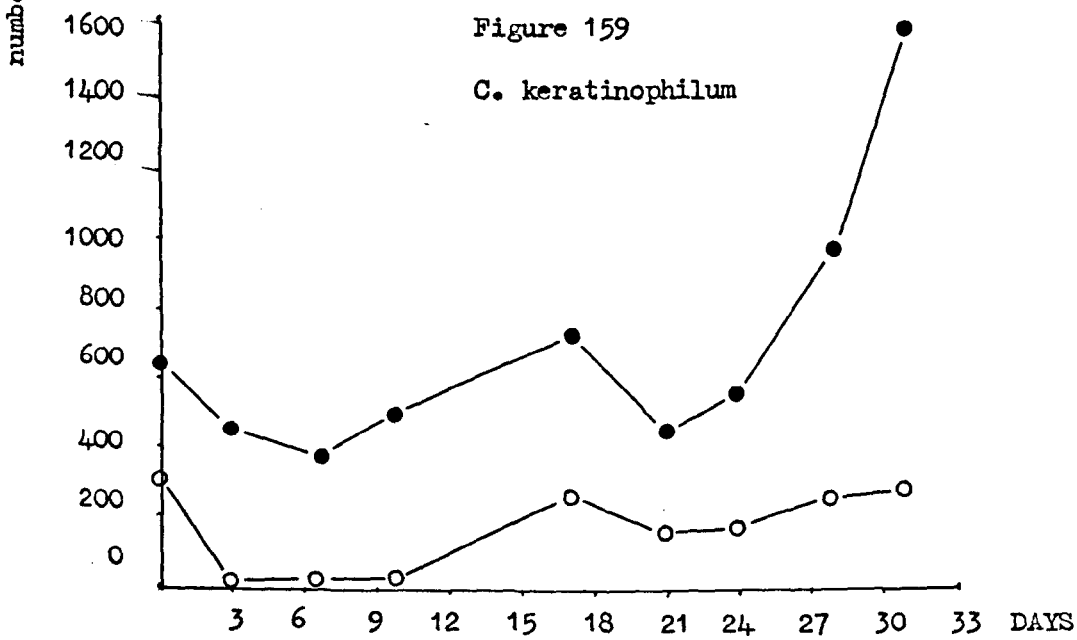


Figure 159
C. keratinophilum
number of propagules
1600
1400
1200
1000
800
600
400
200
0
3
6
9
12
15
18
21
24
27
30
33
DAYS

Figure 160

T. terrestre

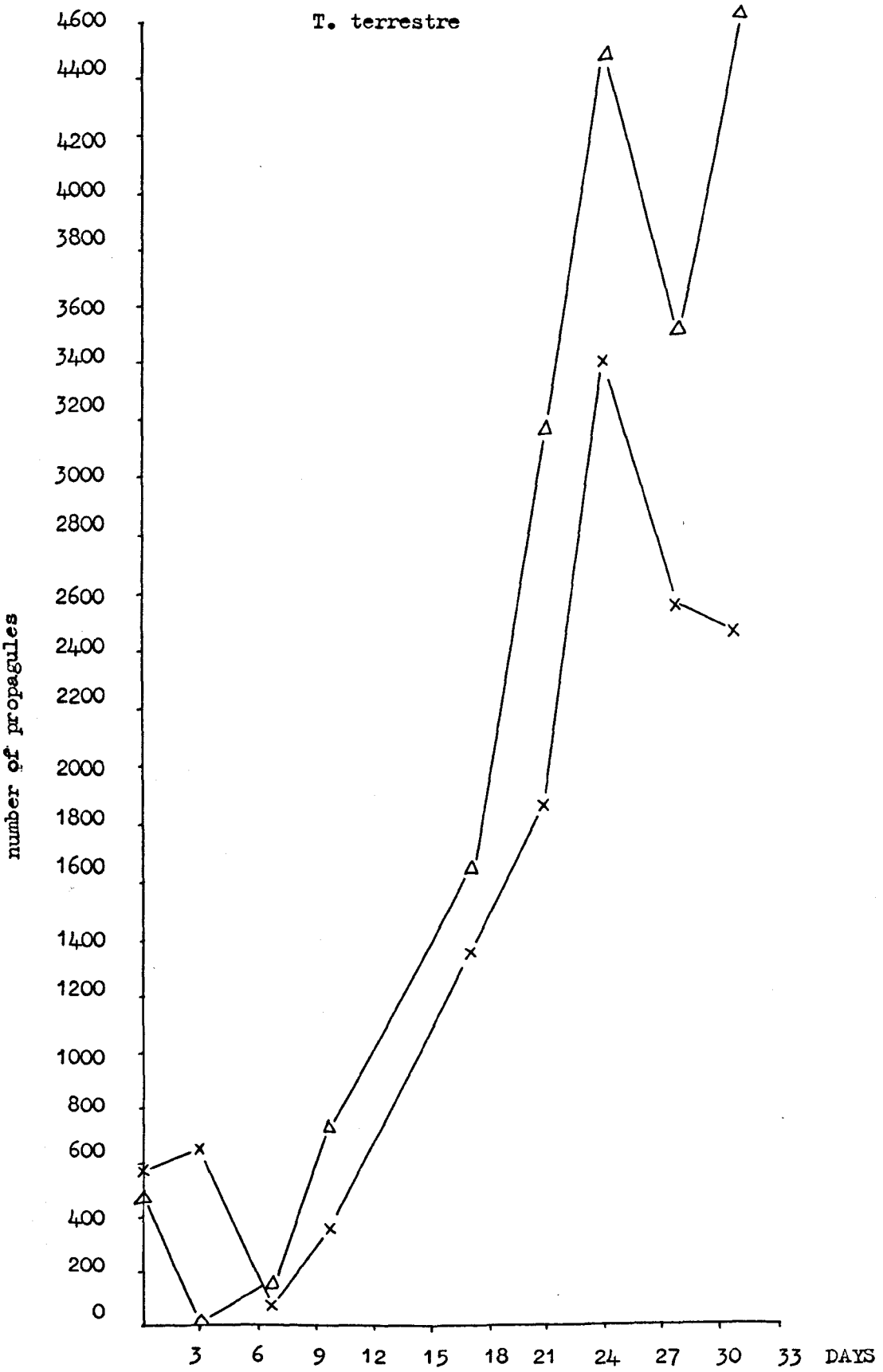
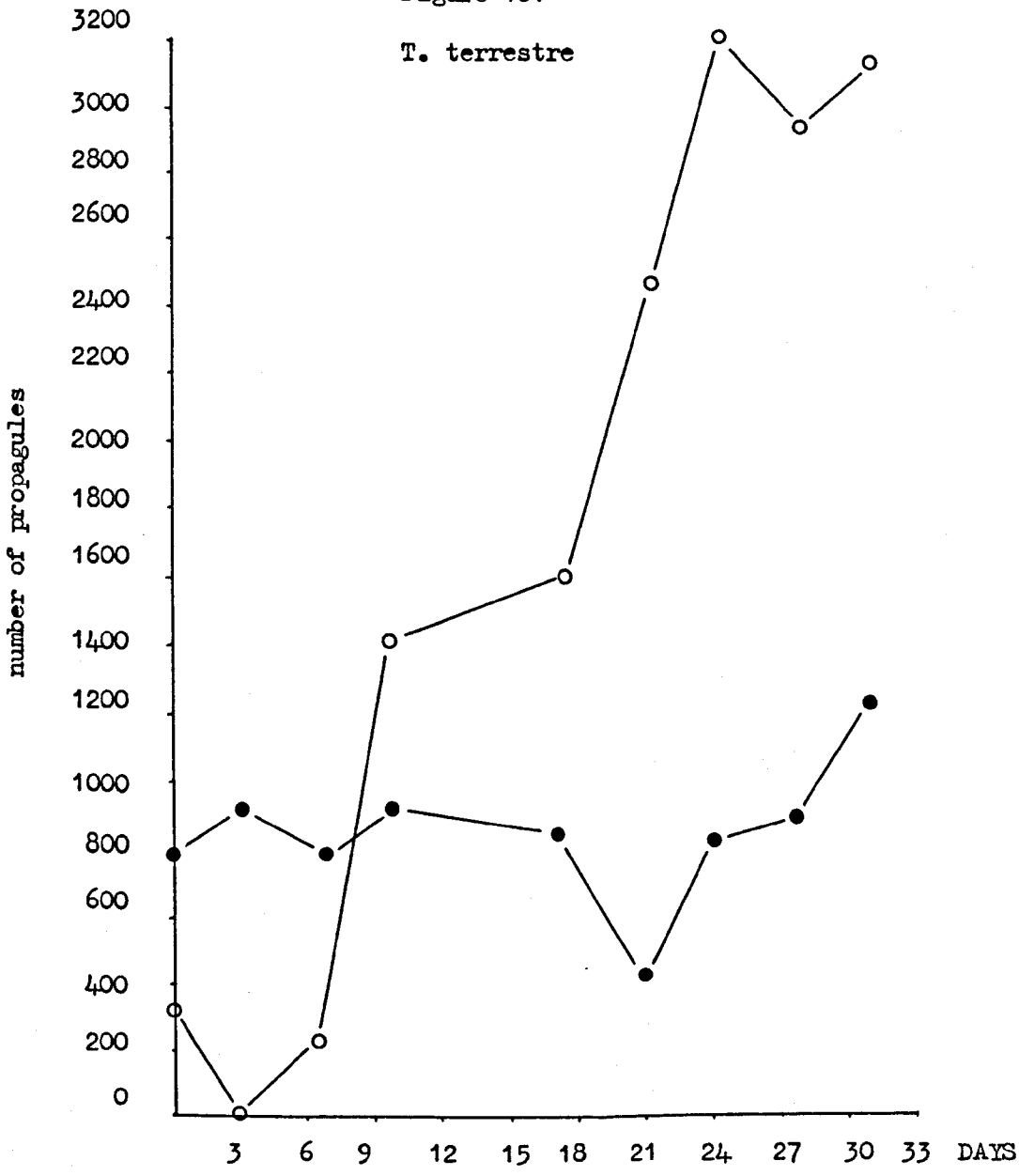


Figure 161

T. terrestre



andwere much higher than those observed for conidial A. uncinatum.

The results of C. keratinophilum were totally different from either T. terrestre or A. uncinatum. Non-sterile soil, with or without hair, always had more propagules than sterile soil, with or without hair. From the 24th day of sampling, a large increase in numbers was seen in non-sterile soil, which, in the case of soils with hair, fell off again at 28 days. Numbers of propagules in sterile soil did not increase above the original observation with the exception of the 28th day in sterile soil with hair, but the numbers fell again on the 31st day.

Results of colonisation tubes.

Numbers of side-arms colonised in 13 weeks.

	Sterile soil	Non-sterile soil
Trichophyton mentagrophytes	7	0
Trichophyton terrestre	10	10
Arthroderma uncinatum	10	10
Microsporum cookei	10	2
Microsporum gypseum	10	5
Chrysosporium keratinophilum	5	2

10 = maximum number of side arms capable of being colonised.

All the keratinophilic fungi were able to grow through sterile soil; only T. mentagrophytes and C. keratinophilum did not colonise 100% of the possible side-arms, but they did colonise 70% and 50% respectively. In non-sterile soil, T. terrestre and A. uncinatum were the most successful fungi; they both colonised 100% of the arms within 13 weeks. Of the other 4 species, T. mentagrophytes did not grow through to any

KEY

•——• Sterile soil + Sabouraud's dextrose agar

•——o Non-sterile soil + Sabouraud's dextrose agar

Δ——Δ Sterile soil + supplemented Sabouraud's dextrose agar

x——x Non-sterile soil + supplemented Sabouraud's dextrose agar

Figure 162

A. uncinatum

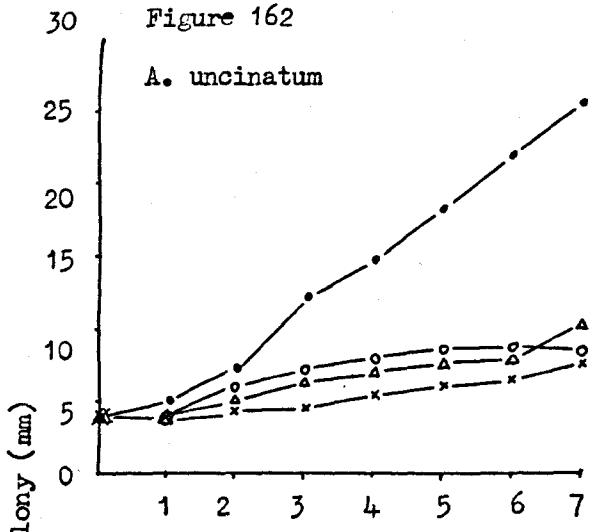


Figure 163

T. mentagrophytes

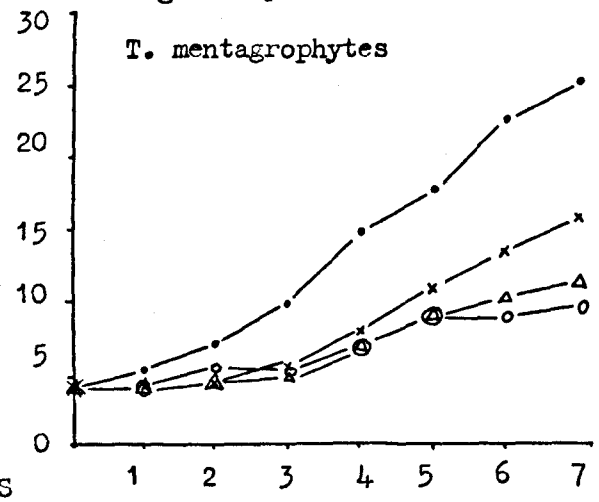


Figure 164

C. keratinophilum

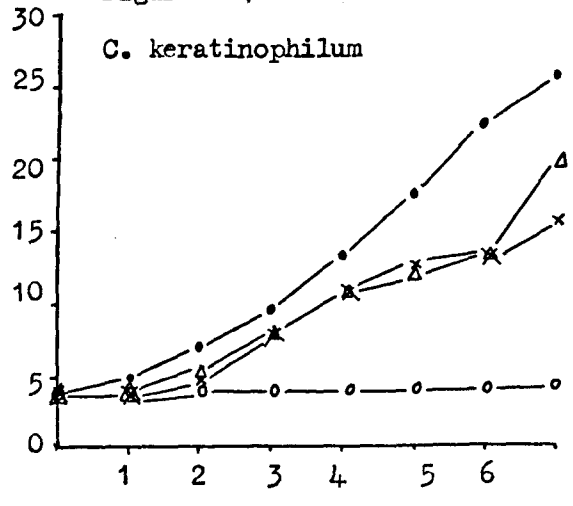


Figure 165

T. terrestre

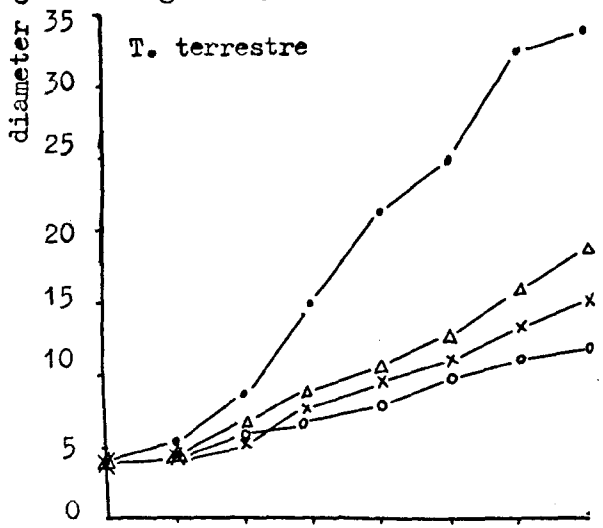


Figure 166

M. cookei

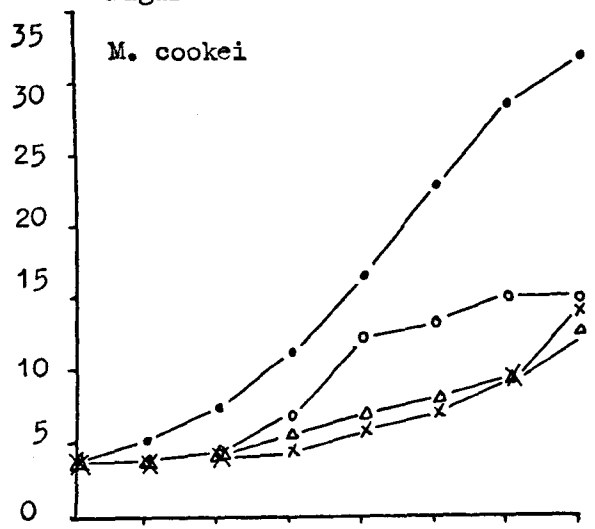
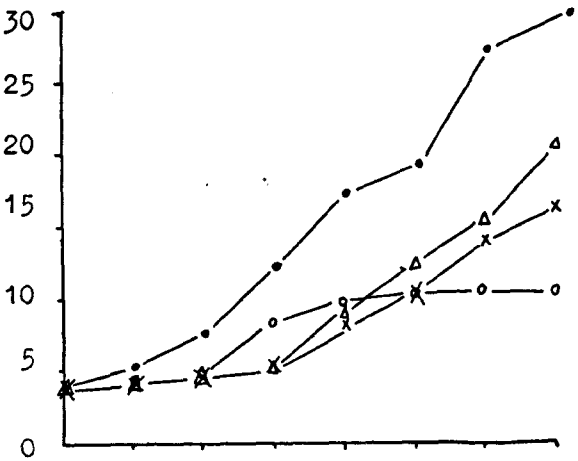


Figure 167

M. gypseum



of the arms, whilst C. keratinophilum, M. cookei and M. gypseum were all slower growing through to each arm, colonising 20%, 20% and 50% of the arms in 13 weeks.

Results of the competitive ability of keratinophilic fungi to colonise an agar plate.

Figures 162 - 167 represent the growth of the fungi on different agars, with or without an inoculum of soil beneath the Cellophane layer.

All species grew best on Sabouraud dextrose agar, with no supplementary antibiotics, inoculated with sterile soil. The results for this combination were outstandingly better than any of the other combinations. Distinctions between the other 3 experiments were not clearly definable. Microsporum gypseum, C. keratinophilum, T. mentagrophytes and T. terrestre grew least when on S.D.A inoculated with non-sterile soil. Microsporum cookei did not grow well on supplemented S.D.A inoculated with either sterile or non-sterile soil.

Trichophyton terrestre, C. keratinophilum and M. gypseum resembled each other in their overall pattern on each combination, whereas M. cookei, A. uncinatum and T. mentagrophytes were all different, but as stated before, variations were very small.

Trichophyton terrestre, M. cookei and M. gypseum were the fastest growers (of the 6 fungi studied), all reaching 30 mm colony diameter or more within 7 days on S.D.A. The other 3 reached 25 mm diameter within the same time on S.D.A inoculated with sterile soil. On the other combinations, T. terrestre, C. keratinophilum and M. gypseum did not reach a diameter over 20 mm within 7 days, whilst M. cookei and T. mentagrophytes did not reach 15 mm diameter, and A. uncinatum did not reach 10 mm within the same time.

Table 14 is a list of perfect and imperfect states of dermatophytes.

Figures 168-206 are scanning electron micrographs of dermatophytes in various stages of development.

TABLE 14

Perfect and imperfect states of several keratinophilic fungi.

