THE MYCOFLORA OF FLEECE WOOL

A Thesis presented in partial fulfilment of the
requirement for the degree of
Master of Science in Microbiology at
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Soo Chin @ Soo Chun Lim
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SUMMARY

A study has been made of the variation in the total population of fungi within sheep fleece *in vivo* with time, and also of the types of moulds and yeasts present within this environment. Fungi were isolated by dilution plating and by the brush technique at the two temperatures of 25C and 37C. Considerably larger numbers of fungi were isolated at the lower temperature. There was no apparent correlation between fungal numbers within fleece wools and the environmental rainfall, either at the time of sampling or on a monthly average basis. The total population of moulds able to grow at 25C did, however, vary with the average monthly environmental temperature. No such correlation existed for moulds growing at 37C nor yeasts at either temperature.

Fungal numbers were unaffected by pretreatment of the sheep with commonly used insecticides variously applied, and seemed more related to general environmental conditions than to mode of pretreatment. Possible factors contributing to variations in the total fungal populations of fleece wools are discussed.

The types of fungi isolated from wools could be grouped into very frequently isolated species, e.g. *Alternaria alternata*, *Phoma spp.*, *Torulopsis candida*, frequent species, e.g. *Mucor racemosus*, *Aspergillus fumigatus*, occasional species, e.g. *Cladosporium herbarum*, *Epicoccum purpurascens*, *Fusarium oxysporum* and very occasional species, e.g. *Aspergillus niger*, *Geotrichum candidum*, *Peyronellaea glomerata*.

A study of the spatial distribution of the fungi within the fleece was made by an impression technique involving both microscopic and cultural examinations. Most fungi were present in the median parts of the staple and numbers decreased towards the tips and basal parts of the fleece. Again, the most frequent types included *Alternaria alternata*, *Fusarium culmorum* and *Phoma spp.*

Of the 68 isolated species, 19 were tested for their ability to degrade autoclaved and propylene oxide sterilised wool *in vitro*. While *Aspergillus niger* and *Penicillium canescens* were consistently unable to degrade either of the wools, most of the tested species degraded both autoclaved and propylene oxide sterilised wool. The degradation of wool by these fungi resulted in the release into the culture medium of cortical cells from the wool fibres. It is postulated

that degradation occurred as a result of the breaking down of cementing materials holding the cortical cells together and did not involve true keratin digestion. Wool degraded by fungi was densely stained by lactophenol cotton blue. It is suggested that this activity could be useful in estimating the ability of fungi to degrade wool, even though undegraded wool was lightly and irregularly stained.

As several fungi were shown to be capable of breaking down wool *in vitro*, further tests were made to determine which of these species are potentially able to grow within fleece *in vivo*. Of those able to grow at skin temperature and which were unaffected by the fatty materials present in wool, *Sordaria fimicola* and *Aureobasidium pullulans* were the two species most likely to grow in the natural fleece environment.

The significance of the work reported here is discussed. Many of the fungi commonly isolated from fleece wools have been reported to cause opportunistic fungal infections in man and animals and are also capable of spoiling refrigerated meats. Thus knowledge of the presence of these fungi in wools is necessary to help avoid problems in public health.

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INTRODUCTION

1. General

Today, as a result of work in many different countries, Veterinary Mycology is a rapidly expanding subject. Knowledge of the role of fungi in causing disease in livestock has greatly increased, but very few attempts have been made to enumerate those fungi associated with healthy animals. Even in the case of humans, it is only in recent years that the mycoflora of adult skin (Taylor, Henney and Ellis, 1973) and interdigital spaces (McGinnis, Rinald, Halde and Hilger, 1975) has been evaluated.

In the case of sheep, most mycological work has been done on actual cases of mycotic or suspected mycotic infections. (Ainsworth & Austwick, 1973). The disease 'Ringworm', for example, although not common on sheep, can be a cause of economic loss (Dawson, 1968). Other fungi, such as Peyronellaea glomerata can cause discoloration of the fleece (Mulcock, 1959) and it has also been suggested (Dempsey, Heath, Hughes & Vivian, 1972) that some member of the flora of sheep skin might be the cause of New Zealand 'Scatter Cockle' on pelts. Thus the mycoflora of wool includes species which may, in certain circumstances, be hazardous to the health of the live sheep as well as causing detrimental changes to the products of the sheep. But again there is a lack of information on the general mycoflora of wool and sheep skin and this was the initiative for the present study.

2. Wool and Sheep Skin As a Substrate For Fungal Growth

The fleece must serve as a natural trap for microorganisms from the general environment, and conditions within the fleece should theoretically be suitable for the growth of a number of fungi (Hayman, 1953; Fraser, 1957). But although the fleece might contain large numbers of deposited spores, the resistant nature of wool keratin and other factors, such as the presence of sebum, might restrict the growth of these fungi within this microenvironment. It is, therefore, necessary to examine the structure of keratin and its potential as a substrate for fungal growth.

2.1 Morphology of sheep skin

Most mammalian skins are similar in structure to human skin, which has been well described by Rothman (1954). Mammalian skin is divided into an outer, thin, cellular epidermis of ectodermal origin and an inner, relatively thick and fibrous, mesodermal dermis or corium.

The epidermis is a stratified structure divided into a horny layer, which is continually being worn away at the surface, and a germinal layer, the function of which is to replace the horny layer. Division in the basal germinal layer causes cells to move outwards to form a thick layer above known as the stratum spinosum because of the appearance given to the cells by intercellular bridges through which pass fine tonofibrils providing cohesion and reinforcement for the epidermis. Above this lies the horny layer.

The horny layer itself is subdivided into three strata representing progressive stages of cornification. The lowest stratum is known as the stratum granulosum because the cells contain granules of keratohyalin. Above this is the stratum lucidum, a clear layer from which the cell boundaries and nuclei have disappeared. The outermost layer is the stratum corneum, consisting of flattened, scale like cells composed of keratin. It is thus only on this outermost layer that fungal spores and fragments are likely to be deposited from external sources.

Beneath the epidermis is the dermis which consists of a dense felt-work of connective tissue in which bundles of collagenous fibres are intermingled with a mesh of elastic fibres (Ryder, 1973). The dermis contains hair follicles, sebaceous glands and sweat glands. Sebum, the secretion of the sebaceous glands, is a mixture of fatty acids and other lipids and is of interest due to its reported fungistatic properties (Baxter & Trotter, 1969). The sebum of sheep contains about 10% of free fatty acids, aliphatic monohydric alcohols (constituents of both monoester waxes and type 1 diester waxes), and a low proportion of triglycerides (Nikkari, 1974). Squalene, which is present in human sebum and has a marked fungistatic activity (Baxter & Trotter, 1969), is absent in sheep but sheep sebum has a higher content of cholesterol and other sterols.

2.2. Morphology of Wool.

The complete wool fibre consists of two parts, that outside the skin and that within the wool follicle where the fibre is undergoing the

final synthetic processes. Only the fully keratinized part outside the skin is here considered as wool and it is on this wool that fungal fragments are most likely to be deposited.

Wool consists of three distinct regions, the cuticle, cortex and medulla (Fig 1). The relative proportions of these three components vary widely from one type of sheep to another.

The cuticle cells (scales) form the outermost layer of the individual fibre and vary in relative size and arrangement in different fibres. The individual scales appear to be connected to one another but it is not known whether this is due to the presence of an intercellular cementing material or the interlocking of keratinous fibrils (Haselmann and Zann, 1951).

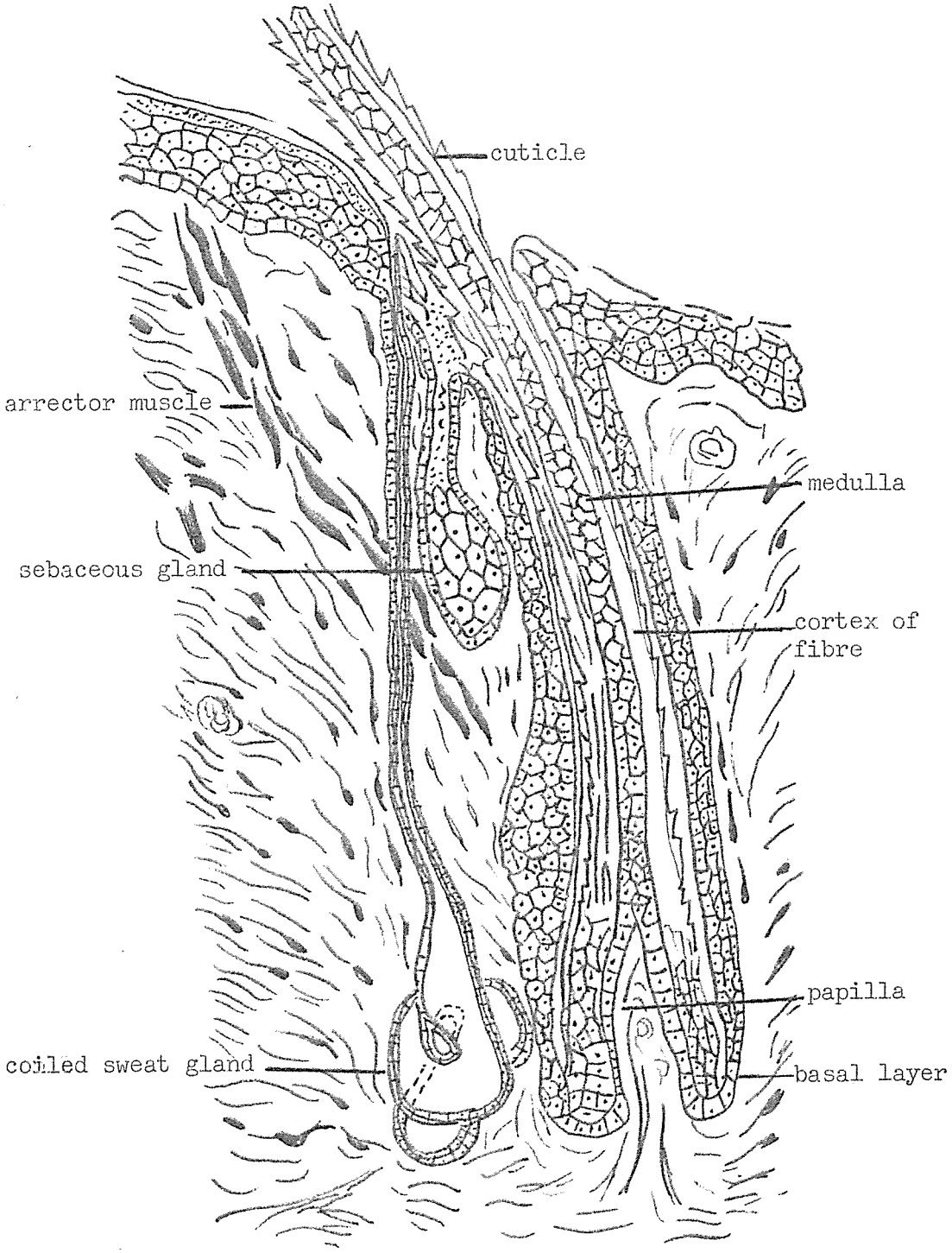
The cortical cells consist of longitudinal units which are of the order of 100 µm long and 4 µm wide and are fully keratinized. Each cell is composed of a number of fibrils which in their turn are made up of still smaller units termed subfibrils. Mercer and Rees (1946) have shown that it is these subfibrils which are the smallest structural units and suggest that they are held together within the actual fibril by a cement which can be slowly digested by enzymes such as pancreatin and trypsin. Although enzymes may only digest a very small proportion of the fibre, this limited attack is sufficient to enable the fibres to be split up into their morphological components.