Perfect form	Imperfect form
Arthroderma uncinatum Dawson and Gentles, 1960	Trichophyton ajelloi [K. ajelloi] Vanbreuseghem, 1952
Arthroderma quadrifidum Dawson and Gentles, 1960	Trichophyton terrestre Durie and Frey, 1956
Nannizzia incurvata Stockdale, 1960	Microsporum gypseum (Bodin) Guiart and Grigorakis, 1928
Nannizzia gypsea Stockdale, 1963	
Nannizzia cajetani Ajello, 1961	Microsporum cookei Ajello, 1959
Arthroderma benhamiae Ajello and Cheng, 1966	Trichophyton mentagrophytes

Figure 168

A. uncinatum

Microconidial cluster

x 8.6K

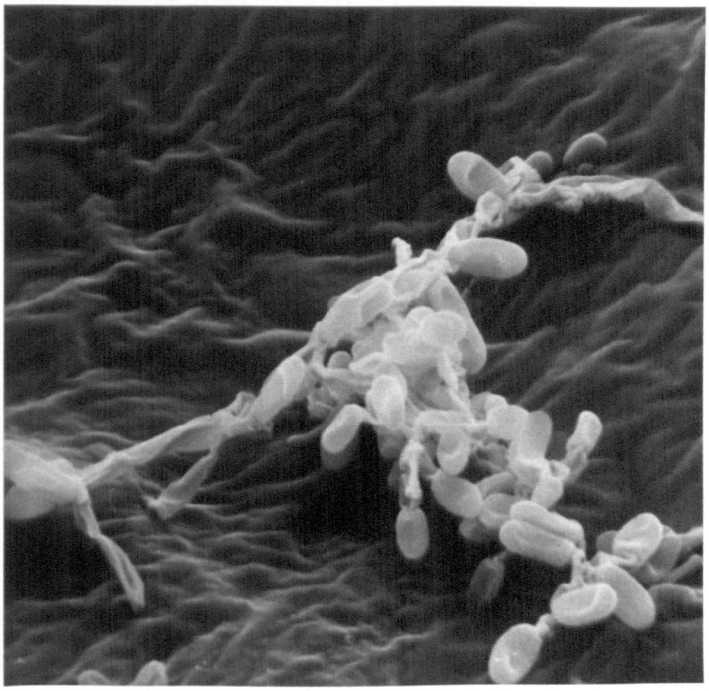
Figure 169

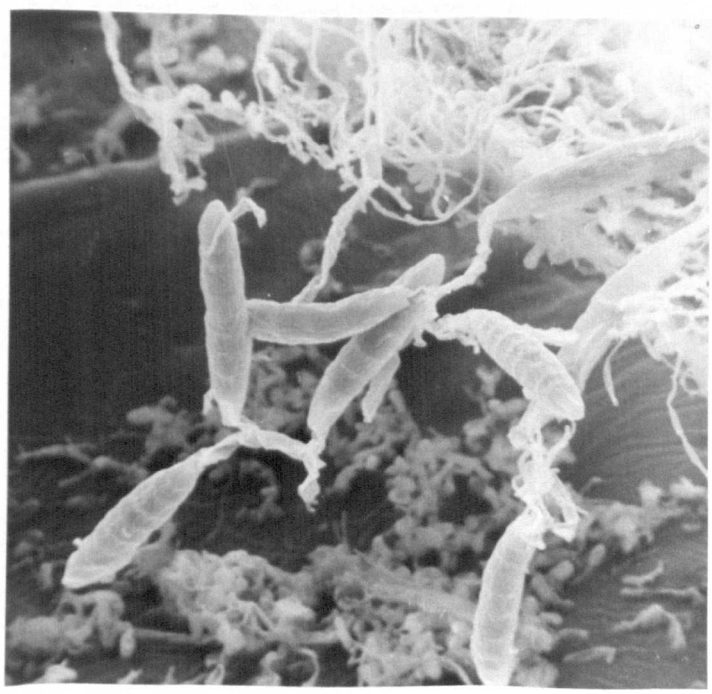
A. uncinatum

Macroconidia

x 4.06K

168





169

Figure 170

A. uncinatum

Macroconidium

x 20.48K

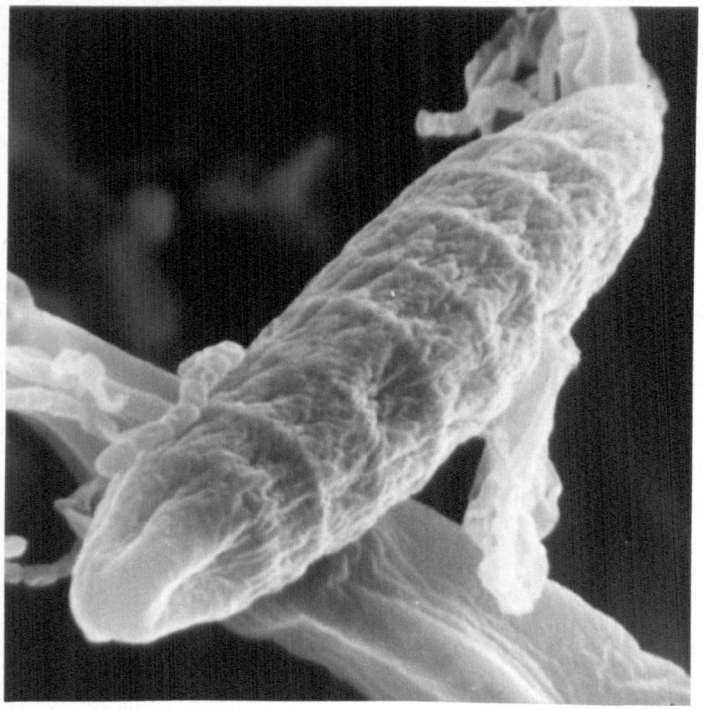
Figure 171

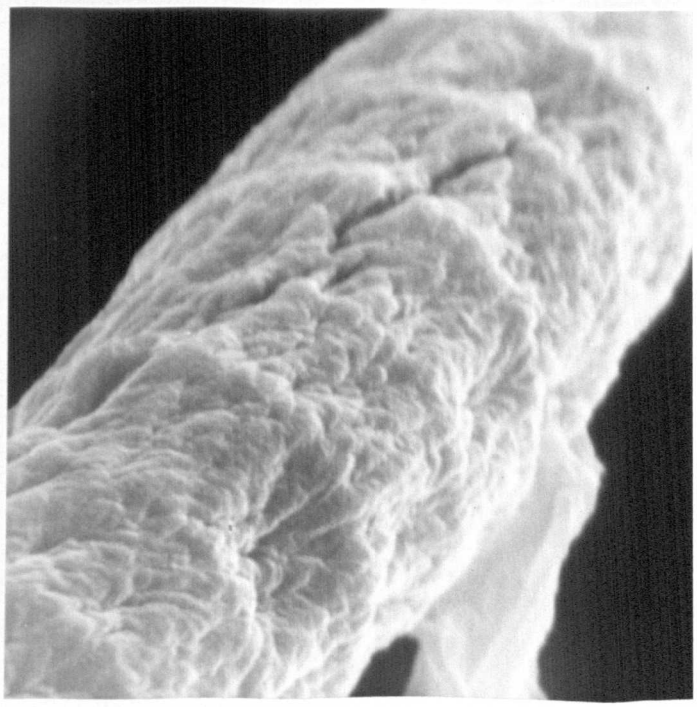
A. uncinatum

Macroconidium

x 41.4K

170





171

Figure 172

A. uncinatum

Young macroconidia

x 8.19K

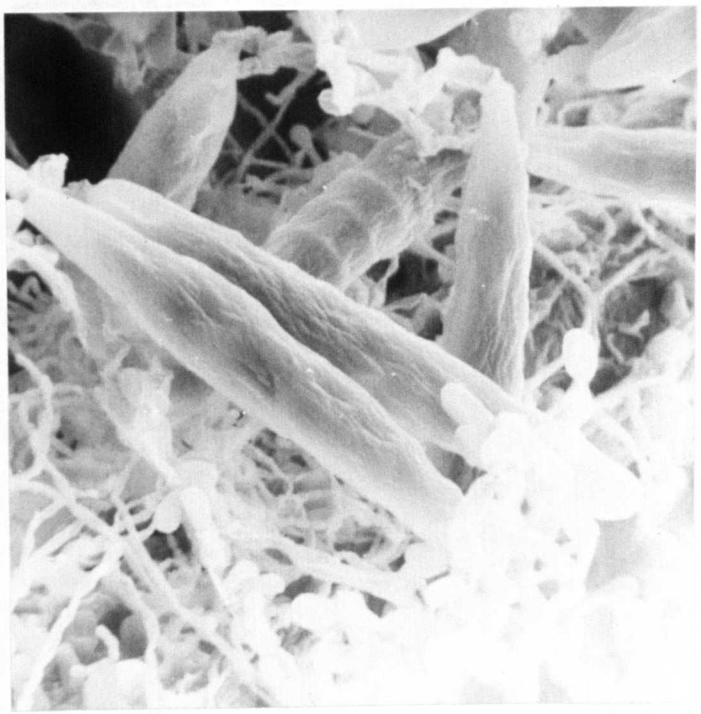
Figure 173

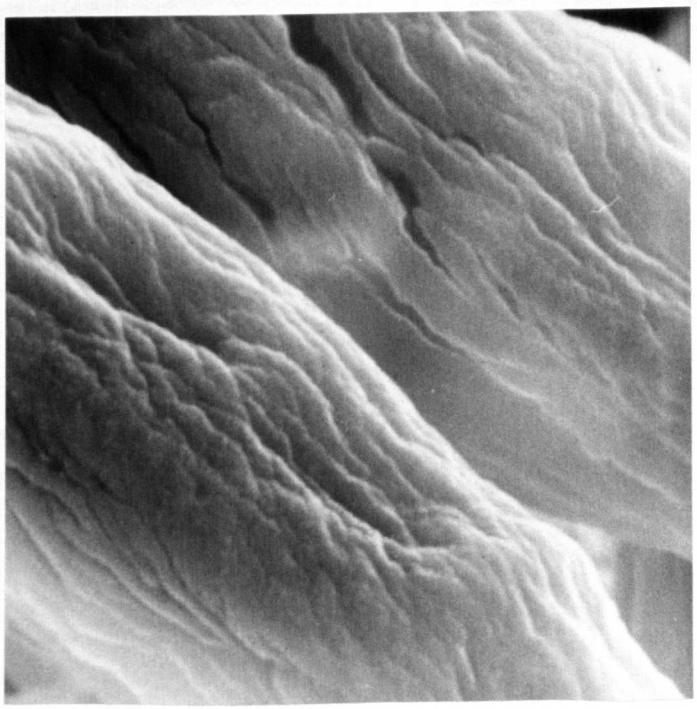
A. uncinatum

Young macroconidia

x 30.95K

172





173

Figure 174

A. uncinatum

Cleistocarp

x 697

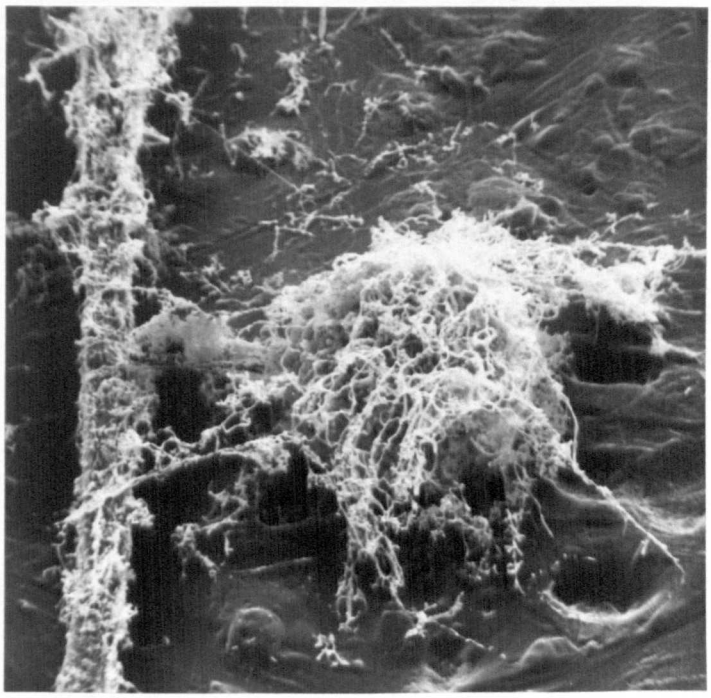
Figure 175

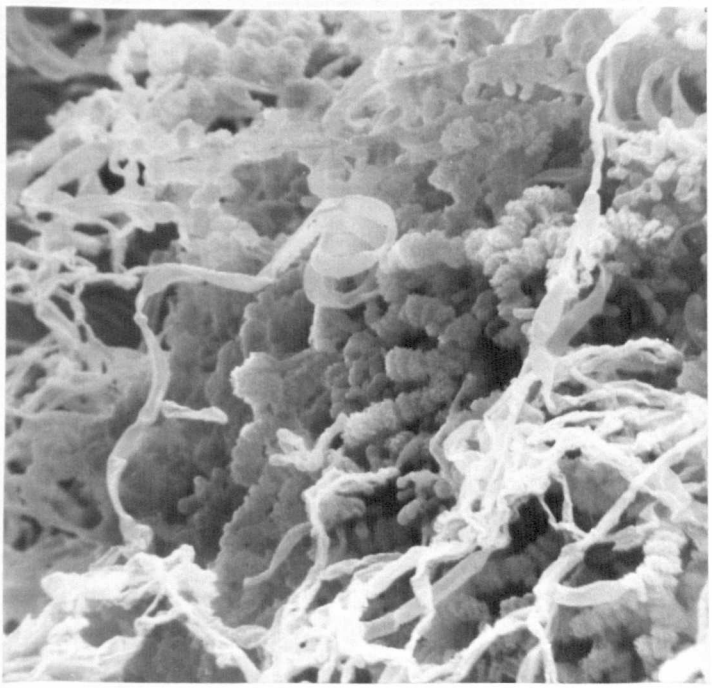
A. uncinatum

Dumbbell-shaped peridial hyphae

x 4.26K

174





175

Figure 176

A. benhamiae

Cleistocarp

x 675

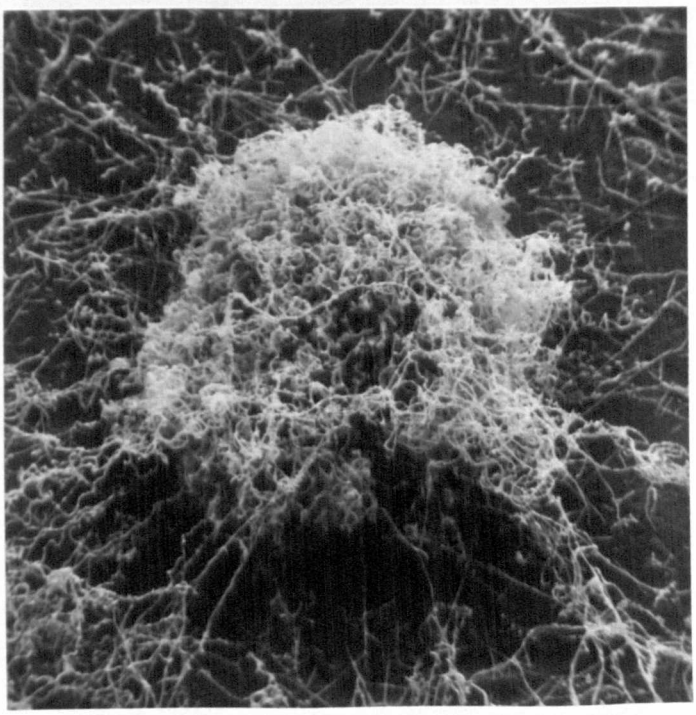
Figure 177

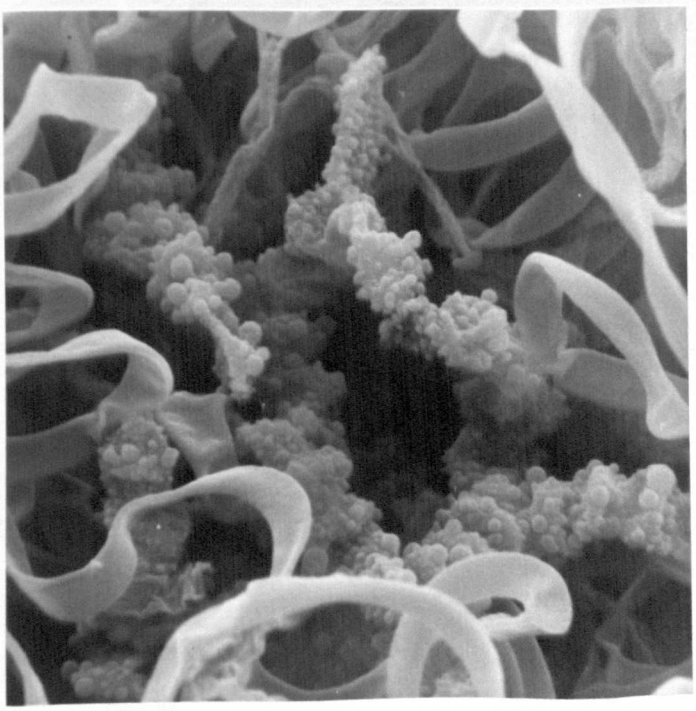
A. benhamiae

Spiral- and dumbbell-shaped peridial hyphae

x 13K

176





177

Figure 178

A. benhamiae

Dumbbell-shaped cells of hypha

x 18.9K

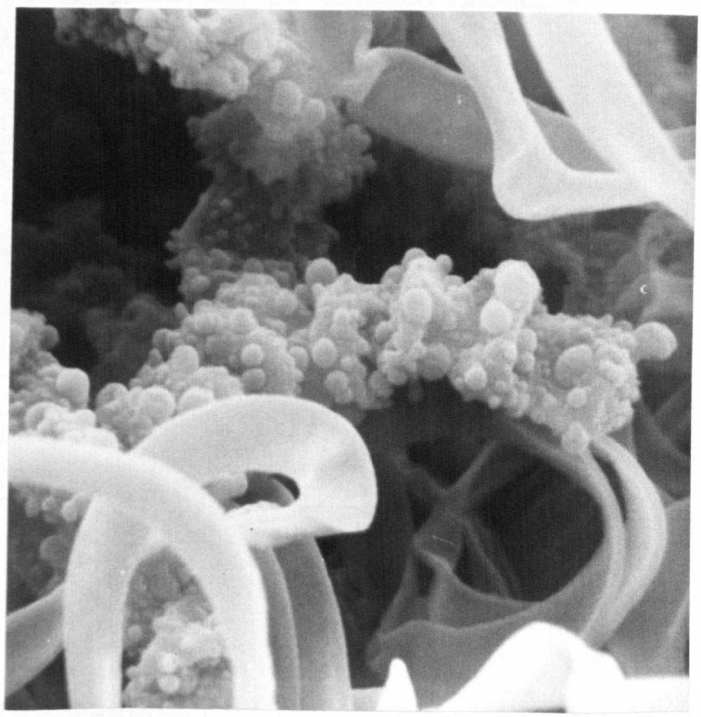
Figure 179

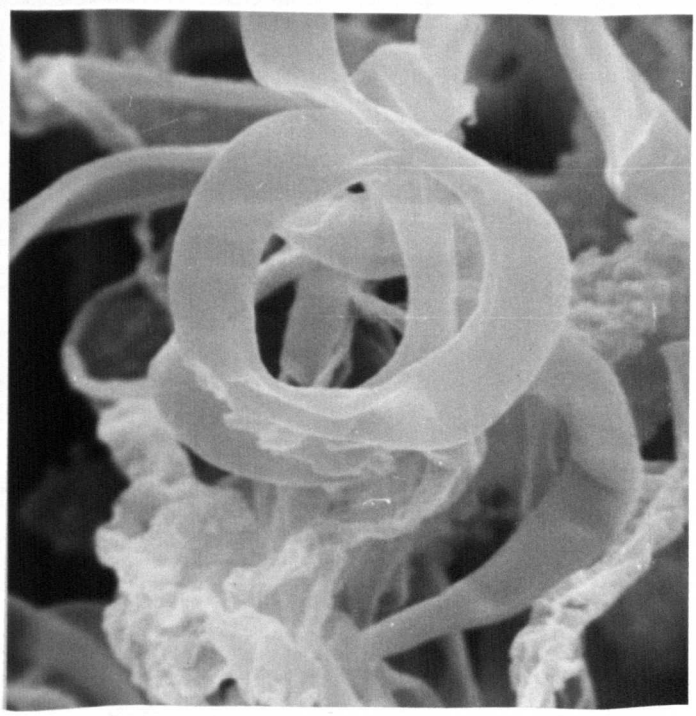
A. benhamiae

Spiral peridial hypha

x 17K

178





179