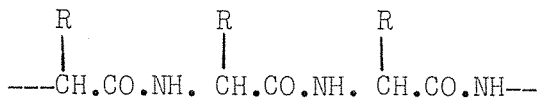
The medulla is a network of air-filled cell walls, or, in some cases a completely hollow tube. Most coarse wools contain a high proportion of medulla but in fine wool the medulla is absent (Alexander, 1963). Very little information is available concerning the chemical composition of the medullary cells, which are readily digested by enzymes which do not attack the cortex of unmodified wool (Baxter, 1968).

2.3 Structure of keratins

Keratins are a class of resistant, insoluble proteins found in the vertebrate epidermis and its appendages. The insolubility, toughness and elasticity of the protein is attributed to the presence of high molecular weight, long chain polypeptides with a large number of reactive side chains.

Fig 1 Longitudinal section, magnified 100x, of a wool follicle in a merino lamb, showing a growing medulated fibre. (Wool Science Review No 6, p 18; 1950).





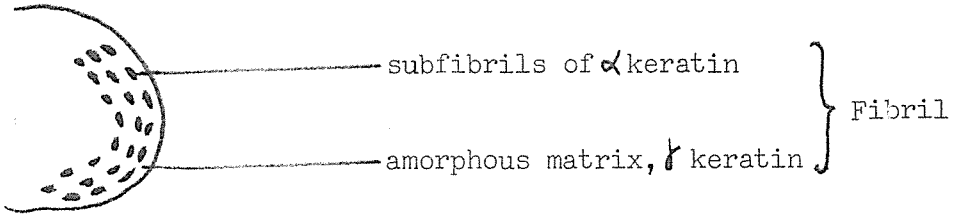
CHR.CONH-- = amino acid residue

R- = side chain

Keratinization is a method of hardening protein relying on the establishment by oxidation of cross-linkages between the cysteine residues in adjacent protein chains to form the characteristic disulphide link or cystine bridge. As well as the disulphide links, other interchain bonding must be present, particularly salt linkages and H-bonding. It is the numerous H-bonds and interchain cross-linking by the sulphur bridges of cystine residues that make keratins so insoluble and stable.

Most chemical analyses indicate the presence of the amino acids glycine, alanine, valine, leucine, isoleucine, phenylalanine, proline, serine, threonine, tyrosine, aspartic acid, glutamic acid, arginine, lysine, histidine, tryptophan, cystine and methionine in various sequences and groupings within the polypeptide chain.

Alexander and Hudson (1963) separated the fibre-forming (α) component (the subfibrils) and the non-fibrous S-rich (γ) component from solutions of wool oxidised with peracetic acid. The α-component consisted of filaments about 60-80 A diameter and indefinite length. This fibrous protein contains less cystine, serine, threonine and proline and more acidic residues than the whole fibre and probably has a molecular weight between 50,000 and 80,000. In wool it probably constitutes about 60% of the total keratin. The γ component is the matrix in which the filaments are embedded and consists of amorphous, shorter chain polypeptides of molecular weight about 10,000, with a higher content of cystine, serine, threonine and proline.



The various keratinized cells contain non-keratinous substances in different amounts, eg. epidermal cells contain 60-70% keratin and mature keratin of the cortex of wool contains all keratin. The non-keratinous components are nuclear and cytoplasmic remnants of proteins, enzymes, peptides, free amino acids, carbohydrates, glycogen, phenols, uric acid, lipids etc. Mineral and trace elements are also present in variety (Carruthers, 1962). Non-keratinous components of keratin have been reported to support the growth of a variety of fungi *in vitro* (Griffin, 1960), and Raubistchik (1961) has suggested that apparently keratinolytic fungi such as the dermatophytes have no need to utilise the keratin itself when invading hair *in vivo* but can grow simply on the non-keratinous components of the hair.

3. Mycology Of Sheep Skin And Wool

3.1 The association of microorganisms with wools and woollen fabrics.

Microorganisms have long been associated with the shorn fleece of sheep and with the spoilage of woollen goods (Barker, 1925). Before the introduction of fungistatic agents in the woollen industry, the principle problem was the 'mildew' which developed during storage and transportation. This condition was found to be caused by a combination of bacterial and fungal action, the organisms acting individually or conjointly depending largely on the moisture content of the wool (Von Bergen, 1963). Fungi, eg. species of *Aspergillus* and *Penicillium*, were the predominant organisms involved and developed on commercial goods which during storage had a 17-19% moisture content.

Burgess (1928) investigated the moulding of fabrics in storage and transit and stressed the role of the chemical agents used in the manufacture of woollen goods in rendering the fabric susceptible to moulding during storage. Looking for the sources of this contamination, Prindle (1935) studied the microbiology of raw wool and reported the changes in the number of bacteria and moulds during the processes of shaking and scouring. He reported that the mould count increased after shaking but that a large number of fungi were either washed off or killed in the scouring process. In contrast, the bacterial count increased throughout the shaking and scouring process but the total number dropped during the drying process.

The occurrence of discolouration and degradation of the fleece on the live animal by microorganisms, mainly bacteria, was investigated by Seddon (1937), Hayman (1953) and Fraser & Mulock, (1956). Pseudomonas aeruginosa, for example, was found to cause green and other discolourations of fleece wool. It has been reported that sheep whose fleeces are susceptible to bacterial discolouration also exhibit a skin which is sensitive to water (Fraser, 1957). The presence of water at the basal ends of the susceptible fleece produces an oedematous skin reaction, which ultimately results in the production of an exudate. Mulcock and Fraser (1958) demonstrated that the total bacterial count in the fleece of Corriedale sheep varied with rainfall. They reported that there was a significant difference between the numbers of bacteria in the fleece of sheep which had been selected as immune and susceptible to fleece discolouration. Mulcock (1959) reported a black discolouration of fleece wool as being due to the growth of a species of Peyronellaea among the fibres of the fleece. The black colour could not be removed with water and detergent nor extracted with solvents. Hyphae were found within the wool fibres, where pycnidia were also found overlying the cortex. Cuticular cells were apparently disrupted as a result of the fungal growth. Further reported examples of the growth of fungi within the keratinised portions of sheep skin are those concerning infections with dermatophytic fungi. The organisms, principally Microsporum canis, produce ramifying mycelium growing through the stratum corneum and occasionally invading the wool follicles (Dawson, 1968). Wool loss may occur and scales may form on the affected animal.

3.2 Degradation of wool by fungi

Early reports on the actual breakdown of wool and woollen fabrics provide conflicting reports, depending on the species of fungus tested and the pretreatment to which the wool had been subjected. Nevertheless, the ability of certain fungal pathogens to grow on human keratins provided a stimulus to investigations into the possibility of the transmission of skin diseases by the wearing of woollen garments.

Burgess (1924) failed to obtain growth of a variety of moulds on commercially scoured wool but in 1928 he did find a few strains of Aspergillus and Penicillium which fruited vigorously on such a substrate.

A variety of other imperfect fungi and yeasts showed less growth on the wool. Burgess called attention to the difficulties and uncertainties of such tests due to the hydrolysis of the wool caused by the discontinuous steaming process employed. Gould and Carter (1931) innoculated small squares of a sweated woollen athletic jersey with Trichophyton interdigitale and reported that the fungus remained viable for some months, although it did not grow on the wool. Berberian (1938), however, obtained good growth of T. interdigitale on autoclaved woollen squares which were kept moist. Hirschman and Humfeld (1940) found T. interdigitale capable of destroying squares of autoclaved wool blanket material when placed on various agar media and emphasized the practical importance of their findings, both in the possible transmission of 'Athlete's Foot', and in the possibility of the period of serviceability of woollen goods being shortened as a result of fungal degradation.

In more recent years the role of soil borne fungi in the natural breakdown of wool keratins has been extensively investigated. Karling (1946, 1947) studied certain chytrids with an ability to utilize keratin. The two species Rhizophydium keratinophilum and Phlytorhiza variabilis are strictly soil inhabitants and both were trapped on keratinous substrates and were found to grow on all common forms of keratin-containing tissue, including wool.

In 1950, White, Mandels and Siu investigated the ability of a few common saprophytic moulds and a series of human pathogens to degrade woollen fabrics. They measured the fungal activity in terms of decline in tensile strength. Degradation was accompanied by an increase in the pH of the medium. Relatively few species could attack woollen fabrics as compared with the range which can attack cotton materials. The investigations of White et al had some inherent drawbacks. One of them recognized was that the wool had been subjected to both industrial processing and autoclaving and hence was partially denatured. Macquade (1964) studied the ability of approximately 100 species of bacteria, Streptomycetes and fungi to decompose wool and developed a method for sterilizing the keratin substrate by rinsing degreased wool in unsterilized acetone, then twice through sterile water and incubating the wool in water overnight at 35C. The fibres were then drained and transferred to germination medium. The wool was removed from the germination medium after incubation, drained, rinsed in water, drained, rinsed in sterile acetone for 15 seconds and the acetone

immediately washed from the samples by rinsing twice in water. This tedious method was reported to avoid denaturation. Mcquade reported that isolates from widely differing orders in the 3 major groups of microorganisms disintegrated wool and suggested that the ability to decompose keratin may be a necessary, but not the only, condition for pathogenecity. Disintegration of the fibres to cortical cells was taken as evidence of damage.

The development by Vanbreusegham (1952) of the keratin-bait technique for the isolation of keratinophilic fungi from soil served as a stimulus for other workers throughout the world to study the occurrence of these fungi in soils and to perform epidemiological studies on the role of soil as a reservoir of pathogenic fungi. The mechanism by which fungi breakdown keratin substrates has also received detailed attention. Although Davidson and Gregory (1934) discovered the characteristic, peg-like, penetrating organs and pits produced on hair attacked by keratinophilic fungi, these structures were described in detail by Vanbreusegham (1952). Barlow and Chattaway (1955) reported that hair was attacked by keratinophilic fungi in stages, these being cuticle lifting, cortical erosion, the formation of penetrating organs and the colonization of the medulla. English (1963) studied the in vitro growth of 9 species of keratinophilic fungi on hair, hedgehog spine, nail and callus. She found that hair digestion is carried out primarily by means of flat fronds of eroding mycelium lifting the cuticular scales and then eroding the cortex. Perforating organs arise from the fronds and in their turn may produce further eroding mycelium growing longitudinally through the cortex.

Although the ability to actually utilise keratin as food material is limited to the truly keratinophilic species, other fungi are able to simply colonise such substrate. Frond-like mycellium, a reaction to the space available for growth (English, 1965), can be formed by a number of non-keratinophilic fungi on hair and other keratinous substrates. Such mycelium can lift the scales of the cuticle and grow longitudinally through the cortex between keratinized layers but they do not erode the keratin. From the frond-like mycelium narrow 'boring hyphae' may develop and penetrate the hair causing longitudinal cracks within the cortex (English, 1965), but again there is no evidence of digestion.

While truly keratinophilic fungi must be capable of actually digesting keratinous substrates as such substrates disappear after a few weeks incubation in in vitro experiments, there has been considerable difficulty in isolating keratinolytic enzymes from these fungi. Mathison (1964) stressed the importance of using purified and unmodified substrates in digestion experiments, for the non-keratinous components of keratins can play a major role in the colonization of these substrates. In 1963, he grew Keratinomyces ajelloi in liquid cultures containing animal wool* and found that after 10 days, the culture fluid became opaque due to the masses of cortical cells floating in it, but that after a further day or two these had completely disappeared and been replaced by little knots of hyphae. Cystine and peptides were demonstrated in the culture filtrates and Mathison considered the presence of these further evidence of the enzymatic attack of the substrate. Similar monitoring of the metabolic products released into culture media as a result of the growth of Microsporium canis and Microsporium gypseum in a basal salts solution containing kanamycin and wool (Peyton, Char & Cawley, 1965), indicated that keratinolysis by dermatophytes is a slow process, in contrast to the rapid keratinolysis observed by Noval and Nickerson (1959) with Streptomyces fradiae. Peyton et al suggested that the presence of keratinolytic activity does not imply the presence of one enzyme capable of such activity but that keratinolysis may be the result of a complex chain of reactions among which reduction of the disulphide linkages occurs.