Figure 180

A. benhamiae

Microconidia

x 11.43K

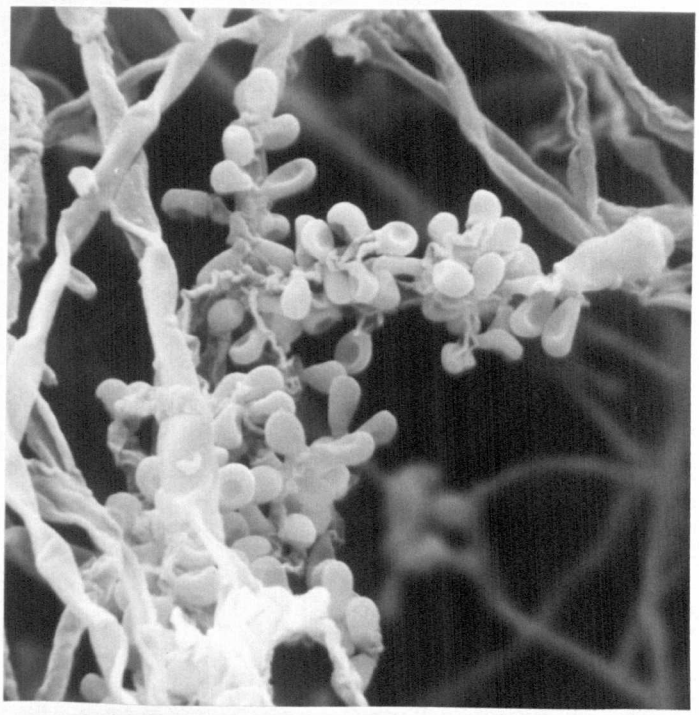
Figure 181

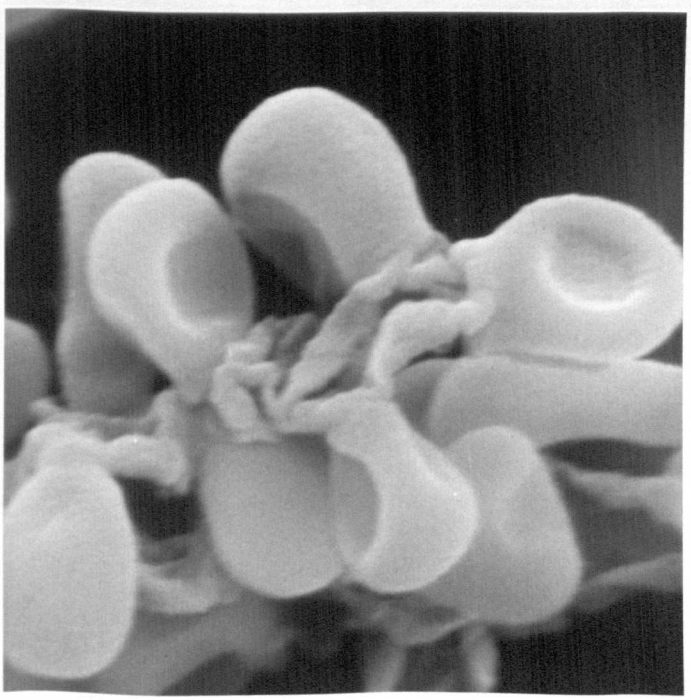
A. benhamiae

Microconidia

x 57.2K

180





181

Figure 182

A. tuberculatum

Conidia

x 5.76K

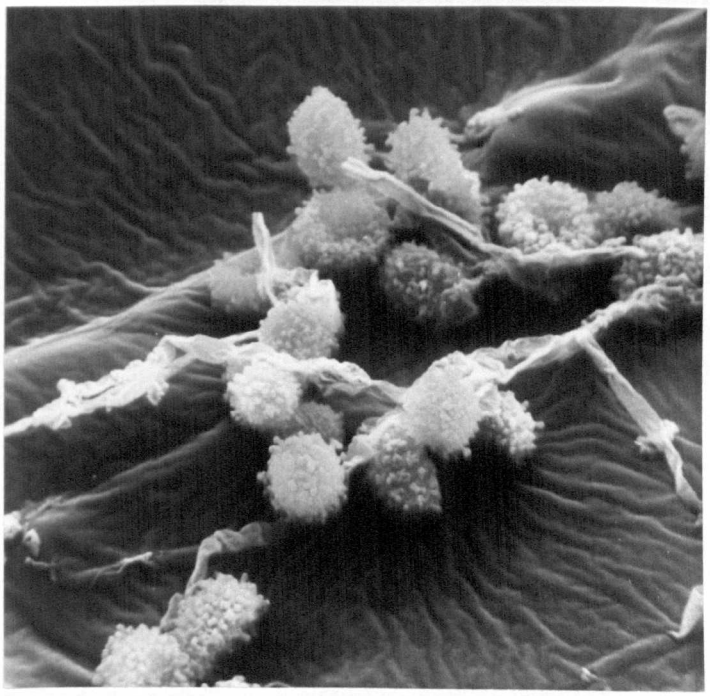
Figure 183

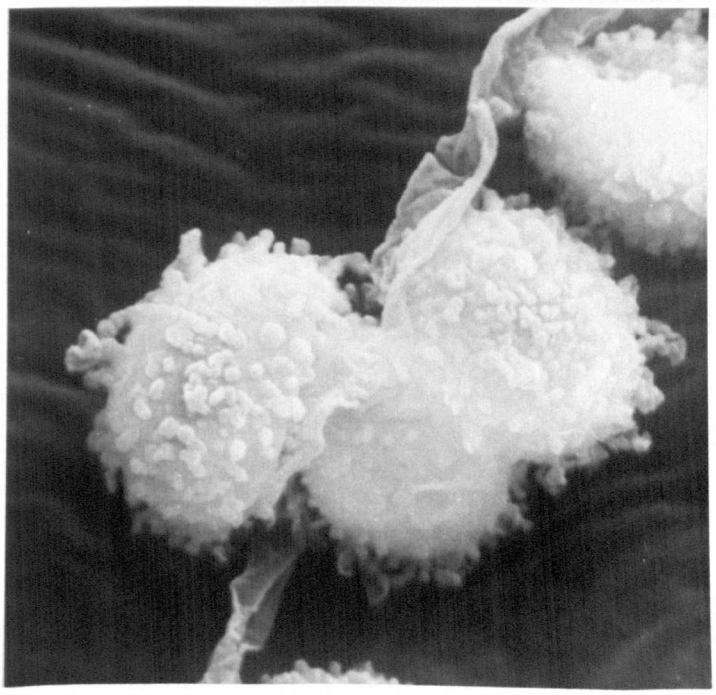
A. tuberculatum

Conidia

x 17.28K

182





183

Figure 184

A. tuberculatum

Cleistocarp

x 720

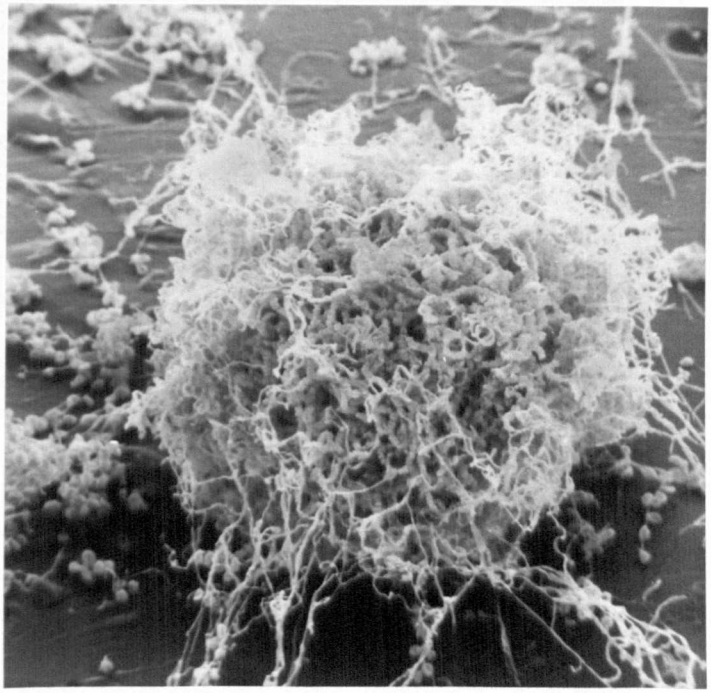
Figure 185

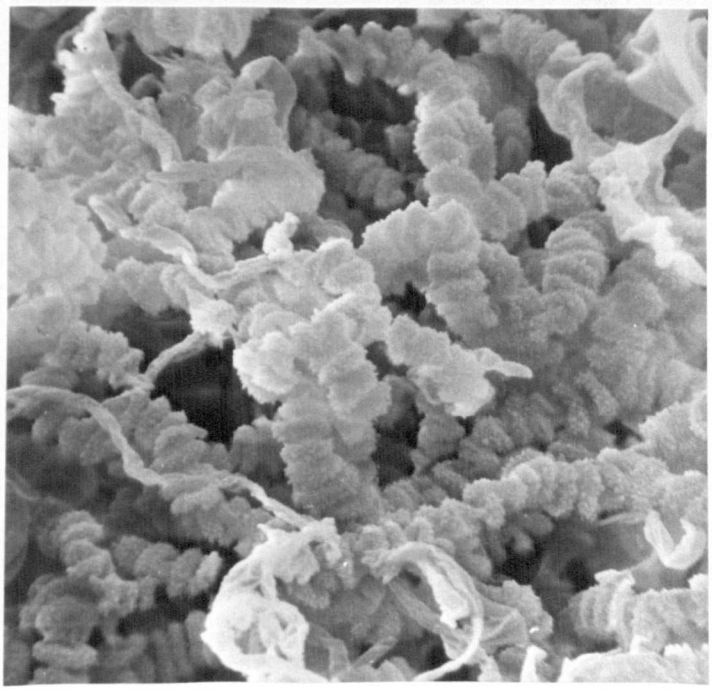
A. tuberculatum

Dumbbell-shaped cells of peridial hyphae

x 5.76K

184





185

Figure 186

A. tuberculatum

Dumbbell-shaped cells of peridial hyphae

x 11.52K

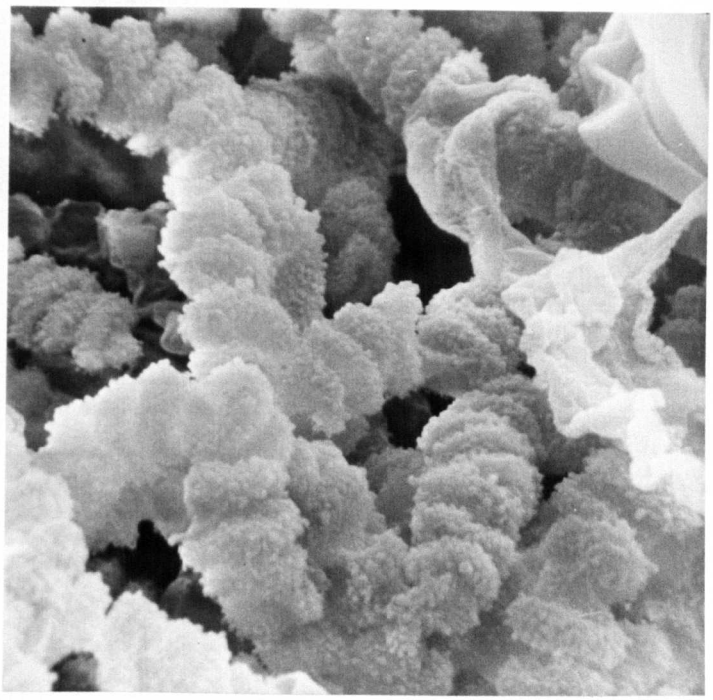
Figure 187

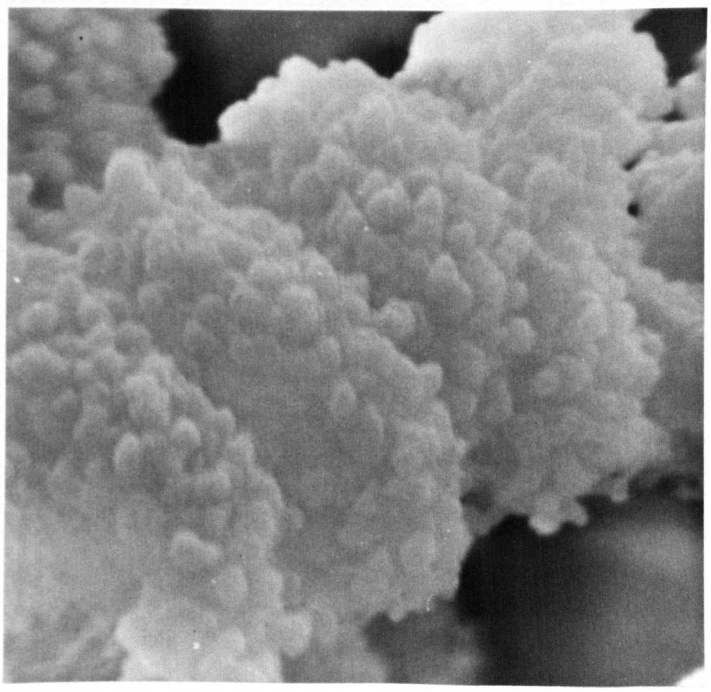
A. tuberculatum

Dumbbell-shaped cells of peridial hyphae

x 57.6K

186





187

Figure 188

N. gypsea

Macroconidia

x 6.3K

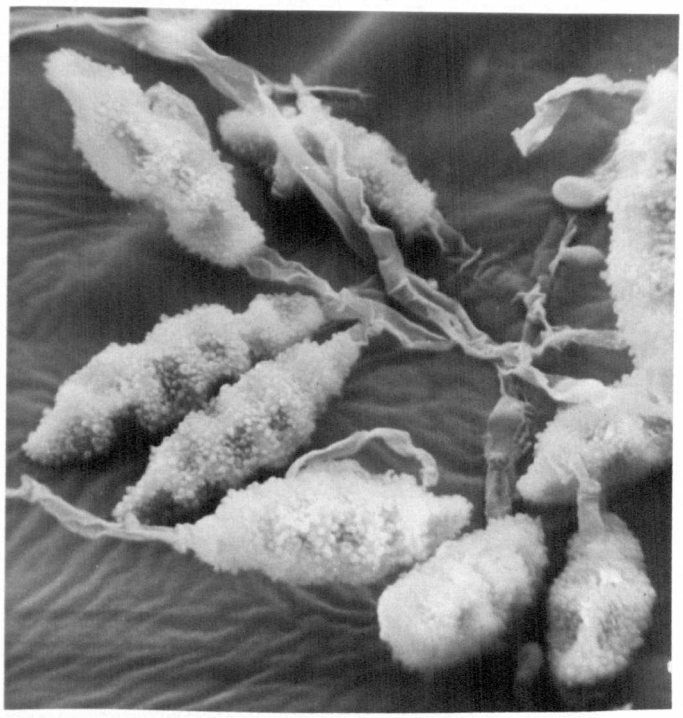
Figure 189

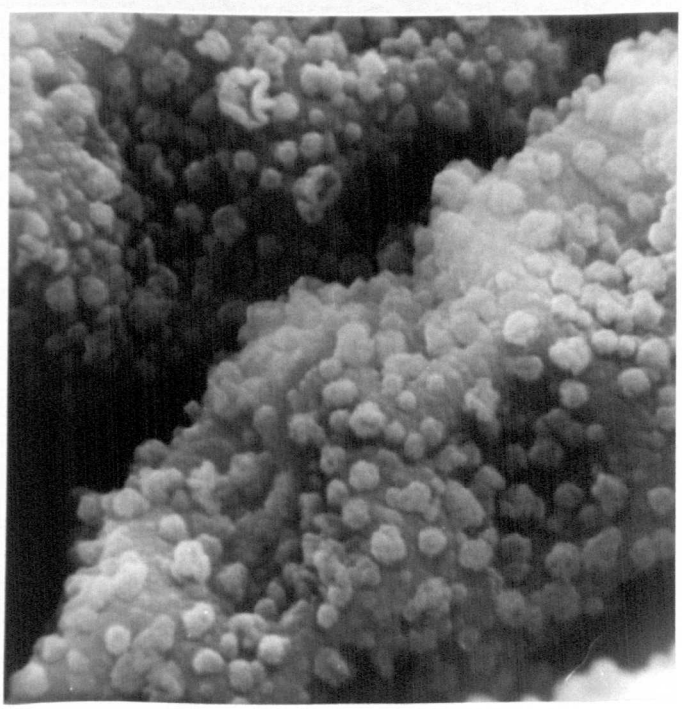
N. gypsea

Macroconidia

x 31.5K

188





189

Figure 190

N. gypsea

Cleistocarp

x 1.125K

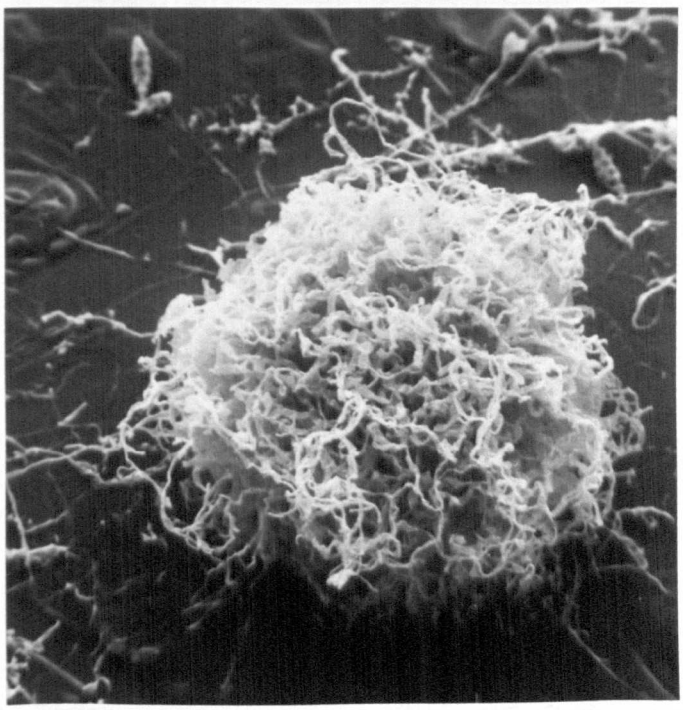
Figure 191

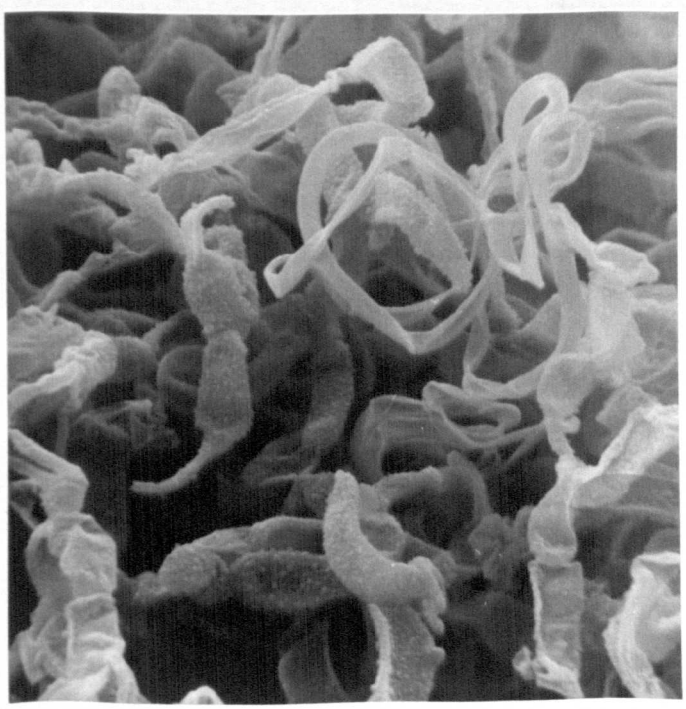
N. gypsea

Spiral and peridial hyphae

x 6.44K

190





191

Figure 192

N. cyrsea

Dumbbell-shaped cells

of peridial hyphae

x 32.4K

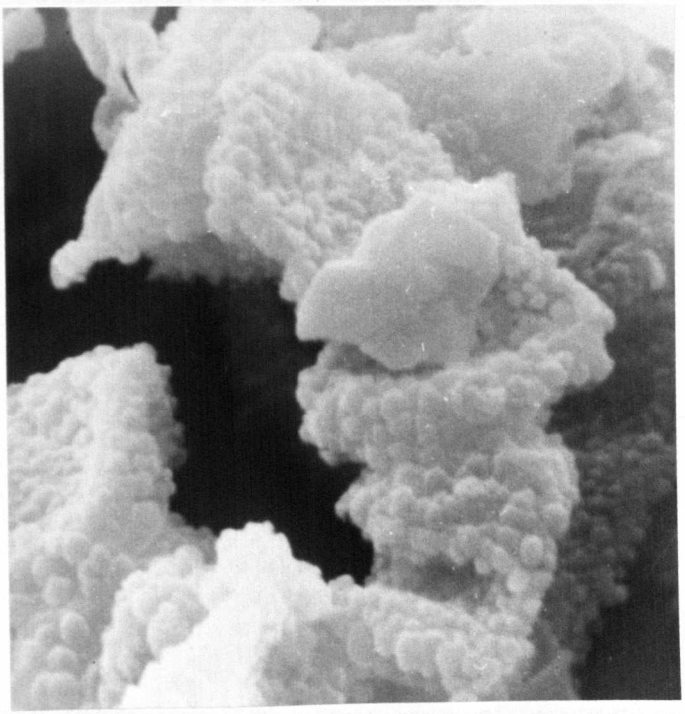
Figure 193

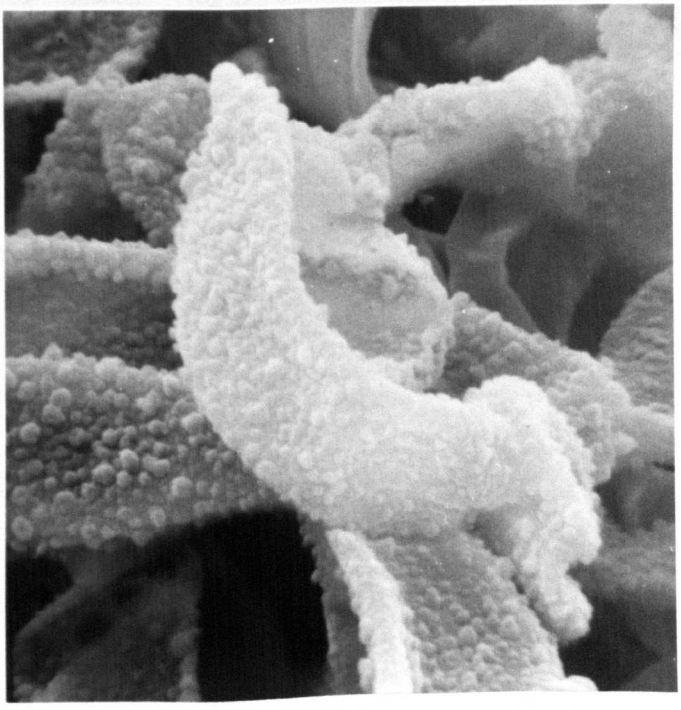
N. cyrsea

Antler-shaped peridial hypha

x 19.35K

192





193

Figure 194

N. gypsea

Spiral peridial hypha

x 12.73K

Figure 195

N. cajetani

Macroconidia

x 2.97K

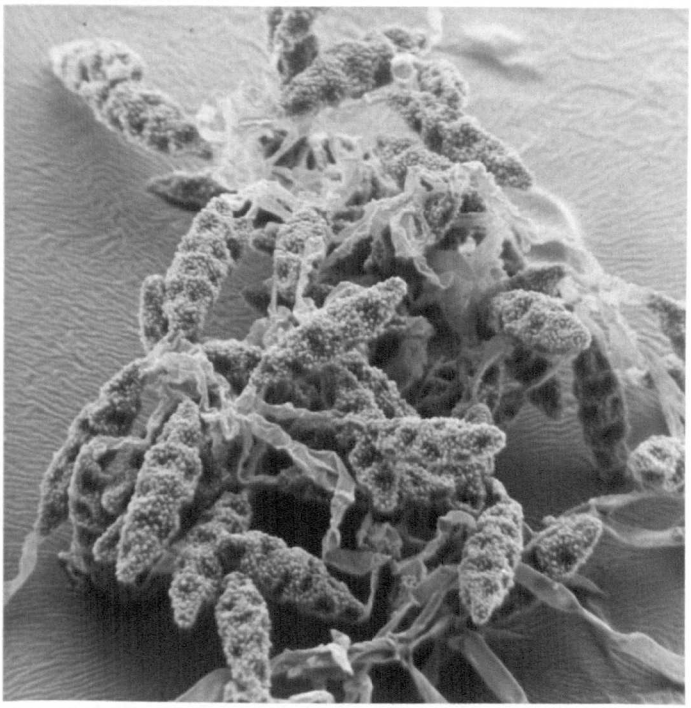
Figure 196

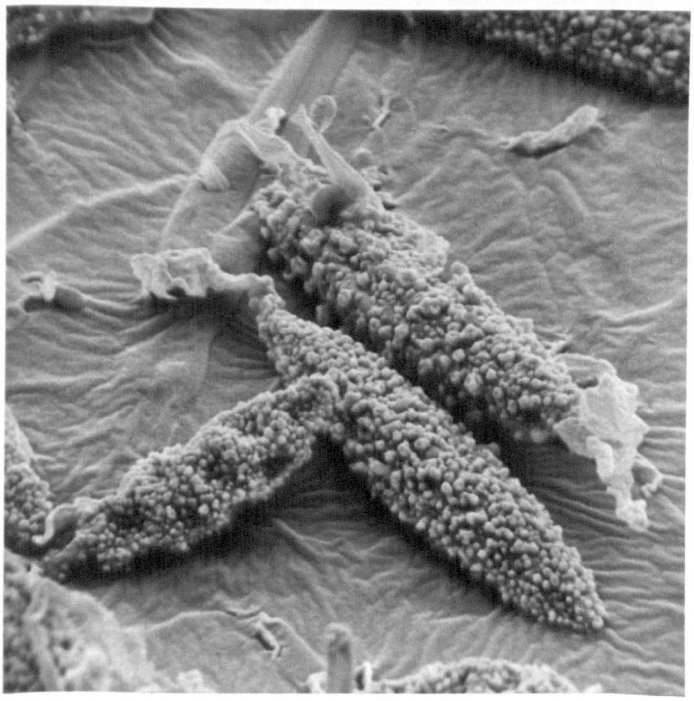
N. cajetani

Macroconidia

x 6.08K

195





196