The existence of an extracellular keratinase elaborated by the dermatophytes was established by Yu, Harman and Blank in 1968. They isolated and purified an extracellular keratinase of Trichophyton mentagrophytes var granulosum which readily digested unautoclaved white guinea pig hair, removing the medulla of the hair, and causing the appearance of many fissures in the cortex. From the mycelium of the same species two cell-bound keratinases were later isolated and purified. Immunodiffusion analysis showed that these two cell-bound keratinases were not identical to each other nor to the extracellular keratinase of the same species (Yu, Harman, Grappel & Blank, 1971).

* Chiroptic wool (Boot's Pure Drug Co. Ltd)

Investigations of the *in vitro* fungal colonization of keratins have therefore provided evidence that wool is potentially able to support the growth of a variety of fungi but the extent to which this occurs on the unshorn fleece is uncertain. Under suitable *in vivo* conditions only some of these fungi seem able to cause disease or damage. Such species would include the keratinolytic dermatophytes causing ringworm, an uncommon disease of sheep, and those such as *Peyronellaea*, able to grow in the moist fleece and causing discolouration and spoilage, again a very rarely reported condition.

4. Aims of Investigation

1. To determine the variations in the total fungal population of fleece wools with time.

2. To investigate any possible correlations between fungal population and macroenvironmental conditions, and to enquire into any possible effect of pretreatment of sheep with commonly used insecticides.

3. To demonstrate the variety of fungal species present in fleece wools *in vivo*.

4. To investigate the distribution of these fungi along the length of the wool fibres, and any possible variation in distribution with time of sampling and pretreatment of the sheep.

5. To study the ability of certain of the isolated fungi to degrade wool *in vitro* and to assess which of these selected species are most likely to grow in fleece *in vivo*.

MATERIALS AND METHODS

1. Source of Specimens

The specimens used in this study were obtained from a group of 68 sheep purchased by the Leather and Shoe Research Association (LASRA) in early April 1974, for continuing studies on the incidence and control of the skin disease of sheep known as "Cockle".

The sheep were predominantly Border Leicester-Romney cross hoggets which had been run on hill country near Shannon. They had been neither dipped nor shorn and comprised both prime and culled stock. On receipt by LASRA at Massey University these hoggets were weighed and drenched with 7.5 cm3 "Nilverm'* (drenching was repeated monthly). The sheep were then randomly assigned to one of the groups listed in Table 1. The individuals in each group were variously treated on 14.4.74, with a range of commercially available insecticidal compounds by either plunge dipping, shower dipping or tip spraying, some remained untreated and some were shorn.

2. Frequency of Sampling

Sampling commenced on 29.4.74 and was repeated twice weekly until 20.8.74 and thereafter monthly until the end of the experimental period on 10.12.74. Sheep from a varying number of the pretreatment groups were available at each sampling session. Details of the numbers of sheep sampled from each group are recorded in Table 2.

3. Sampling Procedure

3.1. Fleece Samples

At each sampling session portions of skin with fleece attached were obtained, as aseptically as possible with sterile scalpels and forceps, from the left upper shoulder and left upper leg of each sheep immediately after slaughter. After sampling, each portion was placed in a clean plastic bag and transported to the laboratory.

* "Nilverm" (4% levasmisole), I.C.I. (N.Z) Ltd., Wellington

Table 1: Initial treatment of the 68 experimental sheep from which samples were obtained

Number of sheep	Treatment	Mode of application
18	Untreated	-
7	Shorn	-
10	Benlate + (0.1% benomyl)	plunge
6	Diazospray * (0.04% diazinon)	plunge
17	'Diazotas' ** (0.04% diazinon)	shower
8	'Diazotas' (0.4% diazinon)	tip spray
1	'Trigon D F F'++ 1 : 1280	spray, left side only
1	water	hosed, left side only

* 'Diazospray'; Cooper, N.Z. Ltd., Auckland.

** 'Diazotas Dipspray & Jetting Fluid' TVL Ltd., Upper Hutt.

\+ 'Benlate'; Neill Croper Ltd., Auckland.

++ 'Trigon DFF; (100% Vcl-13) Cooper, N.Z. Ltd., Auckland.

Table 2: Frequency of sampling from treatment group

Date of Sampling (1974)	Untreated	Shorn	Benlate plunge	Diazospray plunge	Diazotas shower	Diazotas spray	Trigon D	F.F.
29.4	2	1			2			
14.5		1	2	3		2		
28.5	2	1			1		1	1
11.6			2	2		2		
25.6	2	1			2			
9.7		1	2			2		
23.7	2	1			2			
6.8		1	2	1		2		
20.8	2		2		2			
17.9	2				2			
15.10	2				2			
13.11	2				2			
10.12	2				2			

The wool was cut from the skin to within a few mm of the surface and the wool and skin portions processed separately.

All samples were processed as soon as possible but portions of skin and wool from each sample were kept at -12C to serve as a source for further reserch purposes if required.

3.2 Brush sampling

Prior to slaughtering at each sampling session each sheep was brushed with a circular plastic scalp-body massager* over the left shoulder and the left leg, the technique being that described by Baxter (1973). After sampling, the brushes used on each animal were placed in individual clean plastic bags for delivery to the laboratory.

3.3. Air sampling

Monthly samples of the air flora were obtained in the paddock in which the experimental sheep were grazed. The sampling site was approximately 200m from a large wool shed and 250m from a group of pine trees. Isolation of airborne fungi was performed using a Casella slit sampler (Plate 1), sampling 2 000l of air in 3 minutes.

3.4 Weather information

Recordings of rainfall and temperatures for the experimental period were obtained from the Meteorological Station at Massey University.