Figure 197

N. cajetani

Macroconidia

x 12.06K

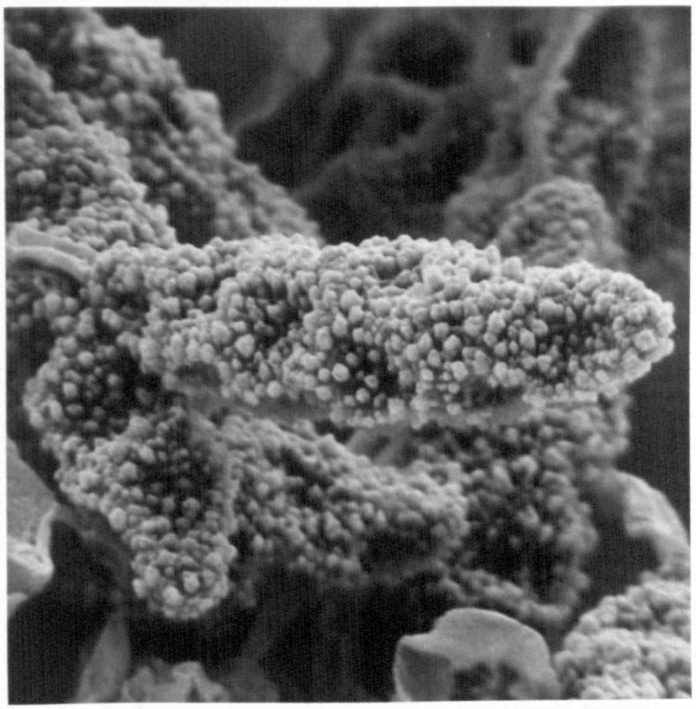
Figure 198

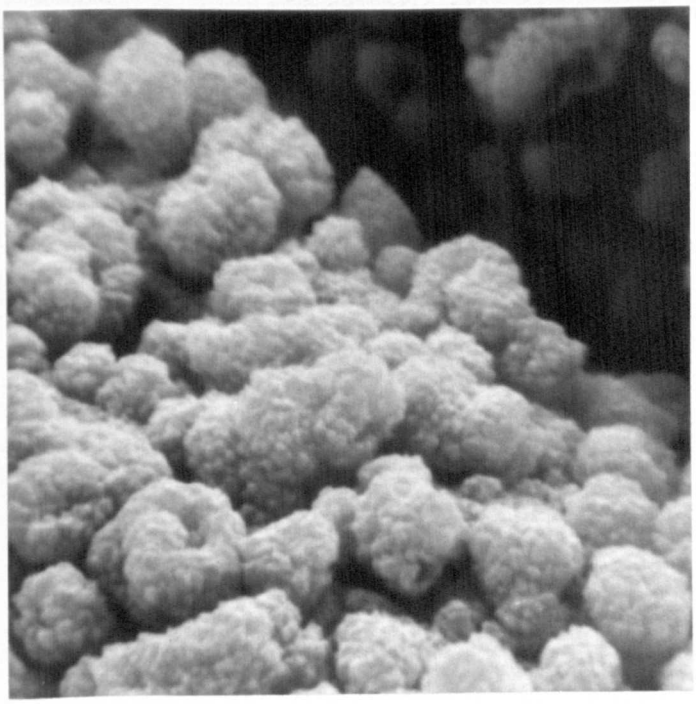
N. cajetani

Macroconidium

x 60.8K

197





198

Figure 199

N. incurvata

Macroconidia

x 12.06K

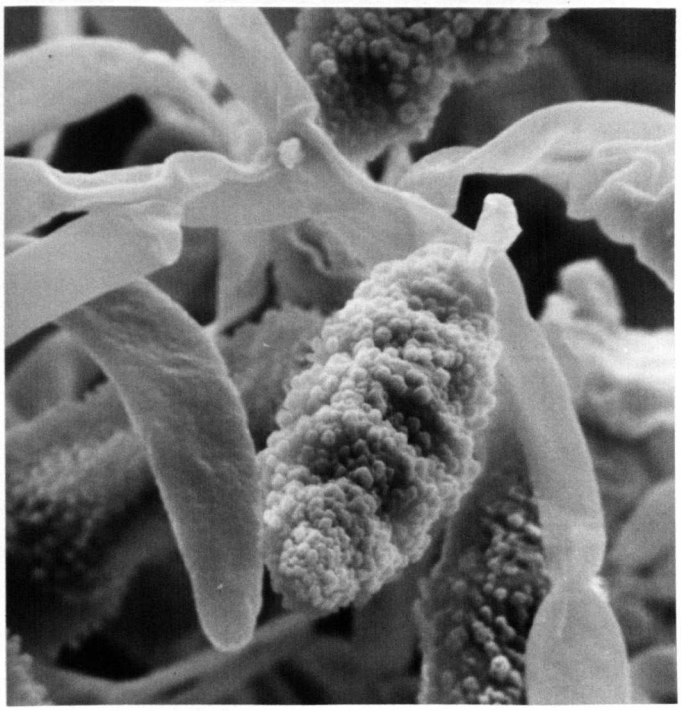
Figure 200

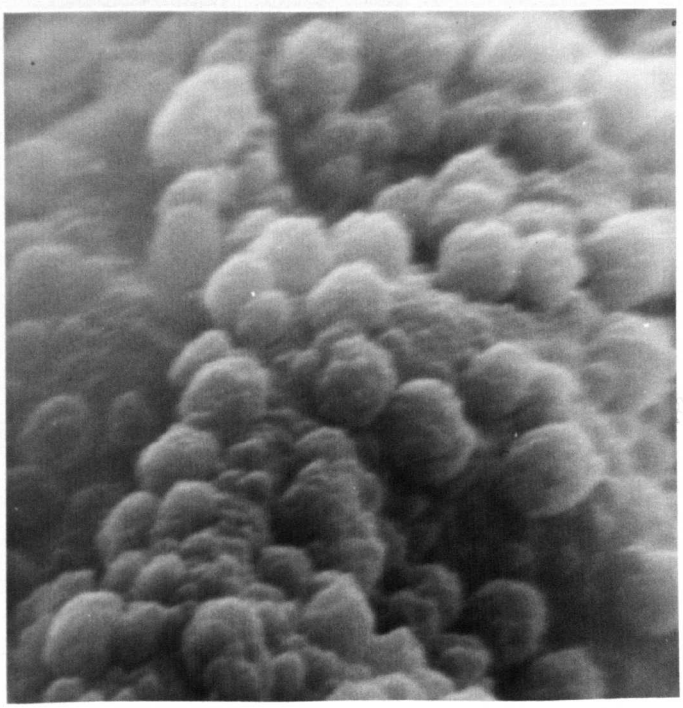
N. incurvata

Macroconidia

x 60.3K

199





200

Figure 201

N. incurvata

Macroconidia and microconidia

x 6.03K

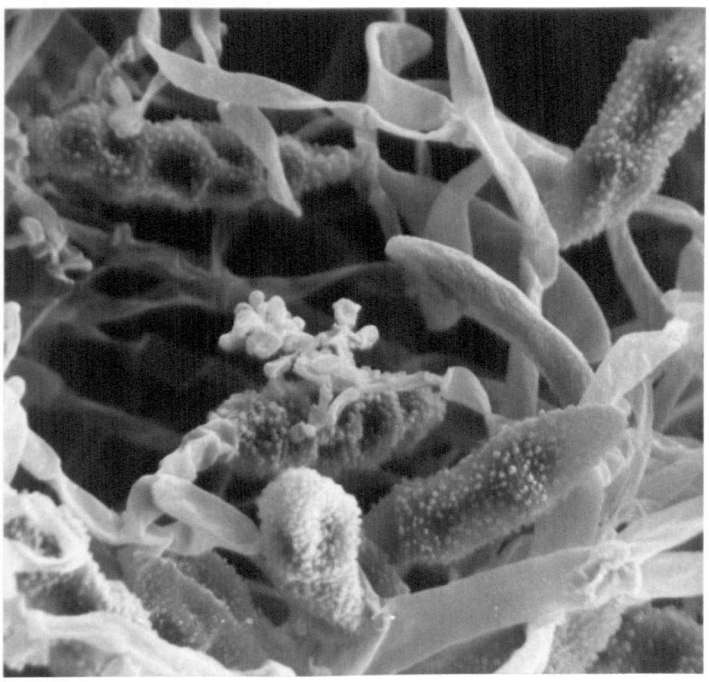
Figure 202

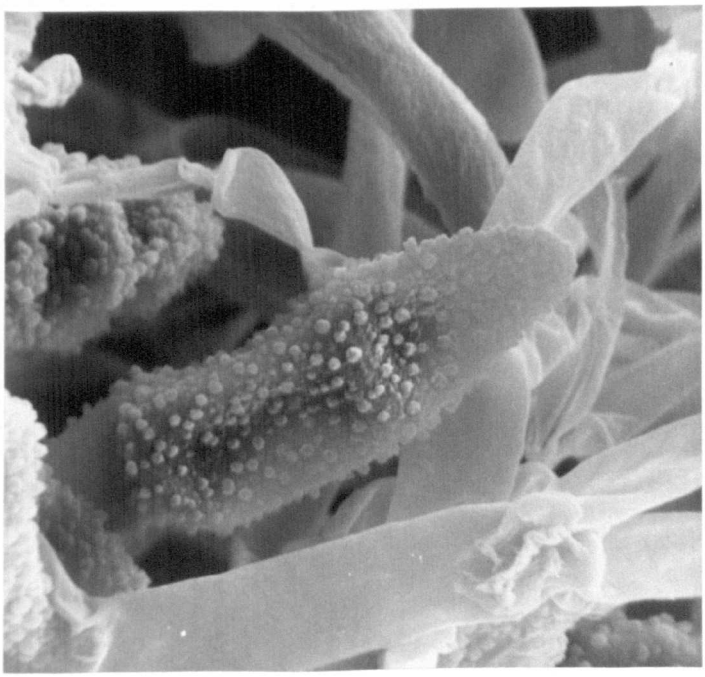
N. incurvata

Macroconidia

x 12,06K

201





202

Figure 203

N. incurvata

Cleistocarp

x 1.21K

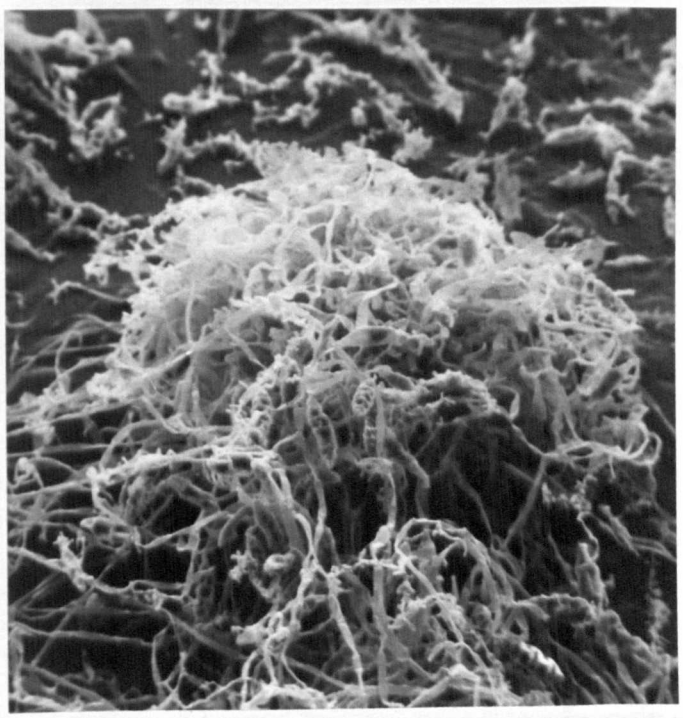
Figure 204

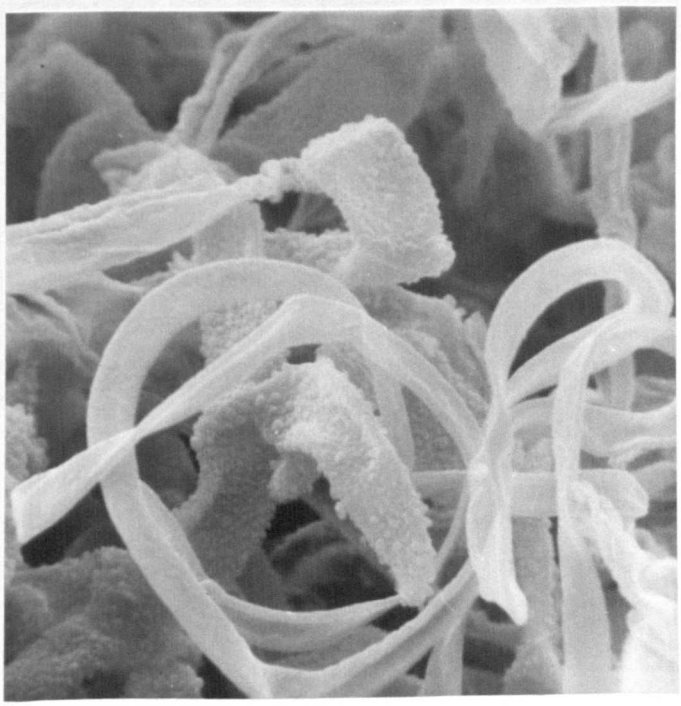
N. incurvata

Spiral and peridial hyphae, and conidia

x 12.60K

203





204

Figure 205

T. mentagrophytes

Microconidia

x 13.5K

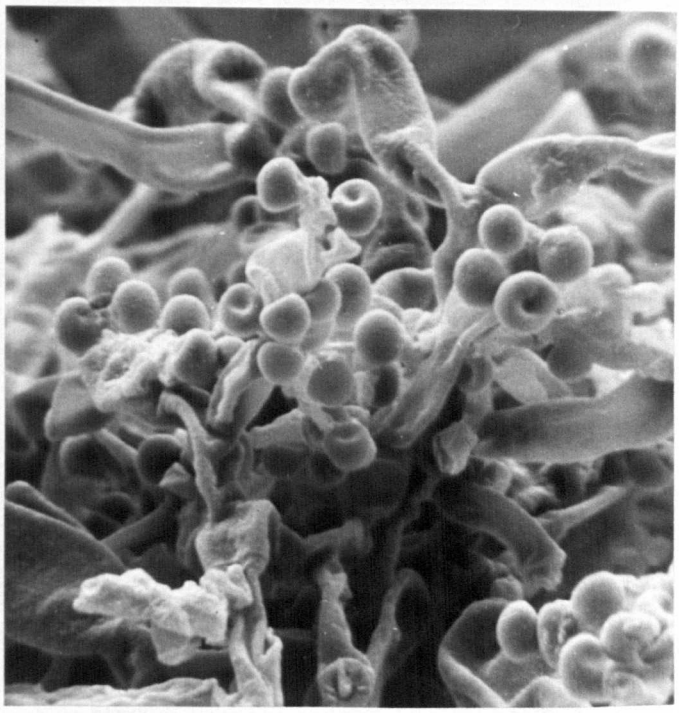
Figure 206

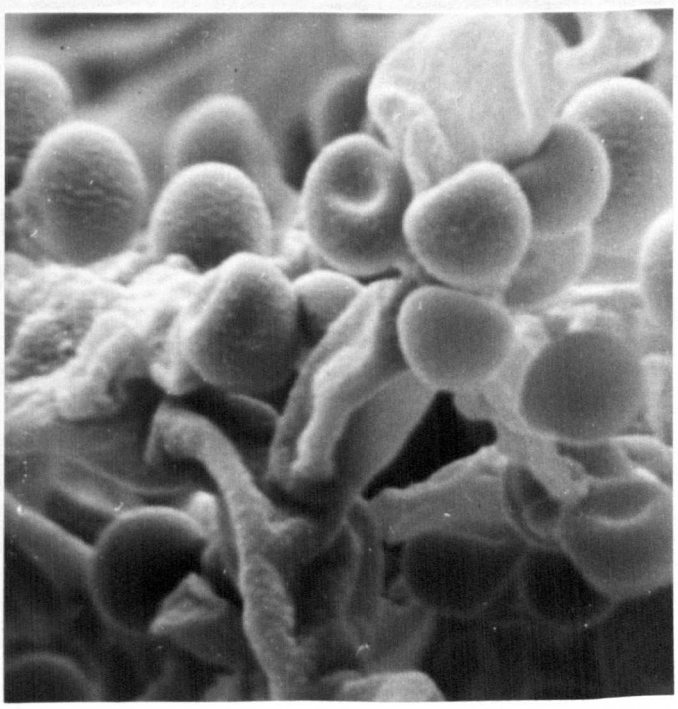
T. mentagrophytes

Microconidia

x 33.75K

205





206

DISCUSSION

All 6 keratinophilic fungi showed typical growth curves, which were functions of temperature, i.e. a linear portion in which growth increased with increased temperature, an optimum range which may be broad in the region of 20 - 25°C, e.g. M. gypseum, or narrow, reaching a peak, i.e. the other 5 species, at 25°C, followed by declining growth as the temperature became higher. None of the fungi grew well at or above 35°C. Only M. gypseum and C. keratinophilum grew at all at 35°C. The results for conidial A. uncinatum, T. terrestre, C. keratinophilum and M. cookei agree with those from Evans (1969) and Pugh and Evans (1970b), although the diameters all seemed to be slightly greater in the present work.