* "Nu-Brush No 2", Consolidated Plastics Ltd., Auckland.

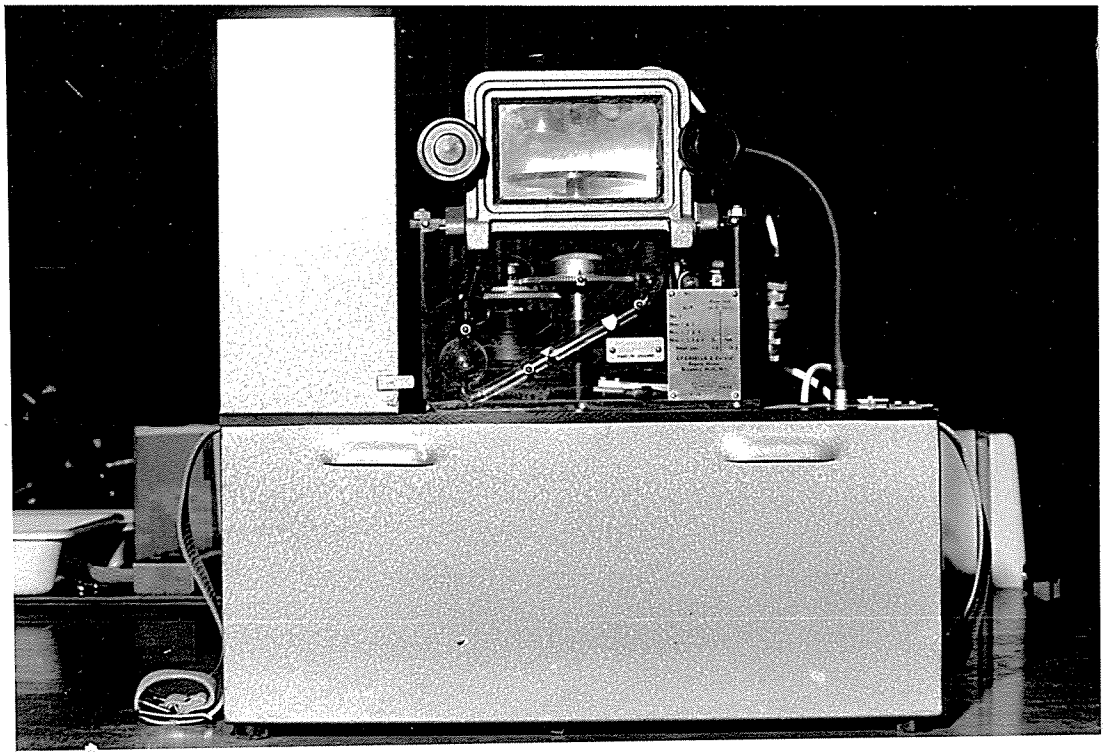


Plate 1: Cassella Slit Sampler

4. General Methods

4.1 Media used

Throughout the experimental period Sabouraud dextrose agar (Difco) containing 0.05% chloramphenicol (SDA) was used for the isolation of both moulds and yeasts.

4.2 Sterilization of materials

4.2.1 Sterilization of general equipment

All test tubes, flasks and bottles for culture media and aqueous solutions which were capable of withstanding a high temperature without decomposition were sterilised by autoclaving at 121C for 15 min.

Heat-labile solutions were sterilised by membrane filtration. The filter assemblies and cellulose acetate discs were sterilised in a pressure cooker at 121C for 10 min.

4.2.2 Sterilization of wool by autoclaving

When required for experiments studying the breakdown of wool, samples of wool were sterilised by autoclaving at 121C for 15 min.

4.2.3 Sterilization of wool by propylene oxide

Samples (5-10g) of wool were placed in glass petri dishes and exposed to propylene oxide in a sealed dessicating jar for 24 hours.

4.2.4 Sterilization of plastic brushes

The brushes, after use, (Methods 3.2) were sterilised by immersion in a 0.1% chlorhexidene solution* for 30 min. They were then cleaned with soap and running hot water. After drying, any residual fibres were picked off the bristles with forceps and the brushes wrapped in clean plastic bags ready for re-use. Occasional checks of the effectiveness of the disinfectant in destroying fungal fragments on the brushes were carried out by sampling cleaned, unused brushes, all with negative results.

4.2.5 Test for sterility of the wools

Small samples of wool sterilized by either autoclaving or propylene oxide were each placed onto SDA and incubated at 25C and 37C for 4-7 days. No growth on the agar plate was taken as indicating that the wools were adequately sterilised.

* "Hibitane", ICI (NZ) Ltd., Wellington

5. Isolation and Counting Techniques

5.1 Dilution plate samples

Standard dilution plating techniques were used to obtain quantitative estimations of the fungi on the wool samples. With the possible exception of some profusely sporulating species of Penicillium and Aspergillus, preliminary experiments indicated that 1:400 dilutions were sufficient to obtain a satisfactory separation of colonies for counting.

A known weight (approximately 1g) of each sample was shaken in $20cm^3$ sterile distilled water on a reciprocating shaker giving a 1cm throw, 5 throws/sec. After 30 minutes shaking, $1cm^3$ of the supernatant was transferred to $9cm^3$ sterile distilled water, mixed thoroughly and $0.5cm^3$ samples from this dilution plated out in quadruplicate onto SDA. The supernatant was allowed to soak into the medium before inverting the plates. Of the 4 plates, 2 were incubated at 25C and 2 at 37C. After 4-7 days the numbers and types of colonies appearing on each plate were recorded and subcultures onto SDA taken to facilitate species identification.

5.2 Brush samples

Each brush sample obtained prior to slaughter of the sheep (Methods 3.2) was used to innoculate 2 petri dishes of SDA. The cultures were incubated at 25C and 37C and examined for growth after 4-7 days. Colonies were subcultured for further identification as necessary.

5.3 Air samples

At each sampling session, a total of 4 plates of SDA were used. The plates were returned to the laboratory and 2 incubated at 25C and 2 at 37C for 4-7 days. The various colonies were then counted and subcultured for further identification as necessary.

6. Identification Methods

6.1 Microscopic examination

Slides for the microscopic examination of fungal growths were prepared according to standard methods, using lactophenol cotton blue as stain. To make semi-permanent preparations, nail varnish was applied between coverslip and slide (Dring, 1971).

Table 3: Fungi Selected for Experimental Purposes and their Taxonomy

Class	Order	Family	Genera	Species	Collection No
Hyphomycetes	Sphaeropsidales	Sphaeropsidaceae	Phoma	Phoma sp	DBM
			Peyronellaea	P. glomerata	V
	Moniliales	Moniliaceae	Aspergillus	A. flavus	3e
				A. niger	B
			Beauveria	B. bassiana	4D
			Paecilomyces	P. marquendii	2WH
			Penicillium	P. canescens	2DD
			Trichoderma	T. glaucum	6X
				T. viride	I
			Trichophyton	T. mentagrophytes	Tm
		De matiaceae	Alternaria	A. cheranthi	3X
			Aureobasidium	A. pullulans	2i
			Cladosporium	C. cladosporioides	2SG
			Pithomyces	P. chartarum	J
		Tuberculariaceae	Epicoccum	E. nigrum	3A
				E. purpurascens	WZ
			Fusarium	F. oxysporum	2r
				F. semitectum	3L
Pyrenomycetes	Sphaeriales	Sordariaceae	Sordaria	S. fimicola	Bmm
		Pseudoeurotiaceae	Pseudoeurotium	P. zonatum	7u

To facilitate the identification of certain species, where ordinary wet mounts disturbed the relationship between conidia and conidiophore, it was necessary to use the slide culture technique (Ajello, Georg , Kaplan & Kaufman, 1963).

6.2 Species Identification

Moulds were identified on the basis of their macroscopic and microscopic morphology on Sabouraud dextrose agar, Potato dextrose agar, Czapek-Dox agar or some other suitable medium. Identification, depending on the species, was according to Ainsworth and Sussman, 1973; von Arx, 1970; Barron, 1968; Benham and Miranda, 1953; Booth, 1971; Barnett and Hunter, 1972; Carmichael, 1962; Dare and Gunnell, 1969; Domsch and Gams, 1970; Ellis, 1968; Gilman, 1959; Raper and Fennell, 1965; Raper and Thom, 1968; and Smith and Brown, 1957.

Yeasts were identified according to the methods of Lodder, 1973, and Ainsworth and Sussman, 1973.

Strains from all sources were allocated a culture collection number. Those used for experimental purposes are listed in Table 3.

7. The Distribution of Fungi Along the Length of the Fleece

7.1 Impression technique

For this investigation wool fibres closely cut from the skin of sheep previously shorn, plunge-dipped in Diazospray or Benlate solution or sprayed in diazotas were used, as well as wool from untreated sheep. The length of the fibres varied from 2-200mm depending on the treatment group from which the samples were taken.

From each sample a small bundle of complete fibres (5-10mm thick) was gently pressed, using aseptic precautions, onto the surface of plain agar contained within a petri dish. A column, approximately 10mm x 70mm was ruled onto the petri dish base and marked B for basal end of the wool fibres, and T for the tip (apical end). The fibre bundle was gently rolled from one side of the column to the other to produce a more thorough impression. The wool fibres were discarded aseptically and the lid of the petri-dish replaced.

The impressed agar column was divided into 2 strips longitudinally with a sterile scalpel and each was equally subdivided into sections 20mm long.

7.1.1 Microscopic examination

The sections from one of the 2 strips were each separately transferred to a slide with the impressed surface upwards. The surface was flooded with lactophenol cotton blue and a coverslip applied. The slide preparation was left for 3-4 hours to allow the stain to diffuse into the agar before microscopic examination.

7.1.2 Cultural examination

The sections from the other strip were individually transferred to SDA in a petri dish which had previously been divided into 6 equal sectors marked on the base and labelled 1-6. The agar section impressed with the basal part of the wool was placed onto number 1 sector and the tip section onto number 6 sector, with the impressed surface downwards (Plates 8 & 9). These cultures were incubated at 27C for 4-5 days. The species of fungi growing from the impressed agar were then recorded.

7.2 Potassium hydroxide examination of skin material

Skin material was cut into thin sections using sterile scalpels and a few sections transferred to a slide, flooded with 15% KOH and a coverslip applied. The preparation was left for a few minutes to allow the KOH to soften the tissues, before microscopic examination.

8. Estimation of Ability of Fungi to Grow on Wool

8.1 Selection of isolates

19 fungal species were selected from all the fungal isolates recovered from the wool samples (Table 3). The isolates were representative genera from the 4 families of the Class Hyphomycetes and also Sordaria fimicola, an Ascomycete. Selection of the fungi was on a random basis although some species have been previously reported to be able to degrade keratins. Trichophyton mentagrophytes was included as an expected positive control in all experiments.

8.2 Degradation of wool

All species selected were examined for their ability to breakdown both autoclaved and propylene oxide sterilised wool in 3 different media. The media used were water-wool liquid medium (0.2 g wool in 5 cm^3 water), a weak yeast-extract-wool liquid medium (2 drops of yeast extract added to the water) and a plain agar-wool medium (bundles of wool pressed into the agar surface of a poured plate).

After innoculation, all cultures were incubated at room temperature and portions of wool from each culture examined after 2 weeks and 4 weeks incubation periods.

8.3 Assessment of degradation

Positive degradation was assessed as the breakdown of the intact structure of the wool fibres along their length, such breakdown resulting in the liberation of cortical cells into the medium (Plate 2).

9. Additional Studies

9.1. Colonisation of human hair

All the 20 species of fungi (Table 3) were also tested for their ability to colonise human hair and to determine if either perforating organs or boring hypae (English, 1965) were formed.