Cochrane (1958) said that there is no single temperature for optimum growth. This is because growth is also dependent upon various other conditions which may in fact be limiting at the optimum temperature. Therefore, the optima given in these experiments are really only valid for the conditions prevailing at the time of the experiment, i.e. time, medium and the method of measurement.

The graphs show that the Microsporum species were faster growing than the other 4 species. Chrysosporium keratinophilum seemed to be intermediate between the Microsporum species and the Trichophyton species.

Maximum diameter of colonies after 2 weeks:-

Microsporum gypseum	85 mm
Microsporum cookei	85 mm
Chrysosporium keratinophilum	75 mm
Trichophyton mentagrophytes	72 mm
Trichophyton terrestre	71 mm
Arthroderma uncinatum	57 mm

At low pH values, enzyme systems may be disrupted and at high pH, metal solubilities may be affected (Cochrane, 1958). (Permeability and some surface phenomena are the main factors affected by pH). Many other parameters in an ecosystem can alter the pH of that system, e.g. temperature, gross changes in the medium and the nitrogen source (Cochrane, 1958).

Cochrane also suggested that many of the broad optima of pH recorded for growth of fungi, reflect the ability of a fungus to raise or lower the pH of an initially unfavourable medium. This can be seen in the table of pH changes in the media containing different carbon and nitrogen sources, in the Appendix. This phenomenon has also been seen by Stockdale (1953b) and by Chesters and Mathison (1963).

All the keratinophilic fungi were found to grow in media over a range of pH values from 4 to 8.

Microsporum cookei and conidial A. uncinatum appear to show a double pH optimum in liquid medium. Fries (1945, 1956) found that for Coprinus, this reflected a pH-dependent unavailability of one or more inorganic elements. This may prove to be the case for these 2 keratinophilic fungi.

It would seem that M. gypseum and C. keratinophilum produce maximum growth in acid liquid media (pH 5), A. uncinatum, T. mentagrophytes and M. cookei in slightly acid media (pH 6) and T. terrestre was able to tolerate weakly acid or weakly alkaline to neutral media (pH 6, 7 and 8). However, all the species grew well over the range 5 to 8, and although this was true for M. gypseum too, the trend was for this species to decrease its dry weight from pH 6 to 8 compared with pH 4 and 5. This tends to agree with the observation of Pugh (1964) that M. gypseum prefers acid soils.

When these 6 keratinophilic fungi under consideration were inoculated into sterile soil of pH value 4, 6.7 or 8, none

of them could be really successfully reisolated from soils of pH value 4, whereas all of them could be isolated from soils of pH 6.7 and 8. It would appear that, although C. keratinophilum, T. terrestre and conidial A. uncinatum can be isolated from soils of pH 4, when there is no competition from other organisms, as in the case of the sterile soil, they appear to lie in the soil in an almost dormant state.

Böhme and Ziegler (1969) found that most soils with low pH values (3.0 - 4.5) did not yield keratinophilic fungi, but at pH 5 and over, most soils contained at least 1 keratinophile. They concluded that "the less acid the soil the higher the probable incidence of keratinophilic fungi" and the results in the present work support this hypothesis. Absence of this type of fungus in strongly acidic soils may be due to inhibition of enzyme activities under these conditions.

It was noticeable that in liquid media T. terrestre had the fastest growth of the Trichophyton species, whereas in solid media, both in pH and temperature experiments, this was one of the fungi which grew most slowly. There was some difficulty in measuring colony diameters of T. terrestre since, although there was only 1 initial inoculum disc, invariably several colonies would arise on the agar plate, whereas on the other plates only one colony arose. Therefore, although T. terrestre produced more colonies on the agar plate and greater dry weight in liquid medium than the other Trichophyton species, the fact that the original colony only was measured creates an anomalous situation. The microconidia of T. terrestre are obviously easily detached from the hyphae, in contrast to the other species. It would seem that for this particular species measurement of colony diameter on an agar plate gives a false impression of the growth of T. terrestre.

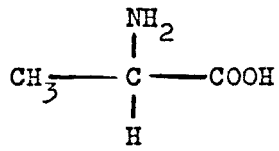
Cochrane (1958) states that linear measurement of a

colony on agar has its uses. Although it is not sufficiently accurate to assess the relative utilisability of different carbon sources it is thought that it is good for studying the influence of temperature and may be so for the effect of pH or toxic agents, although this has not been confirmed as yet.

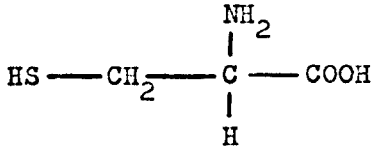
Brancato and Golding (1953) considered that the diameter of a mould colony was a reliable measure of growth, because there was rarely any difficulty in measuring diameters and because there is no acceleration of growth rate with time, because only apical cells of hyphae multiply.

A carbon source is vital to the metabolism of any fungus because, firstly it provides essential material for producing the basic structures of cells and secondly carbon compounds are the organism's main energy source. However, provision of just a carbon source is not sufficient for the 3 keratinophilic fungi,which were studied in this work, to grow well. This can be seen from the results of growth in a basal medium supplemented with one of 6 carbon sources. Growth was never more than 0.06 g dry weight of mycelium / 100 ml of medium. A nitrogen source is the other major essential factor necessary for good growth, i.e. it is needed for assimilation of amino acids and proteins, the synthesis of specific peptides and metabolism of purines and pyrimidines. From the results of basal medium and a nitrogen source in the form of a nitrate or amino acid, it can be seen that C. keratinophilum can grow well with the addition of phenylalanine, cysteine and proline. Trichophyton terrestre can grow well with cysteine and phenylalanine, while conidial A. uncinatum grew well with proline alanine and phenylalanine. However, it can be seen from the structure of these 4 amino acids, that they can provide both carbon and nitrogen requirements. (See overleaf for structure of these amino acids).

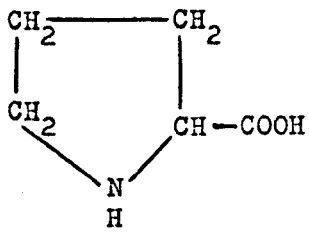
Alanine:-



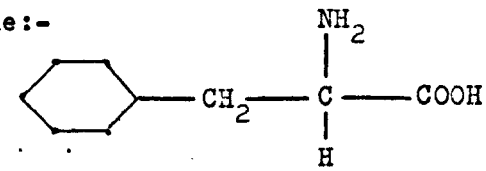
Cysteine:-



Proline:-



L-Phenylalanine:-



The controls also indicate that the nitrogen source played a major role in producing good growth.

Weidman and Spring (1928) are thought to have been the first to employ chemically pure compounds to make media and they found that the carbohydrate source of fungi did not appear to be critical. Mosher, Saunders, Kingery and Williams (1936) discovered that T. interdigitale was able to grow well without

carbohydrates if proper amino acids were provided.

D-glucose is considered to be the most important hexose biologically, therefore it is not surprising that all 3 keratinophilic fungi were able to utilise it well for growth. Fructose is thought to be almost equivalent to glucose and this is reflected in the fact that fructose also produced good growth in T. terrestre, C. keratinophilum and A. uncinatum. Maltose is the oligosaccharide most readily used by nearly all fungi. Trichophyton terrestre and A. uncinatum are not exceptions to this, since maltose was the third carbon source which was used most by these 2 fungi. Chrysosporium keratinophilum, on the other hand preferred starch and sucrose before maltose. Sucrose and starch are both considered to be good carbon sources for fungi, but sucrose is not normally as readily available as maltose. Starch on the other hand, is hydrolysed primarily to maltose, and good growth on starch is frequently accompanied by good growth on maltose. This appears to be the case for T. terrestre and A. uncinatum, but C. keratinophilum preferred starch to maltose as a carbon source. Lactose is generally thought to be a poor source of carbon and this was true for all 3 keratinophilic fungi; they all utilised it least of all 6 carbon sources under investigation.

Hejtmánek (1961) found T. ajelloi used glucose, fructose, galactose, arabinose, maltose, sucrose, lactose, raffinose, mannite, sorbite and inulin in decreasing order. Apart from a reversal in order of utilisability of fructose and glucose and the addition of use of starch, the present results agree with this order of preferential use.

Hejtmánek (1960) also reported that T. ajelloi assimilated nitrogen sources in the following decreasing order: asparagine, casein hydrolysate, glutamic acid, urea, $(NH_4)_2SO_4$, NH_4NO_3 and KNO_2. The present results do not indicate an agreement with the results published by Hejtmánek for nitrogen sources.

Nitrates are a good source of nitrogen for most fungi, but it appears that dermatophytes are an exception to this. Stockdale (1953) found that no dermatophytes were capable of using nitrate-nitrogen and that growth on ammonium salts was poor. In the case of NH_4NO_3 it is possible that the fungi can use either ion, but a drop in pH is supposed to demonstrate the utilisation of the ammonium ion. In general, there was a fall in pH after growth in the medium containing NH_4NO_3. The exceptions were A. uncinatum and T. terrestre with lactose, T. terrestre and C. keratinophilum with fructose and C. keratinophilum with glucose. In media containing $(NH_4)_2SO_4$, all combinations showed a drop in pH after growth. Hopkins and Iwamoto (1923) found dermatophytes fermented glucose and fructose with the production of acid when NH_4NO_3 was the nitrogen source. Arthroderma uncinatum and T. terrestre have been shown in the present work to do this, but C. keratinophilum has not.

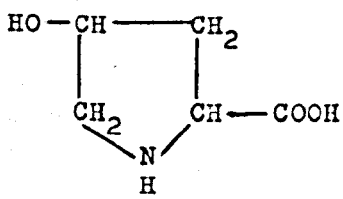
Although generalisations about the use of amino acids have been made, there are always exceptions. Usually glycine, asparagine, glutamic acid and aspartic acid are considered to support good growth, but the 3 keratinophilic fungi studied utilised these 4 amino acids to varying degrees. Arthroderma uncinatum and T. terrestre used aspartic acid to support good growth, whereas asparagine and glycine only produced half as much growth for A. uncinatum and glycine produced half as much for T. terrestre. The other combinations did not produce good growth. Chrysosporium keratinophilum on the other hand, only used glycine of the 4 to produce good growth. Stockdale (1953) pointed out that a comparison of availability of amino acids to different dermatophytes was difficult, but concluded that leucine, arginine, tyrosine and glutamic acid were easily available to most dermatophytes. In the present work, this was not found to be so for glutamic acid at all. Leucine was only used by C. keratinophilum, tyrosine was not studied, but arginine was used for good growth.

The amino acids which did seem to be good promoters of growth for all 3 keratinophilic fungi are phenylalanine, proline, serine, arginine, alanine, glycine, NH_4NO_3 and urea. Those which produced good growth for 2 of the 3 were aspartic acid and valine for A. uncinatum and T. terrestre and cysteine for T. terrestre and C. keratinophilum. This utilisation of cysteine is thought to signify requirement of sulphur rather than nitrogen (Cochrane, 1958). Raubitschek (1962) found cysteine inhibited the dermatophytes that he studied and suggested that the absence of cysteine from keratinised epithelial structures may be related to the keratinophilia of dermatophytes. Although T. terrestre and C. keratinophilum were able to use cysteine, it does not necessarily mean they are unable to penetrate keratinised substrates.

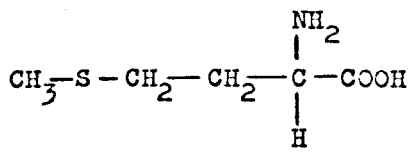
Ragot (1966) found that the optimal nitrogen source for T. ajelloi was phenylalanine and that this species only used glucose, fructose, mannose and to a small extent trehalose.

Hydroxyproline has been found to be inhibitory to Trichophyton species and other dermatophytic fungi (Robbins and McVeigh, 1946; Georg, 1949; Johnson and Grimm, 1951). Stockdale (1953) reported that methionine was inhibitory to some Trichophyton species. Hydroxyproline and methionine were the 2 least used amino acids. Glutamic acid and $(NH_4)_2SO_4$ were also used less than the majority of the amino acids by all 3 keratinophilic fungi. These 3 fungi obviously derive little energy from the carbon of these amino acids.

Hydroxyproline:-



Methionine:-



Rothman (1954) suggested that the ability of parasitic hyphomycetes to parasitise only keratinised tissue may be due to the fact that they are obliged to confine themselves to keratin since hydroxyproline is present in skin and surroundings but not in keratin itself.

Table 15 shows the availability of amino acids with either glucose or fructose as the carbon source.

Comments on methodology

On occasions, the inability of a fungus to utilise a certain carbon or nitrogen source is determined by the pH of the medium. A fungus may even produce organic acids, which in a poorly buffered medium, lower the pH so far that growth is hindered or prevented. To avoid this, media can be buffered by adding phosphate buffer, solid $CaCO_3$ or another alkali.

Burack and Knight (1958) found the pH of a medium with a dermatophyte growing in it rose from 5.6 to 7.0 or above and that the increase of ammonia closely followed the increase in pH. This causes the ink blue to lose its colour in agar with dermatophytes growing on it; it is also the reason DTM turns from yellow to red when a dermatophyte is growing on the medium. Goldfarb and Herrman (1956) discovered that the ability of dermatophytes to change the pH of Sabouraud's medium parallelled their ability to deaminate amino acids. They also considered the effect of the rise in pH upon the progress of fungal infections in man, i.e. the acid of the skin upon the dermatophyte and the effect of the alkali production of the fungus upon the acid. This is another reason people are able to rid themselves of fungal infections without any sort of treatment.

In the past, studies on the nutrition of T. mentagrophytes have been contradictory (Robbins and Ma, 1945; Nickerson, 1947; Georg, 1948; Bocobo and Benham, 1949; Giblett and Henry, 1950;

TABLE 15

Availability of amino acids with 2 different carbon sources.

a) glucose

Arthroderma uncinatum

Proline serine phenylalanine arginine urea asparagine aspartic acid glycine alanine

Chrysosporium keratinophilum

Phenylalanine glycine = serine proline arginine leucine alanine cysteine urea valine

Trichophyton terrestre

Proline alanine arginine urea phenylalanine serine aspartic acid = glycine = valine

b) fructose

Arthroderma uncinatum

Phenylalanine serine aspartic acid urea = arginine glycine valine proline $(NH_4)_2SO_4$

Chrysosporium keratinophilum

Proline phenylalanine glycine arginine serine alanine tryptophan NH_4NO_3 leucine

Trichophyton terrestre

Aspartic acid proline serine alanine phenylalanine glycine arginine cysteine urea

McVeigh and Campbell, 1950; Bereston, 1952; Silva and Benham, 1952 and Stockdale, 1953). Differing experimental conditions account for these conflicting reports and therefore criteria for evaluating growth, the chemical constituents of the media and the size of inocula should all be defined if any valid comparison of work is to be made.

Although all chemicals used should be of analytical reagent quality, this does not always guarantee purity. Biotin, a growth factor for fungi was found in starch, sucrose and maltose (but not fructose and glucose) by Wilson (1942). Other growth factors for fungi have been found in many carbon compounds, including glucose (Lockwood, Ward, May, Herrick and O'Neill, 1934).

Some fungi need other factors for growth besides amino acids and carbohydrates, i.e. they are deficient for certain factors, while other fungi are able to grow without these growth factors or else assimilate them from the food source provided. It is not always possible to obtain vitamin-free sources, but it is generally accepted that A. uncinatum is not deficient for any vitamins or growth factors (Mathison, 1961). It also seems that T. terrestre and C. keratinophilum are not deficient for any growth factor or vitamin, because growth occurred under most instances. Some fungi, e.g. M. gypseum can synthesise their own amino acids (Johnson and Grimm, 1951). Archibald and Reiss (1948) considered implications that the pathogenic fungi,which they studied,were able to use amino nitrogen of any 1 of several amino acids to synthesise other amino acids,necessary as building blocks in formation of protoplasmic proteins,characteristic of each individual species.

It was established that all the fungicides, except Triarimol, inhibited the growth of the 5 keratinophilic fungi studied, to half, if not considerably more than, that of the control at 100 ppm of fungicide in liquid medium.

Table 16 shows which fungicides completely inhibited each fungus at 100 ppm in liquid medium. Arthroderma uncinatum was the only fungus studied which was completely inhibited by all fungicides at 100 ppm. It was also the fungus which was least resistant to all fungicides at concentrations as low as 25 ppm.

Microsporum gypseum and M. cookei, T. terrestre and C. keratinophilum appeared to be the most resistant to several fungicides, but these fungi were not resistant to the same fungicides. Only dicloran was commonly inhibitory to all 4.

Of the 6 fungicides, only thiram was completely effective against all 5 fungi. Dicloran was the one compound to be wholly inhibitory to only one fungus, i.e. A. uncinatum. Milcol totally inhibited all fungi except T. terrestre, and captan and formaldehyde inhibited all but C. keratinophilum. Triarimol inhibited all but the Microsporum species. In fact, in the case of M. gypseum, at low concentrations the fungus was stimulated and even at 200 ppm the growth of this species was scarcely inhibited. At 100 ppm this was also the situation for M. cookei.

No stimulation by lower concentrations was observed in solid medium and the fungicides, in general, were tolerated better in solid media, e.g. A. uncinatum, T. terrestre, M. cookei and M. gypseum. The results for C. keratinophilum were fairly similar in both types of media.

There have been studies in the past upon the salinity requirements of marine fungi (Ritchie, 1959; Te Strake, 1959; Borut and Johnson, 1962; Jones and Jennings, 1964,) and although most true marine fungi (defined by Vishniac, 1955 and

TABLE 1b

Fungicides which completely inhibited at 100 ppm in liquid medium.

A. u	M. g	M. c	C. k	T. t
Thiram	Thiram	Thiram	Thiram	Thiram
Milcol	Milcol	Milcol	Milcol	
Captan	Captan	Captan		Captan
Formaldehyde	Formaldehyde	Formaldehyde		Formaldehyde
Triarimol			Triarimol	Triarimol
Dicloran				

A. u = Arthroderma uncinatum ; M. g = Microsporum gypseum ; M. c = Microsporum cookei ;

C. k = Chrysosporium keratinophilum ; T. t = Trichophyton terrestre

Ritchie, 1960) decrease in numbers with a decrease in salinity, and below 1% salinity few survive (Jones, Byrne and Alderman, 1971), other fungi are unable to survive above 2.5% salinity. Jones, Byrne and Alderman,(1971) reviewed the literature concerning ecological studies of marine and freshwater fungi, but no mention was ever made of human pathogenic or keratinophilic fungi. Whether this was because no search was made for them (because they are not easy to isolate from agar plates) or that the sea water adversely affects keratinophilic fungi is unknown. However, Pugh (1971) stated that conidial A. uncinatum is absent from coastal soils, and Orrù, Pinetti and Aste (1968) found that NaCl could hinder the growth of Epidermophyton floccosum, M. gypseum and 8 Trichophyton species, but A. curreyi, Chrysosporium species, Ctenomyces serratus, M. gypseum, M. cookei and T. terrestre have been isolated from coastal soils by Pugh and Mathison (1962). Gip and Paldrok (1966) also isolated T. terrestre from beach sands and it would seem,from in vitro work,that T. terrestre is the keratinophilic fungus which is least affected by sea water, whereas growth of A. uncinatum, M. cookei, M. gypseum and C. keratinophilum is hindered to some extent by sea water. The percentage isolation of these fungi varies throughout the soils andthis may be dependent on the amount and nature of organic matter in the soil and the degree of inundation of the sands by sea water (Pugh, 1962). Pugh and Mathison (1962) found from their preliminary studies that C. serratus was much less tolerant of high salt concentration than A. curreyi.

The patterns of growth in liquid and solid media resembled one another more or less exactly, but the sea water did not entirely prevent growth. Double strength sea water almost achieved that end, with the exception of M. gypseum and T. terrestre which could still tolerate that concentration. Dzawachiszwili, Landau, Newcomer and Plunkett (1964) found that M. gypseum was

hindered by sea water, but not until concentrations of NaCl were at 3.4% was growth cut to half the control. Only at 6.8% NaCl was M. gypseum truly inhibited.

A similar pattern was seen in the results of reisolation of keratinophilic fungi from inoculated sterile loamy soil. Chrysosporium keratinophilum and M. cookei were most hindered in their growth, A. uncinatum was intermediate between them, while M. gypseum and T. terrestre were still isolated with comparatively high frequency. In the sterile sands, conidial A. uncinatum and C. keratinophilum were reisolated with least percentage colonisation (the latter was not isolated at all), whereas T. terrestre, M. gypseum and M. cookei were all isolated at about the same percentage , but even then not in high percentages.

Results may differ considerably from growth patterns of the same fungi when availability of either carbon or nitrogen or both is limiting compared with conditions of sufficient of these. Temperature can also affect the results (Ritchie, 1957, 1959; Hughes, 1960).

The fact that these fungi are able to grow in the laboratory on media containing sea water means that they fulfil one of the requirements necessary for classification of a marine organism. Dzawachiszwili, Landau, Newcomer and Plunkett (1964), suggest that this raises the possibility of their survival in marine environments. Vishniac (1955) and Ritchie, (1960) also considered that this was true. Pugh and Mathison (1962) found that C. serratus and A. curreyi could both sporulate profusely on strips of dogfish egg cases and chitin from cuttlefish "bone", both of which are found in abundance at Gibraltar Point. Adaptation to various levels of salinity has been observed by Ohtsuki (1953).

Only C. keratinophilum, T. terrestre and A. uncinatum were isolated from the university and farm soils. Arthroderma uncinatum and C. keratinophilum were inhibited in their isolation by sea water concentrations and T. terrestre was not.

Chrysosporium keratinophilum and T. terrestre were not abundant in the potato field when watered with sterile distilled water. Arthroderma uncinatum, on the other hand, appeared to increase slightly in the potato field compared with the hedge soils. It is possible that the addition of turkey debris encouraged the growth of this species which in turn provided too much competition for the survival of C. keratinophilum and T. terrestre.

All species were isolated least on greasy wool-baited soil watered with sea water and most on degreased wool watered with sterile distilled water. This was also found by Pugh (unpublished material).

Using the fluorescent antibody technique, it has been demonstrated that conidial A. uncinatum is capable of growing through non-sterile soil. Since it can do so, A. uncinatum is an active soil inhabitant, i.e. it has the ability to compete with other saprophytes in the soil to reach and utilise a keratin source. The colonisation of the uninoculated baits could have arisen from the fungus on the inoculated bait and/or from the surrounding soil. However, when an inoculum of A. uncinatum was added to either sterile or non-sterile soil without a keratin source no growth was seen to have taken place. This could be due to fungistasis or it might be that amino acids and sugars were being used by "sugar" fungi (the primary colonisers) and prevented growth of A. uncinatum in non-sterile soil. There is also a possibility that something essential for the growth of A. uncinatum in sterile soil had been destroyed by autoclaving.

Arthroderma uncinatum does not produce keratinase in the absence of keratin (Jill Doe, personal communication) and although it would seem that some mechanism, other than random growth, is important for the detection of keratin by A. uncinatum, organisms such as Onygena equina (Ward, 1901), Streptomyces fradiae Waksman and Curtis or Actinomyces citreus Krainsky (Waksman 1919) can utilise keratin, and the breakdown products may diffuse through the soil thereby stimulating the growth of A. uncinatum. Mathison (1964) found that such organisms were apparently more efficient keratin decomposers than dermatophytes and he felt that "an important factor in determining the keratinolytic ability of an organism may be the capacity to deaminate amino acids resulting from proteolysis of non-keratinised residues, thereby creating alkaline conditions sufficient to bring about partial denaturation of the keratin".

From experiments concerning assimilation of keratin in pure culture, Hejtmánek (1959) considered that T. ajelloi appeared to be a natural destroyer of keratinous remains in soil. Baxter and Mann (1969) found that, in their electron microscope studies of the invasion of hair in vitro, T. ajelloi has much less observable keratinolytic ability than T. mentagrophytes. They considered that, because the perforating organ of T. ajelloi was much larger than that of T. mentagrophytes, T. ajelloi possibly relies more on mechanical action for its colonisation and disintegration of hair. Weary, Canby and Cawley (1965) also considered this possible. The paucity of mitochondria in T. ajelloi was thought to reflect its lower enzymic activity. Anglesea and Swift (1971) reported that T. ajelloi had a much higher competitive saprophytic ability in relation to keratin substrates than it did on sugar-rich media and they considered that this fungus should be regarded as keratinophilic as defined by Mathison (1964). The present work substantiates this idea,

since A. uncinatum was rarely isolated from soil crumb plates.

Spores and cleistocarps of A. uncinatum were found to be present in the soil smears and although Hill and Gray (1967) stated that fluorescent antisera will not distinguish between living and dead cells, (or if they are living, give no idea of their physiological activity), germinating spores of A. uncinatum have been seen. This provides evidence of viability. Thus, in the absence of keratin, A. uncinatum appears to survive in the form of ungerminated spores and/or cleistocarps, but in the presence of keratin the fungus can be detected in the mycelial state. Even if the fungus cannot find a keratin source it would be able to use the amino acids and sugars in the soil, provided the other soil inhabitants did not compete too well for this source.

Novák and Galgóczy (1966) considered that T. mentagrophytes had some possibility of soil inhabitancy. Grin and Ožegovic (1963) found that it was possible to reisolate this fungus from non-sterile soil after 85 days. Novák and Galgóczy (1966) reported that T. mentagrophytes was able to live for some time when inoculated into sterile or non-sterile soil and Ožegovic (1970) found it could survive > 6 years in non-sterile and sterile soil. Novák and Galgóczy questioned the publications of Lurie and Borok (1955), Rodriguez (1958) and Evolceanu, Alteras and Cojocaru (1962) relating to the isolation of T. mentagrophytes from soil, because of several difficulties, which they discuss, in the identification of T. mentagrophytes in soil. Schönborn (1966) found that this fungus could be reisolated from non-sterile soil after 22 months and from sterile soil after 4 years, 8 months. She also reported that T. mentagrophytes survived in the form of its spores which she proved by vital staining with fluorescent acridine orange and she also saw germination take place in sterile soil but not non-sterile.

Since non-specific staining was seen involving other species of fungi besides T. mentagrophytes, it is thought that the fluorescent antibody technique is of little use in determining the status of this species in soil. It may be possible to eliminate non-specific staining by obtaining a better immunological response from another rabbit or possibly another host. Some rabbits do produce poor responses to the fungi injected into them (Axelsen and Faux, personal communication) and it is considered best to discard them and repeat the experiment with another host. Financial reasons prevented this course of action.

Difficulties with immunisation have been discussed by Seeliger (1960) and methods of overcoming this are mentioned. He also stressed that "there is no such thing as absolute antigenic specificity". Despite various methods of immunisation and intensive treatment of animals with antigens,no antibodies may become demonstrable even after a prolonged course of immunisation, resulting in what is termed "immunological paralysis" - antibodies are produced, but because they are absorbed by antigens in tissues they pass undetected. This is followed by destruction of the antibody-antigen complex. It is possible to overcome this by short-term immunisation using relatively low dosages of antigen.

Holland and Choo (1970) found that only fully immunised animals gave a reliable antiserum which would give reproducible results.

Choo and Holland (1970) attempted to obtain a specific antiserum from Ophiobolus graminis by cross absorption, but in each case it resulted in overall diminution in the intensity of fluorescence, i.e. there was a loss of antibodies for O. graminis because they were also antibodies for other antigens. Only by studying the wall structure of T. mentagrophytes and other

related fungi, or the ones with which the serum cross-reacted, would it be possible to say whether this is what happened in the case of T. mentagrophytes. Choo and Holland (1970) also discovered that removal of $(NH_4)_2SO_4$ from γ globulin may give rise to non-specific staining.

Since germinating spores of T. mentagrophytes inoculated into non-sterile soil were detected by the fluorescent antibody technique, there is evidence that the species can at least grow in non-sterile soil even if the growth is not prolonged, i.e. this work agrees with that of Baxter (1966) who thought that T. mentagrophytes was capable of survival in non-sterile soil, even if only for a limited period.

Baxter (1966) found that T. ajelloi competitively inhibited the growth of T. mentagrophytes on keratin baits, if both fungi were present in a soil incubated at only 26°C, but not at 37°C. Since these slides were incubated at 26° it would seem that one would not expect T. mentagrophytes to compete in non-sterile soil for a keratin bait if A. uncinatum was known to be present, which in this case it was, because it had been isolated before by the hair-baiting technique.

Comments on methodology

Non-specific staining can be minimised by a slightly expensive method using gelatin-rhodamine conjugates (Bohlool and Schmidt, 1968) which serve as a counter stain as well as preventing non-specific staining. "The gelatin apparently adsorbs to soil and tissue, blocking sites of non-specific adsorption; the dye conjugated to the gelatin imparts an orange-brown background fluorescence to the soil or tissue in good contrast to the yellow green of a fluorescein-labelled antibody.

Although whole serum can be labelled, Eren and Pramer (1966) considered it undesirable; less fluorochrome is wasted

and the probability of non-specific staining is minimised if antibody protein is separated from the other serum proteins and then labelled.

Kaplan and Kaufman (1961) considered that the problem of autofluorescence could be eliminated through the use of proper filters, e.g. a combination of a 5113 corning glass primary filter, (3mm) and a Wratten ZA secondary filter (2mm) but other studies may show other combinations to be superior.

As Nairn (1961) said " the method is perhaps the most elegant and specific of all the histochemical techniques; its only limitations are those of immunology itself".

Since both geophilic and zoophilic fungi have been isolated from soil, the question of their method of survival has aroused great interest. Although these fungi utilise keratin, quite frequently they are isolated from soils which have little or no supply of keratin (Griffin, 1960; Pugh and Mathison, 1962). Some species, like A. curreyi, are isolated from soil, but appear to be unable to compete successfully for keratin substrates (Pugh, 1964) in non-sterile soil, but survive in soil as cleistocarps. Trichophyton mentagrophytes has also been isolated from soil (Lurie and Borok, 1955; Evolceanu, Alteras and Cojocaru, 1962; Baxter, 1965; Garg, 1966; Al-Doory, 1967), but Baxter considered that this species was unable to survive for long periods of time. Evolceanu, Alteras and Cojocaru,(1962) and Lurie and Borok (1955), on the other hand, thought that T. mentagrophytes was able to grow and compete successfully as a saprophyte in soil. Gentles (1971) reported that T. mentagrophytes could survive for at least 33 months in keratin enriched fallow land, but only 12 months in unenriched subsurface soil. However, McGinnis and Hilger (1972) showed that T. mentagrophytes was unable to compete as a

saprophyte in non-sterile soil (with no bait or keratin source). It was incapable of growing through non-sterile soil and could not survive for long periods of time in non-sterile soil either, although it could do so in sterile soil. Enrichment of soil with hair or callus provided a source of keratin which enabled T. mentagrophytes "to compete" as a saprophyte.

Using an adaptation of the McGinnis and Hilger (1972) technique, it was found in the present work that T. terrestre, C. keratinophilum and A. uncinatum were all capable of competitive saprophytic growth in non-sterile soil, because propagules increased. They were also able to survive in soils with no keratin. Proof of this survival was the fact that the number of propagules reisolated remained about the same throughout the sampling period. The observation that these species can survive when no keratin source is provided can be seen from the results of isolations of soil crumb plates. Although Anglesea and Swift (1971) suggest that T. ajelloi competes better for keratin than a sugar-rich medium, and this is true for T. terrestre and C. keratinophilum too, the spores still remain viable in the soil, because some of them, albeit a few, were able to germinate on agar plates.

Chrysosporium keratinophilum behaved differently from T. terrestre and A. uncinatum in sterile soil. The number of propagules with or without hair did not increase, and it would seem that C. keratinophilum did not proliferate in sterile soil. It is possible that although C. keratinophilum propagules remained viable, a necessary stimulus from other sources had been destroyed when the soil was autoclaved. Alteras (1971) found that C. keratinophilum was the most resistant of fungi and could be isolated from stored non-sterile soil after 10 years. Trichophyton ajelloi and T. terrestre were isolated from stored non-sterile soil after 8 years.

Balabanoff and Usunov (1970) found that T. terrestre and T. ajelloi were able to remain viable in sterile soil in buried nylon bags for an indefinite period in the soil.

In non-sterile soil, (with or without hair, C. keratinophilum was found in greater numbers than in sterile soil, in contrast to T. terrestre and A. uncinatum, and this agrees with the work of Alteras (1971), although he studied the keratinophilic fungi for a much longer period of time.

The results from the colonisation tubes demonstrated that all the species investigated were capable of growing through sterile soil, but only T. terrestre, A. uncinatum and M. gypseum were truly able to compete successfully, within 13 weeks, for the keratin in the side-arms of tubes containing non-sterile soil. Microsporum cookei and C. keratinophilum were able to grow and compete in non-sterile soil, but not very successfully, whereas T. mentagrophytes was unable to compete at all.

In colonisation tubes, it was found that A. uncinatum, T. terrestre, M. gypseum, M. cookei and C. keratinophilum were able to grow through non-sterile soil and colonise bait in the side-arms, but the method of detection of the keratin is unknown. It is possible that the fungus, when inoculated onto the bait in the first side-arm obtains enough energy from this initial bait to grow as far as the second bait and so on along the tube. The fungi may be able to utilise the amino acids or sugars in the soil to produce growth to reach a keratin source. It is not known from the results of the colonisation tubes if any of the keratinophilic fungi can grow through soil if no keratin is supplied. The only way to determine this in colonisation tubes would be to inoculate a bait at one end of the tube and after a certain length of time add bait at the other end of the tube to see whether the inoculated fungus grew onto it. However, this would in effect, defeat the object, because

keratin would then be present. If the colonisation of the bait were fairly rapid then one could perhaps assume that the fungus had grown the length of the tube whilst there was no keratin. If colonisation of the bait took place slowly the assumption might be that the fungus had not grown along the tube until, by some means, it had detected that there was a bait at the other end of the tube. This could have been by utilising the breakdown products of keratin (by S. fradiae, O. equinum or A. citreus) which had infiltrated soil spaces along the tube. It would be possible to use the fluorescent antibody technique to determine this, but so far, within the time that A. uncinatum has been studied, no growth was detected in either non-sterile or sterile soil without keratin. This may be because, within that time, in sterile soil, fungistasis was a limiting factor. On the other hand, it may have been because other soil fungi were utilising the sugars and amino acids, thereby preventing A. uncinatum from growing.

A further set of experiments with micropipettes was studied, but the results were not as clear-cut as those from colonisation tubes, in that none of the fungi grew in the non-sterile soil micropipettes. Oxygen tension or the moisture level in the micropipettes may have been the limiting factors in this experiment.

The experiments which were based on work by Wastie (1961) showed that the six dermatophytes were slightly inhibited by the addition of chloramphenicol and cycloheximide to S.D.A. Chrysosporium keratinophilum and M. gypseum were least affected, but in no instance was growth prevented. The only time when growth was completely inhibited was when C. keratinophilum was inoculated onto Cellophane over non-sterile soil on non-supplemented S.D.A. In this case, it would appear that either easily diffusible fungistatic substances passed through the Cellophane, or fungi

from the non-sterile soil competed more successfully for the agar substrate, thereby preventing growth of C. keratinophilum. When no antibiotics were added to S.D.A, the growth of soil saprophytes from the non-sterile soil inoculum was not inhibited beneath the Cellophane. When antibiotics were added to S.D.A, growth of C. keratinophilum was considerably better over a non-sterile soil inoculum.

All the other fungi studied were able to grow over a non-sterile soil inoculum on non-supplemented S.D.A. Wastie (1961) found that the outcome of the struggle for colonisation of the agar plate is largely decided by the relative linear growth rates of the competitors before significant antibiotic or fungistatic effects are produced. The growth rates of M. cookei, M. gypseum and T. terrestre were all faster than those of A. uncinatum, T. mentagrophytes and C. keratinophilum and this was reflected in the results showing the former 3 to be least affected by the non-sterile soil inoculum, and the latter 3 to be most affected by the competition from the soil saprophytes in the inoculum.

GENERAL DISCUSSION

The decrease in incidence of conidial A. uncinatum in the swimming pool coincided with the opening of the pool to the public. Outside the swimming pool, a minimum of conidial A. uncinatum was seen in July, August and September. Therefore, the decrease of conidial A. uncinatum at this sampling site may just be the normal seasonal fluctuation and may not be due to any effect of the chlorinated water spilt onto the soils. Despite the presence of bathers providing a keratin source, no increase of conidial A. uncinatum was seen in the swimming pools, in contrast to the result in the hedgehog pens. This may be because the grass was cut at least once a fortnight and the grass cuttings, which were removed, may have contained any keratin deposited by bathers and hence was also removed. The spring-summer decrease of conidial A. uncinatum was also seen outside the hedgehog pens, but inside the pens an increase was seen when the hedgehogs were present. Chrysosporium keratinophilum, M. cookei and M. gypseum also increased at this time and all 4 species decreased in incidence when the hedgehogs were removed. Since a similar increase was not seen outside the hedgehog pens at this time, it can perhaps be considered that the increase in keratin, in the form of quills and scales, was the reason for the increase in these species. In contrast, cleistocarpic A. uncinatum and T. terrestre both showed an increase outside the pens at this time and therefore could be considered uninfluenced by the increase in keratin inside the pens. A similar dependence on keratin was seen in Ctenomyces serratus, Arthroderma curreyi and C. keratinophilum, but not in M. cookei, M. gypseum and T. terrestre in the coastal ecosystem studied.

Chrysosporium keratinophilum and T. terrestre were isolated most in winter and least in summer and appear to be temperate zone inhabitants. Conidial A. uncinatum appears to be the same, although this species was isolated more or less in the same percentage frequency throughout the year, with the exception

of the decrease in summer. However, since the documented literature and the present work are not in complete agreement, definite conclusions have not been reached about the seasonal distribution of keratinophilic fungi.

Microsporum cookei and M. gypseum were found to prefer warmer temperatures and this would explain why they were rarely isolated. Although little work has been carried out concerning the effect of temperature upon M. cookei, it does appear to show a similar distribution to M. gypseum (Otčenášek, Dvořák and Kunert, 1967).

Sabouraud (1910) was the first to find that the optimum temperature for the development of dermatophytes is in the range 15 - 30°C. In the experimental work, none of the 6 keratinophilic fungi grew well at or above 35°C, (and this agrees with Pugh and Evans, 1970b), although M. gypseum and C. keratinophilum were able to grow to a certain extent at 35°C. All 6 fungi showed optimum growth at 25°C. Saëz and Battesti (1970) found 38°C was the maximum temperature for growth of M. gypseum, while Gentles, Dawson and Brown (1964) found the perfect state of M. gypseum formed at 30°C and good vegetation and asexual growth takes place at temperatures up to and including 34°C. Kadisch (1929) found 40°C was the maximum for T. mentagrophytes, but both these fungi grew better at slightly lower temperatures than the maxima. The optimum growth of T. terrestre, A. uncinatum and M. cookei took place at less than 30°C, (Gentles, Dawson and Brown, 1964). The temperature of the skin surface is slightly less than 35°C and this may explain why M. gypseum and T. mentagrophytes are more commonly found parasitising tissues of man and animals than are the other 3 species.

Several workers suggested that T. terrestre is unable to compete against conidial A. uncinatum (English, 1964) or only increases in numbers when other keratinophilic fungi fall in numbers (Pugh and Evans, 1970a). The results of isolations from both hedgehog pens and swimming pool suggests that these

authors may be correct in their assumptions, especially since T. terrestre was found almost ubiquitously at Gibraltar Point where few other species were discovered. The ability to tolerate sea water concentrations and to grow without a dependence on keratin confers upon T. terrestre the capacity to grow well when other keratinophilic species were absent.

Only M. gypseum of the truly pathogenic dermatophytes has been isolated from either the hedgehog pens or the swimming pool soils and it would appear that the soils in these regions are only minor reservoirs of infection.

The distribution of keratinophilic fungi in soils of differing pH values has been studied and soils of pH values lower and higher than 6 have been recorded for conidial A. uncinatum. The optimum pH of soil for A. uncinatum was on the acid side of neutral, but it grew very little in soils of pH 4.

This discovery was supported by results of growth of A. uncinatum in liquid cultures, where a slightly acid medium (pH 6) was found to be optimum. Trichophyton terrestre showed optimum growth in soils of pH 8 and tolerated liquid medium of pH 6, 7 or 8. Microsporum gypseum, M. cookei and C. keratinophilum have been found mostly in weakly alkaline soils and whereas M. cookei in liquid media grew best at pH 6, M. gypseum and C. keratinophilum produced maximum growth at pH 5. Pugh (1966), Mohapatra and Gugnani (1964) and Steinerova and Buchvald (1967) thought M. gypseum preferred acid soils and although growth in liquid medium supports this theory, reisolations of M. gypseum were much higher in soils of pH 6.7 and 8 than pH 4. The apparent inability to reisolate M. gypseum from soils of pH 4 may be due to inhibition of enzyme activities under these conditions.

The 3 limiting factors of greatest importance at Gibraltar Point appear to be the paucity of keratin, the washing of sands

by sea water and the pH of the soils/sands. The factors affecting T. terrestre have already been discussed. Microsporum cookei was also able to tolerate sea water concentrations and this explains why it was able to grow at and below high water mark. Although M. cookei was thought to be stimulated by keratin in the hedgehog pens, this source of nutriment is not vital for the existence of the fungus in the soil, judging by the fact that the highest incidence of all species except M. cookei was in bird traps at Gibraltar Point, where there was a relatively large supply of keratin compared with other areas at this ecosystem.

Conidial A. uncinatum and M. gypseum were isolated in low frequency at Gibraltar Point. They are able to grow in sea water media, as were all the keratinophilic fungi, therefore the sea water seemed to play little part in determining the flora of the sands. Both species can grow in alkaline soils and therefore, keratin would seem to be the vital limiting factor. This was emphasised for conidial A. uncinatum in hedgehog pens, but not for M. gypseum.

Microsporum gypseum and T. terrestre were the only 2 species which could tolerate well the double strength sea water and they were readily reisolated from inoculated soils watered with sea water. Chrysosporium keratinophilum, in contrast, was the species most affected by sea water when attempting to reisolate it from soils; in fact, C. keratinophilum was not reisolated from inoculated sandy soils. This would explain why this fungus was only found in bird traps at Gibraltar Point. Conidial A. uncinatum was only isolated where the sands were not covered by sea water at any time. This can be explained by the fact that A. uncinatum was difficult to reisolate when soils were watered with sea water.

Although Pugh and Mathison (1962) reported that A. curreyi could tolerate salt concentrations more than Ctenomyces serratus

could, in the present work A. curreyi was only found in bird traps whereas C. serratus was found at both bird traps and high water mark. The effect of the concentrations of sea water, used in this present research, upon C. serratus and A. curreyi would be an interesting aspect to study.

In isolations from the farm soils, C. keratinophilum and conidial A. uncinatum were inhibited by sea water and T. terrestre was not.

Although an increase in tinea pedis was seen after 2 terms at the university, the incidence decreased again after 1 complete year to almost the initial incidence. One reason for the increase could be because those who played rugby, football, badminton and squash took more showers after sports than those who played tennis, athletics and swimming in the first 2 terms. Evidence to support this is supplied by the fact that the incidence of tinea pedis amongst the former players fell again in October 1973, but in the latter group, the incidence had increased. The showers in halls of residence and at the sports centre did seem to be an important source of cross-infection. Another reason could be because the weather is warmer in May than in October, and created a warmer environment for feet in enclosed shoes.

The removal of hosiery in summer months and the tendency to walk around in sandals can help to minimise tinea pedis in summer. In fact, this appeared to be true for the men, but since no such observation was made for women it was considered that, even though they had higher hygiene standards than men, they came into greater contact with tinea pedis in summer than the men did.

The incidence of tinea pedis was found to be greater in men than in women and it was interesting to note that male hedgehogs were infected with T. erinacei to a greater extent than female

hedgehogs were (Morris and English, 1969). The suggestion that women are just less susceptible to disease than men are, would seem to apply to hedgehogs too.

The relative incidence of T. mentagrophytes var interdigitale and T. rubrum has been discussed, but in this present work the former was isolated on most occasions (98%) followed by T. rubrum and E. floccosum (1% each).

It was interesting to note that both feet were less infected than one foot (26 : 76) and that the right foot was infected more than the left (42 : 34). Tentative suggestions have been made for this pattern of infection, but no definite conclusions were drawn.

When the nutrition of C. keratinophilum, T. terrestre and A. uncinatum was studied, the combinations of carbon and nitrogen which produced optimum growth for the 3 species were:-

C. keratinophilum

phenylalanine with fructose and glucose
proline with fructose
glycine and glucose
serine and glucose

T. terrestre

alanine with glucose and fructose
aspartic acid with fructose
proline with fructose and glucose

A. uncinatum

phenylalanine with fructose
aspartic acid with maltose
proline and glucose
serine with starch and fructose

Fructose and glucose were the 2 carbon sources which promoted optimum growth. Trichophyton terrestre and A. uncinatum

utilised maltose next in preference to sucrose, lactose or starch, whereas C. keratinophilum preferred sucrose and starch before maltose. None of the species could use lactose for good growth. Stockdale (1953) reported that dermatophytes were incapable of using nitrate nitrogen; these 3 species were not exceptions to this observation judging by the results of the present work.

Since A. uncinatum and T. terrestre were shown to produce acid when they were utilising fructose or glucose with NH_4NO_3 as the nitrogen source, they clearly have greater connections with ringworm fungi (Hopkins and Iwamoto, 1923) than C. keratinophilum does, since it did not produce acid.

Hydroxyproline and methionine were found to be the 2 amino acids least used by all 3 species.

Although no vitamins were added, growth did occur in most of the combinations of carbon and nitrogen sources. Therefore, it would seem that none of the 3 species was deficient for any vitamin or growth factors which were unsupplied.

An immediate drop in the percentage colonisation of wool baits by conidial A. uncinatum was seen after fungicide treatment of soils at Rothampsted, and this would be the expected result when it is known that, in vitro, conidial A. uncinatum was inhibited by all fungicides, at a concentration of 100 ppm. This species was also the most susceptible to fungicides at concentrations as low as 25 ppm, of all the keratinophilic fungi studied. The percentage colonisation recovered later (50 - 80 days) to be either virtually the same as the control or more. This can be explained by the fact that the fungicides would be leached from the soil and would no longer be in contact with the fungi. Keratinophilic fungi growing on solid media were able to tolerate the fungicides

better than in liquid media, probably because the fungi were not completely immersed by the medium. After the fungicides have filtered through the soils, the keratinophilic fungi seem to recover quite quickly, perhaps quicker than other soil fungi with which they might compete, and increase to a higher percentage colonisation on greasy wool than degreased wool. As this is the reverse of the normal isolation pattern, it would be interesting to compare the effect of grease on keratinophilic fungi, grease and fungicides on the fungi and the adsorption of the active ingredient in the fungicides by the grease.

The effect of the fungicides on the isolation of C. keratinophilum was difficult to determine because of fluctuations, but in vitro, C. keratinophilum has been found to be the most resistant to several fungicides. Trichophyton terrestre and M. cookei were also found to be quite resistant to fungicides in vitro, but they were not isolated very much from Rothampsted and Sutton Bonington control soils anyway.

Triarimol, a systemic fungicide active in the field against Venturia of apple and pear, was the least effective of the fungicides studied, while thiram was the most effective. Milcol, captan and formaldehyde inhibited all but one of the 5 species of keratinophilic fungi. Triarimol stimulated M. gypseum at low concentrations and even at 200 ppm for M. gypseum and 100 ppm for M. cookei, growth was not truly hindered.

Although Zentmyer (1955) found that materials selected as effective fungicides in screening tests in agar varied greatly in their performance in soil, this was not found to be the case in the present work. The results of spraying soils and in vitro work, using agar media, were in general agreement. If the fungi are in continual contact with the fungicide then the chances of inhibitions are greater, e.g. in liquid culture. This would also agree with the fact that in order to kill dermatophytes on feet,

contact with a solution of sodium hypochlorite, ~~e.g. in foot baths at swimming pools, is necessarily long~~ would need to be prolonged and why breaking off treatment too soon does not produce a cure of tinea pedis.

The fluorescent antibody technique has been found to be a useful tool in the ecological studies of conidial A. uncinatum, but not in a study of T. mentagrophytes in soil. Problems arose with non-specific staining in the latter case, but methods of overcoming this have been discussed.

Durie and Frey (1962) considered that only Gordon (1953) had given definite proof of the saprophytic existence of a dermatophyte, viz M. gypseum by finding macroconidia in the soil. Macroconidia of A. uncinatum have been seen in soil smears using the fluorescent antibody technique and using the spore flotation technique of Usunov (1967), therefore it would seem that the techniques have demonstrated the saprophytic existence in soil of A. uncinatum. Durie and Frey (1962) were uncertain whether the dermatophytes lead an active or dormant life in soil. If they were dormant then there would be a deep layer of keratin on the soil surface (Somerville and Marples, 1967), but on the other hand, Griffin (1960) thought that because the fungi seemed to be ecologically restricted to keratin in soil, it was difficult to imagine them able to survive on keratin alone, because there was so little in places where they were found, even allowing for long periods of dormancy.

Taking into consideration the fluorescent antibody technique, the results from the adaptation of McGinnis and Hilger's experiment, results from soil colonisation tubes and the influence of competition and fungistatic substances, it would appear that A. uncinatum, T. terrestre and C. keratinophilum were able to grow through soil to colonise a keratin bait and they were also able to survive in sterile or non-sterile soil

without keratin. Microsporum gypseum was, in addition to T. terrestre and A. uncinatum, capable of growing quite quickly through non-sterile soil to colonise a keratin bait, but C. keratinophilum and M. cookei took quite a long time. Trichophyton mentagrophytes on the other hand, was unable to grow through non-sterile soil with a keratin bait. A few contradictory situations arose, e.g. T. terrestre was able to grow through non-sterile soil without keratinophilic fungi, when keratin was present, but when other keratinophilic fungi were present, it was not so prolific. Chrysosporium keratinophilum was inhibited when growing over a non-sterile inoculum on Cellophane and yet, growth had been good in non-sterile soil in the other experiments, although this was not true in non-sterile soil in colonisation tubes. In addition, C. keratinophilum did not proliferate in sterile soil in the McGinnis and Hilger (1972) experiment, despite other evidence that it could grow in sterile soil. In the sterile soil, something destroyed by autoclaving could be the reason why C. keratinophilum did not proliferate and in fact, the non-sterile soil used in the colonisation tubes was first sterilised and then reinoculated with non-keratinophilic fungi. Therefore, whatever had been added to make the soil non-sterile, did not replace the influence which is necessary for good growth of C. keratinophilum. This may also prove to be the case with M. cookei, which also showed poor performance in colonisation tubes with non-sterile soil. However, this species was not studied in either the fluorescent antibody technique or by the McGinnis and Hilger experiment. Further work on this aspect would be required to determine more about the growth of Microsporum species in soil.

APPENDIX I

Fungal species isolated by the hairbaiting technique

* Acrospeira [Monodictys] levis

Alternaria tenuis

Arthrobotrys atra

* Arthroderma uncinatum

Aspergillus spp.

Botryotrichum piluliferum

Cephalosporium acremonium

Chaetomium sp.

* Chrysosporium keratinophilum

* Diheterospora catenulata

Fusarium sp.

Gliomastix murorum

Helminthosporium sp.

Humicola grisea

* Microsporum cookei

* M. gypseum

Mortierella sp.

Paecilomyces farinosus

Penicillium spp.

Rhizopus sp.

Sordaria sp.

Stachybotrys atra

Stemphylium botryosum

Thamnidium elegans

Thermomyces lanuginosus

* Trichocladium asperum

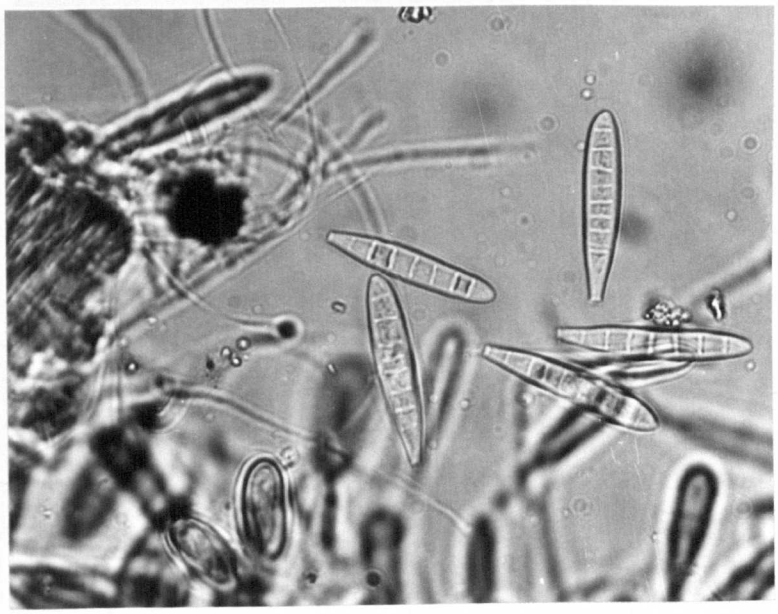
Trichothecium roseum

Trichophyton terrestre

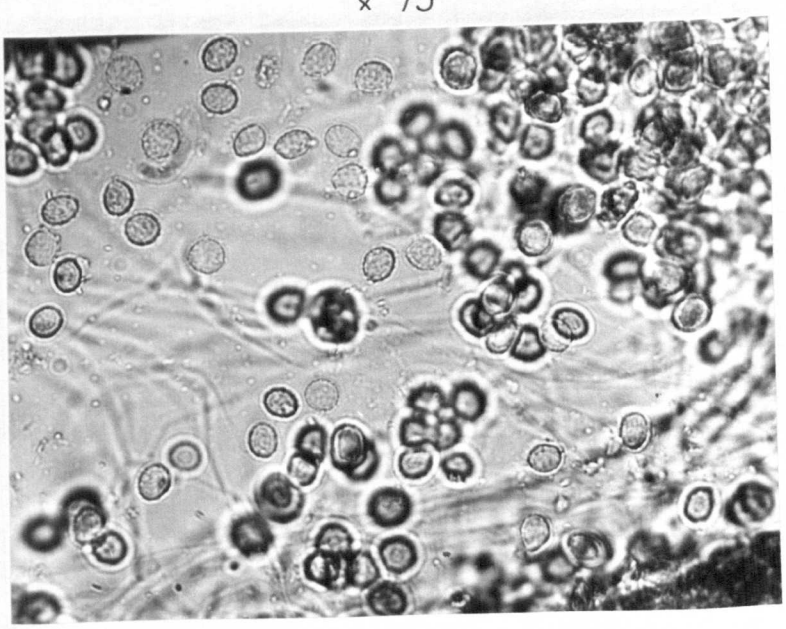
Verticillium albo-atrum

* See figures overleaf

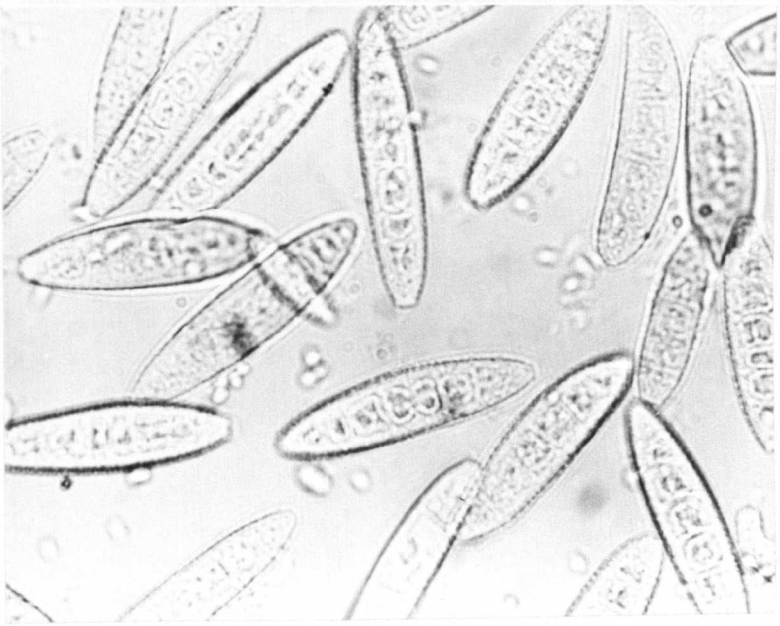
ARTHRODERMA UNCINATUM
× 75



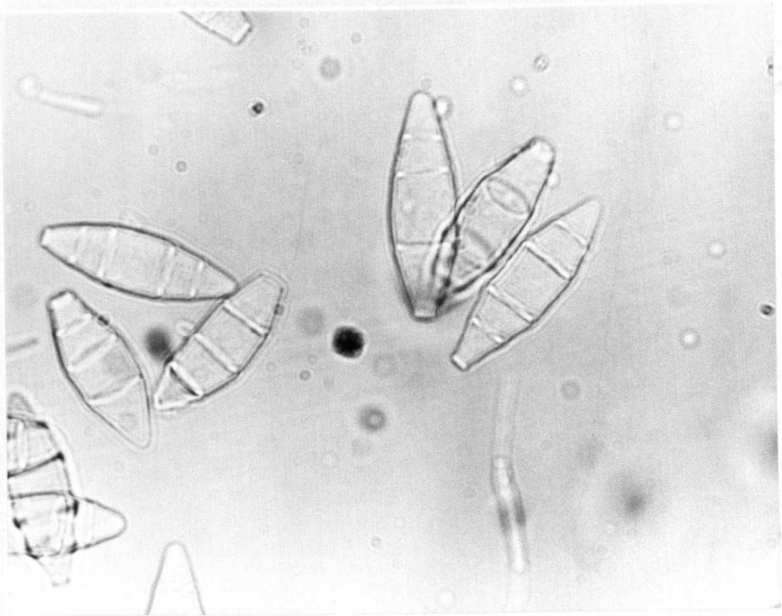
CHRYSOSPORIUM KERATINOPHILUM
× 75



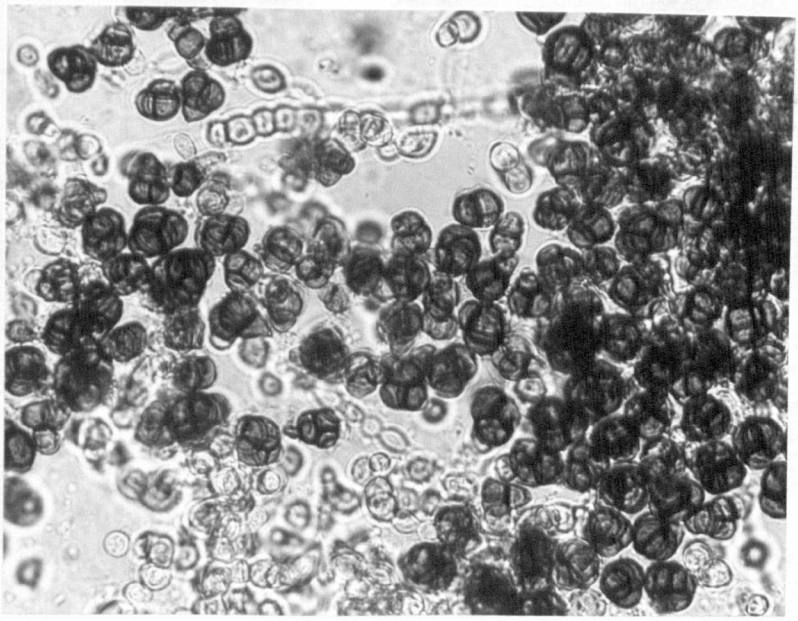
MICROSPORUM COOKEI
× 120



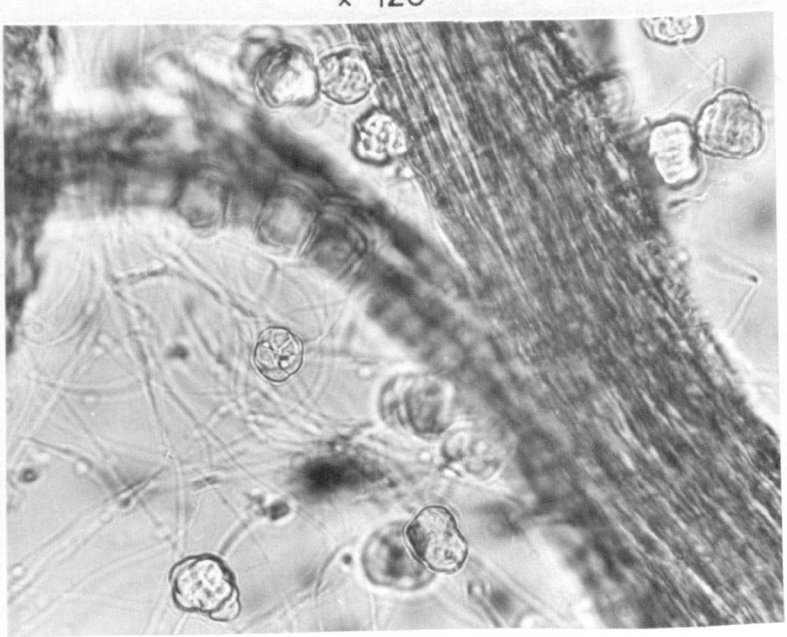
MICROSPORUM GYPSEUM
× 120



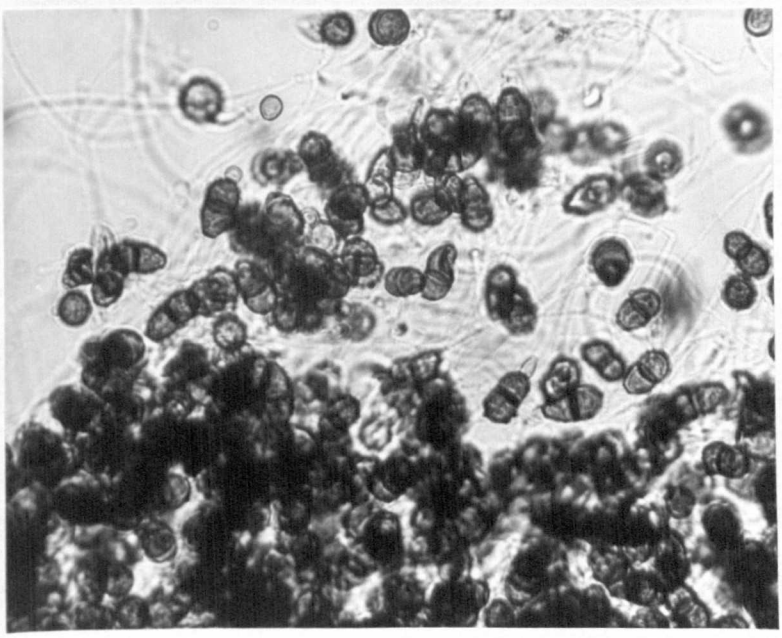
MONODICTYS LEVIS
× 75



DIHETEROSPORA CATENULATA
× 120



TRICHOCLADIUM ASPERUM
× 75



APPENDIX II

Fungal species isolated on soil crumb plates

Alternaria tenuis

Arthroderma uncinatum

Aspergillus spp.

Botrytis cinerea

Doratomyces stemonitis

Gliomastix murorum

Mortierella sp.

Paecilomyces farinosus

Penicillium spp.

P. lilacinum

Scopulariopsis brevicaulis

Trichocladium asperum

Trichophyton terrestre

Verticillium albo-atrum

APPENDIX III

Fungal species isolated from hedgehog nest material

Alternaria tenuis

Aspergillus spp.

A. niger

Epicoccum nigrum

Fusarium sp.

Mortierella sp.

Penicillium spp.

P. lilacinum

APPENDIX IV

Fungal species isolated from hedgehogs

Alternaria tenuis

Arthrobotrys conoides

Aspergillus spp.

Mortierella sp.

Penicillium sp.

P. lilacinum

Sporobolomyces sp.

Trichophyton erinacei

APPENDIX V

pH of soils

Rothampsted		Sutton Bonington	
Fungicide treatment	pH	Fungicide treatment	pH
Captan	6.3	Milcol	7.5
	4.5		6.7
	7.4		6.8
	5.3		6.5
Dicloran	6.1		6.1
	5.0	Dicloran	6.8
	5.9		6.9
	7.3		7.3
Formaldehyde	5.2		6.0
	6.8		5.9
	5.1	Captan	6.8
	7.3		6.7
Quintozene	5.6		5.3
	7.3		5.6
	5.2		6.4
	5.0	Triarimol	6.8
Thiram	5.2		7.9
	4.7		7.0
	5.8		5.1
	5.0		6.0
Control	4.9	Control	6.7
	5.1		6.8
	6.0		6.5
	5.5		6.1
			6.0

APPENDIX VI

Geophilic dermatophytes andtheir hosts.

Arthroderma curreyi

hedgehog	English (1964)
rodents marsupials	Rees (1967)
birds	Pugh (1964, 1965, 1972), Rees (1966 & 1967)

Arthroderma tuberculatum

man	Keddie, Shadomy and Barfatani (1963)
rodents marsupials	Rees (1965)
birds	Kuehn (1960), Carmichael (1962), Rees (1966 & 1967) and Hubálek (1970)

Chrysosporium sp.

wild animals	Knudtson and Robertstad (1970)
fox	Alteras, Nesterov and Ciolofan (1966)
rodents marsupials reptiles	Rees (1965)
birds	Watling (1963), Pugh (1965, 1966b, 1972), Rees (1966 & 1967), Pugh and Evans (1970a) and Hubálek (1974).

Ctenomyces serratus

marsupials	Rees (1965)
birds	Pugh (1964, 1965, 1966, 1972) and Rees (1966 & 1967)

Trichophyton ajelloi

cattle	Dvořák and Otčenášek (1964)
man	Vanbreuseghem, Ghislain and Wellens (1956), Rakhmanov, Fedorova and Yashkul' (1969), Refai and Ali (1970)
horse	Rieth and El-Fiki (1959), Pier and Hughes (1961), Dvořák and Otčenášek (1964)
squirrel	Alteras, Nesterov and Ciolofan (1966)
dog	Menges and Georg (1957a), Dvořák and Otčenášek (1964)
opossum	Marples (1961) and Smith (1968)
rat mouse	Smith (1968)
guinea pig	Bühlmann and Rieth (1962) and Dvořák and Otčenášek (1964)
marsupials	Rees (1965)
small mammals	Otčenášek and Dvořák (1962)
birds	Apinis (1964), Pugh (1966), Pugh and Evans (1970a) and Hubálek (1972)
vole bison	Knudtson and Robertstad (1970)

Trichophyton terrestre

horse	Dawson and Gentles (1961)
birds	English (1964), Pugh (1965, 1966), Pugh and Evans (1970a) and Hubálek (1970)
roebuck stag badger polecat mole	Alteras, Nesterov and Ciolofan (1966)

cattle buffaloes	Gupta, Singh and Singh (1969)
dog	Gip and Martin (1964) and Connole (1965)
antelope mink	Knudtson and Robertstad (1970)
cat	Connole (1965) and Gentles, Dawson and Connole (1965)
opossum rat mouse	Marples (1967)
hedgehog	Marples (1961), Marples and Smith (1962), English (1964)
small birds small mammals	Otčenášek and Dvořák (1963)

Microsporum cookei

birds	Rees (1967), Pugh and Evans (1970a) and Hubálek (1972)
man	Shik (1965), Schick (1966, 1968), Lundell (1969) and Frey (1971)
baboon	Mariat and Tapia (1966)
opossum	Ajello (1959), Marples (1961) and Smith (1968)
rabbit	Ajello (1959) and Rees (1965)
skunk racoon mouse	Ajello (1959)
rat	Ajello (1959) and Ridley (1961)
hedgehog	Smith (1968)
rodents marsupials reptiles	Rees (1965)
small animals	Londero (1962) and English (1967)

Microsporum gypseum

coati	Findlay, Roux and Simson (1971)
rodents	McKeever, Menges, Kaplan and Ajello (1958)
birds	Pugh (1966), Rees (1967) and Hubálek (1972)
parrot	Alteras and Cojocaru (1970)
rabbit	Weisbroth and Scher (1971)
monkey	Ajello (1953), Dvořák and Otčenášek (1964), Gugnani, Randhawa and Shrivastav (1971)
tiger fowl	Ajello (1953)
cattle	Gupta, Singh and Singh (1969)
horse	Ajello (1953), Thorold (1953), Kaplan, Georg and Fosnaugh (1956), Kaplan, Hopping and Georg (1957), Dvořák and Otčenášek (1964), Carter (1967), Connole (1967), Kaben and Ritscher (1968) and Gupta, Singh and Singh (1969)
hens tiger rabbit mouse	Dvořák and Otčenášek (1964)
chamois	Alteras, Nesterov and Ciolofan (1968)
donkey	Menges and Georg (1957) and Dvořák and Otčenášek (1964)
pig	Gierloff and Katić (1961), Dvořák and Otčenášek (1964) and Koehne (1972)
dog	Ajello (1953), Menges and Georg (1957b), Kaplan and Ivens (1961), Dvořák and Otčenášek (1964), Keep and Pile (1965), Fischman, Londero and Santiago (1966), Al-Doory, Vice and Olin (1968) and Gugnani, Randhawa and Shrivastav (1971)

cat	Kaplan, Georg and Bromley (1957), Kaplan and Ivens (1961) and Dvořák and Otčenášek (1964)
gerbil	Taylor, Radcliffe and van Peenen (1963)
rat	Smith, Menges and Georg (1957), Ridley (1961), Taylor, Radcliffe and van Peenen (1963) and Dvořák and Otčenášek (1964).
guinea pig	Dvořák and Otčenášek (1964), Gugnani, Randhawa and Shrivastav (1971)
man	Whittle (1954), Alsop and Prior (1961), Klokke (1962a & b), Stockdale (1961), Sonck and Lundell (1962), Kaben and Moldenhauer (1963), Smith and Marples (1964), Bensch and Gemeinhardt (1966), Gip and Hersle (1966), Chmel (1966), Herpay and Rieth (1966), Mata and Mayorga (1966), Immel (1967), Magalhães and Boesch (1968), Steinerová and Dubrovova (1969), Pöhler and Schönborn (1970), Chmel and Buchvald (1970), Kadlec and Podivinska (1970), Baran (1971) Simordova (1971), Schönborn and Pöhler (1972), Percebois and Vadot (1972) and Belukha and Lukyanova (1972).

APPENDIX VII

pH changes caused by A. uncinatum in media containing different carbon and nitrogen sources.

	No carbon		Fructose		Glucose		Lactose		Maltose		Sucrose		Starch	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Alanine	7.4	8.4	5.0	7.4	6.7	6.3	6.8	8.3	6.7	8.4	7.4	5.1	7.2	8.4
$(NH_4)_2SO_4$	6.4	6.0	5.9	2.1	6.3	3.2	6.4	5.9	6.4	5.5	6.4	5.4	6.4	5.2
NH_4NO_3	6.8	6.4	5.3	4.2	6.3	5.0	6.4	7.0	6.4	5.8	6.4	5.9	6.5	6.0
Arginine	8.8	7.9	6.2	7.0	6.7	6.2	7.2	7.1	7.0	7.1	9.6	8.4	9.5	7.9
Asparagine	7.4	8.0	5.4	5.9	6.4	7.7	6.5	5.0	6.3	7.6	7.7	7.9	7.3	7.2
Hydroxyproline	7.6	7.7	5.9	5.0	6.8	6.1	6.9	6.8	6.8	6.0	7.6	7.0	7.2	7.5
Aspartic acid	7.1	7.4	3.6	9.4	3.6	9.3	3.6	5.5	3.6	8.3	3.6	2.9	3.6	4.1
Cysteine	6.7	6.9	5.9	9.4	5.8	9.3	6.1	5.6	6.1	4.0	6.6	5.8	6.6	5.5
Glutamic acid	3.7	3.8	3.7	4.0	3.7	3.8	3.7	8.5	3.7	7.9	3.7	3.9	3.7	3.8
Glycine	8.2	8.8	5.6	8,6	7.4	8.6	7.6	8.5	7.5	8.9	8.4	8.7	7.9	8.6
Leucine	8.1	8.0	6.2	6.6	7.5	7.2	7.7	7.6	7.6	7.3	8.2	8.2	8.0	8.1
Methionine	8.0	7.6	7.5	6.0	7.5	7.0	7.9	7.3	7.8	5.1	8.0	7.5	8.0	7.3
Phenylalanine	8.0	8.2	7.3	6.9	5.7	7.1	7.8	8.2	7.5	8.1	8.0	8.1	8.0	8.2
Proline	8.2	8.3	6.6	7.1	6.4	7.4	7.0	6.6	7.0	7.5	7.9	8.5	7.0	8.5
Serine	7.8	8.0	7.0	7.7	7.4	8.4	7.6	8.5	7.5	7.6	7.7	8.1	7.7	4.9
Tryptophan	8.3	8.2	7.0	6.2	7.5	7.0	7.7	7.3	7.6	6.9	8.3	8.0	8.1	7.6
Urea	9.0	8.2	7.6	6.7	8.6	7.4	8.4	7.8	8.2	7.9	9.0	8.2	8.9	8.3
Valine	8.1	8.0	7.2	6.5	7.6	6.9	7.9	7.5	7.8	7.5	8.4	7.8	8.3	7.4

a = before growth, b = after growth

APPENDIX VII (continued)

pH changes caused by C. keratinophilum in media containing different carbon and nitrogen sources.

	No carbon		Fructose		Glucose		Lactose		Maltose		Sucrose		Starch	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Alanine	7.4	8.8	5.0	8.5	6.7	8.3	6.8	8.5	6.7	8.6	7.4	8.7	7.2	8.9
$(NH_4)_2SO_4$	6.4	5.0	5.9	3.4	6.3	3.3	6.4	4.8	6.4	5.4	6.4	5.4	6.4	4.7
NH_4NO_3	6.8	6.7	5.3	8.2	6.3	8.3	6.4	4.9	6.4	3.6	6.4	6.0	6.5	2.8
Arginine	8.8	8.8	6.2	5.6	6.7	6.1	7.2	8.5	7.0	8.5	9.6	9.0	9.5	8.6
Asparagine	7.4	8.0	5.4	7.5	6.4	7.6	6.5	7.8	6.3	7.7	7.7	8.1	7.3	8.0
Hydroxyproline	7.6	7.8	5.9	5.9	6.8	6.6	6.9	6.9	6.8	6.7	7.6	7.8	7.2	7.7
Aspartic acid	7.1	6.5	3.6	3.9	3.6	3.6	3.6	6,7	3.6	3.7	3.6	3.8	3.6	3.8
Cysteine	6.7	4.2	5.9	10.9	5.8	10.2	6.1	9.5	6.1	5.0	6.6	9.3	6.6	5.5
Glutamic acid	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.6	3.7	3.7	3.7	3.9
Glycine	8.2	9.0	5.6	8.2	7.4	8.0	7.6	9.0	7.5	9.0	8.4	9.0	7.9	9.1
Leucine	8.1	8.1	6.2	6.2	7.5	6.5	7.7	7.5	7.6	7.4	8.2	8.1	8.0	8.1
Methionine	8.0	7.5	7.5	6.1	7.5	7.1	7.9	7.1	7.8	7.1	8.0	7.5	8.0	7.5
Phenylalanine	8.0	8.3	7.3	5.9	5.7	7.7	7.8	7.8	7.5	8.2	8.0	8.4	8.0	5.1
Proline	8.2	8.3	6.6	5.9	6.4	4.8	7.0	7.5	7.0	8.2	7.9	8.3	7.0	7.6
Serine	7.8	8.7	7.0	8.0	7.4	7.7	7.6	8.6	7.5	8.5	7.9	7.7	7.7	7.7
Tryptophan	8.3	8.0	7.0	6.5	7.5	7.2	7.7	7.4	7.6	7.0	8.3	7.2	8.1	8.1
Urea	9,0	8.6	7.6	7.7	8.6	8.7	8.4	8.3	8.2	8.8	9.0	8.5	8.9	8.6
Valine	8.1	8.1	7.2	6.6	7.6	7.0	7.9	6.0	7.8	7.3	8.4	8.0	8.3	8.0

a = before growth, b = after growth

APPENDIX VII (continued)

pH changes caused by T. terrestre in media containing different carbon and nitrogen sources.

	No carbon		Fructose		Glucose		Lactose		Maltose		Sucrose		Starch	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Alanine	7.4	8.7	5.0	7.1	6.7	6.5	6.8	8.7	6.7	8.6	7.4	8.7	7.2	8.6
$(NH_4)_2SO_4$	6.4	5.2	5.9	3.0	6.3	3.0	6.4	4.9	6.4	3.6	6.4	4.5	6.4	4.4
NH_4NO_3	6.8	6.0	5.3	8.5	6.3	3.6	6.4	6.6	6.4	3.0	6.4	4.5	6.5	4.3
Arginine	8.8	8.9	6.2	6.8	6.7	6.7	7.2	8.4	7.0	7.9	9.6	8.1	9.5	7.7
Asparagine	7.4	8.0	5.4	8.0	6.4	8.2	6.5	8.0	6.3	7.9	7.7	8.1	7.3	8.2
Hydroxyproline	7.6	7.5	5.9	5.6	6.8	6.7	6.9	6.9	6.8	6.6	7.6	7.7	7.2	7.6
Aspartic acid	7.1	4.4	3.6	5.4	3.6	3.7	3.6	7.2	3.6	3.7	3.6	3.6	3.7	3.7
Cysteine	6.7	9.4	5.9	2.7	5.8	10.4	6.1	3.1	6.1	2.7	6.6	6.2	6.6	5.0
Glutamic acid	3.7	3.7	3.7	3.6	3.7	3.7	3.7	3.7	3.7	3.6	3.7	3.7	3.7	3.7
Glycine	8.2	8.6	5.6	8.1	7.4	8.6	7.6	8.6	7.5	8.8	8.4	8.8	7.9	8.9
Leucine	8.1	8.1	6.2	6.8	7.5	7.3	7.7	7.5	7.6	7.4	8.2	8.0	8.0	8.1
Methionine	8.0	7.7	7.5	5.7	7.4	7.2	7.9	7.5	7.8	7.4	8.0	7.7	8.0	7.6
Phenylalanine	8.0	8.5	7.3	7.2	5.7	5.5	7.8	8.4	7.4	8.2	8.0	8.4	8.0	8.3
Proline	8.2	8.3	6.6	7.0	6.4	6.4	7.0	7.5	7.0	8.3	7.9	8.5	7.0	8.3
Serine	7.8	8.7	7.0	7.0	7.4	7.7	7.6	8.5	7.5	8.7	7.9	8.6	7.7	8.6
Tryptophan	8.3	8.2	7.0	4.5	7.5	4.2	7.7	7.5	7.6	6.9	8.3	8.1	8.1	7.8
Urea	9.0	8.2	7.6	8.1	8.6	5.5	8.4	8.0	8.2	8.4	9.0	8.4	8.9	8.5
Valine	8.1	4.6	7.2	6.1	7.6	8.0	7.9	7.9	7.8	6.6	8.4	8.0	8.3	7.9

a = before growth, b = after growth

APPENDIX VIII

Media and solutions.

Supplemented Sabouraud's dextrose agar.

Dextrose	20 g
Peptone	10 g
Agar	20 g
Chloramphenicol	40 mg
Cycloheximide	500 mg
Distilled water	1000 ml

Modified DTM

Dextrose	20 g
Peptone	10 g
Agar	20 g
Chloramphenicol	40 mg
Cycloheximeide	500 mg
Distilled water	1000 ml
Phenol red	40 ml
0.8M HCl	6 ml

Ink blue medium

Dextrose	20 g
Peptone	10 g
Agar	20 g
Chloramphenicol	40 mg
Cycloheximide	500 mg
Ink blue	50 mg
Distilled water	1000 ml

Physiological saline

NaCl	0.9 g
KCl	0.01 g
$CaCl_2$ anhydrous	0.02 g
distilled water	100 ml

Phosphate-buffered saline

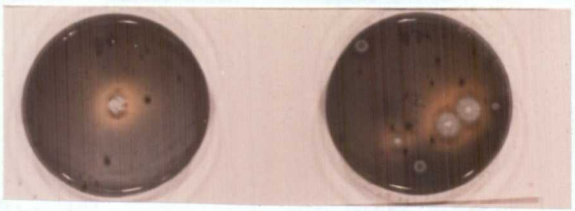
NaCl	8.5 g
Na_2HPO_4	1.07 g
$NaH_2PO_4 \cdot 2H_2O$	0.39 g
distilled water	1000 ml

Sea water K3

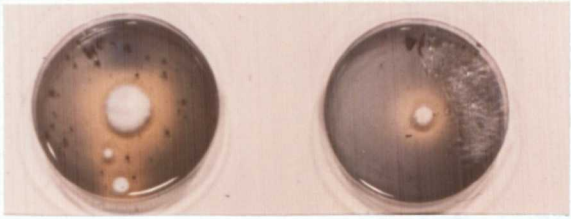
NaCl	360 g
Na_2SO_4	60 g
$MgCl_2 \cdot 6H_2O$	165 g
$CaCl_2 \cdot 6H_2O$	30 g
KCl	10.5 g
$NaHCO_3$	3.0 g
KBr	1.5 g
$SrCl_2 \cdot 6H_2O$	0.6 g
H_3BO_3	0.45 g
NaF	0.045 g
NaSi	0.075 g
distilled water	1000 ml

APPENDIX IX

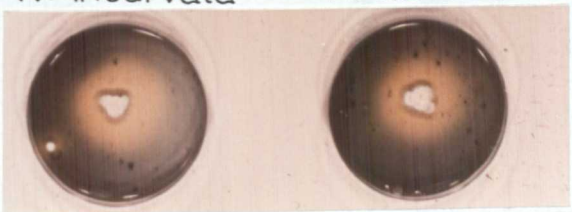
Dermatophytes which decolour ink blue medium



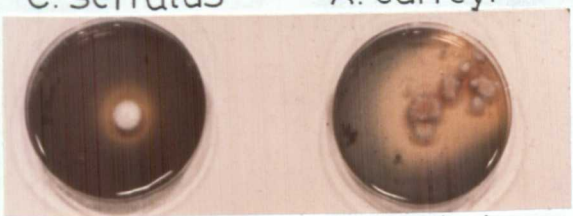
A. quadrifidum A. benhamiae



N. incurvata A. flavescens

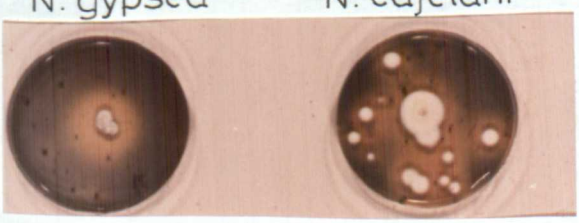


C. serratus A. curreyi



A. uncinatum M. cookei

N. gypsea N. cajetani



C. keratinophilum T. terrestre

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