Bundles of short lengths of fair human hair, obtained from a healthy male, were placed in a weak yeast extract liquid medium in glass petri dishes and autoclaved. After sterilisation each was innoculated with one species of test fungus. All cultures were incubated at room temperature and observed for hair colonisation at 2 weeks and 4 weeks incubation periods.

9.2. Ability of fungi to grow at skin temperature

The ability of each of the fungal species used in the degradation experiments to grow at skin temperature was determined by incubating cultures on SDA at 37C for 7 days. The area of growth and the total area of agar surface was then measured. Ability to grow at skin temperature was then assessed as the ratio, area of fungal growth : area of agar surface.

9.3 The effect of wool fats on fungal growth

Wool samples, cut to convenient dimensions, were weighed and Soxhlet extracted for 24 hours with diethyl ether. Excess ether was removed by distillation, the remainder by evaporation, and the extracted fat weighed.

The plate technique of Baxter and Trotter (1969) was used to estimate the effect of wool fats on the growth of the selected fungi. The required amount of fat to make a final concentration of 0.2% in agar was dissolved in a minimum quantity of diethyl ether.

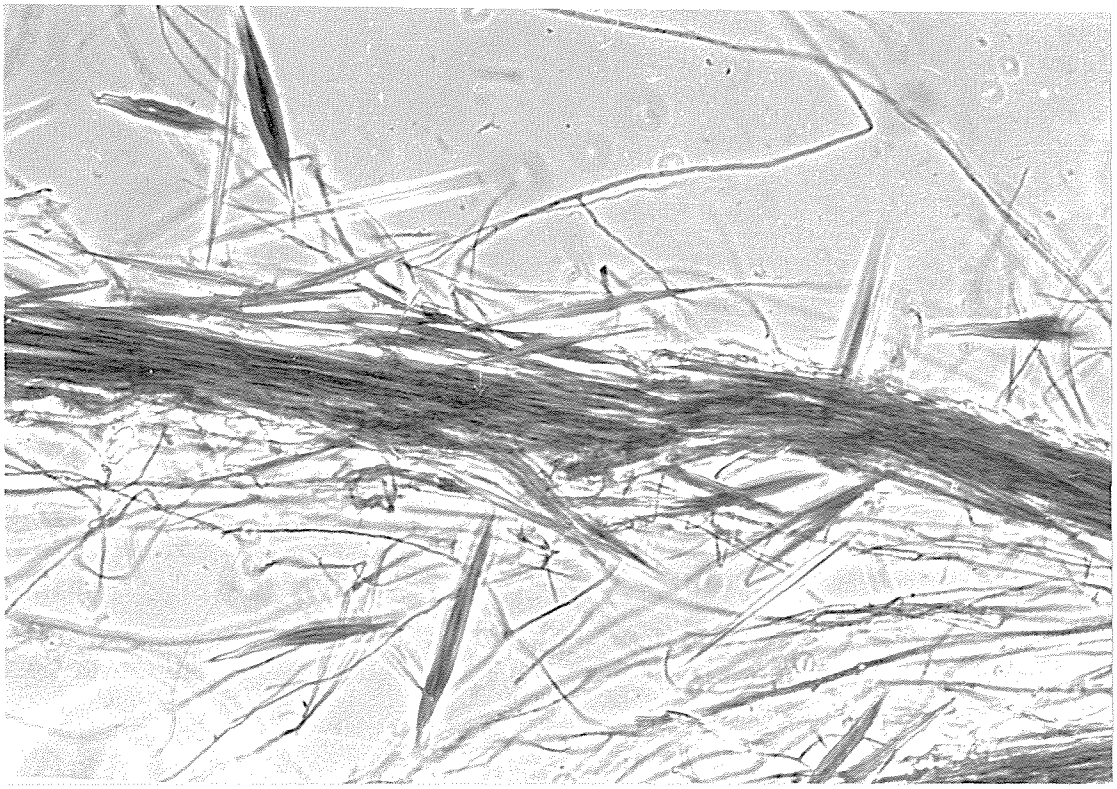


Plate 2: Liberation of cortical cells from a wool fibre following the *in vitro* growth of a fungus (*Trichophyton mentagrophytes*) on the wool

The solution was added to molten, but cooled, SDA. The agar was left to stand before autoclaving, until the ether had evaporated. Using sterile technique, half the medium of a set poured plate of SDA was removed and replaced with SDA containing the test fat. After setting, the plate was innoculated at the mid point with the test fungus and incubated at 25C for 1 week. The radii of fungal growth from the point of innoculation were measured on both the control (Rc) and the fat (Rf) sides. The inhibition could then be estimated:

$$\% \text{ inhibition} = \frac{Rc - Rf}{Rc} \times 100$$

9.4 Proteolytic activity of the selected fungi

The technique used was that of Frazer (1923) as modified by Baxter (1968). Three plates, each containing 15 cm^3 of sterile nutrient agar containing 4% gelatin were innoculated with each of the 20 selected fungi. The plates were incubated at 27C. After 5 days the plates were flooded with a solution of $HgCl_2$ 15g, conc HCl 20cm^3 and water 100cm^3. A clear region around the colonies indicated that the gelatin had been hydrolysed. The radii of the colony and the extent of gelatin hydrolysis were measured and an estimation of proteolytic activity was obtained as

$$\text{Proteolytic activity} = \frac{\text{Extent of hydrolysis}}{\text{Colony radius}}$$

RESULTS

1. The Fungal Population of Fleece Wool

1.1 Variations in total population of fleece wool with time of sampling

Mould and yeast counts for wools obtained at each sampling period from 29.4.74 until 10.12.74 (Methods 2) are recorded in Table 4. Counts are expressed as the average colony forming units (C.F.U.) per gram wool and are recorded separately for the two incubation temperatures of 25C and 37C. The average counts were determined from samples obtained from the 4 to 8 sheep available at each sampling date. Two samples (shoulder and leg) had been obtained from each animal but as there was little variation between these sites, the average count was determined for each animal.

1.1.1 Incubation temperature 25C

At 25C mould counts tended to be lowest during the winter months June-October and highest during the autumn and summer months of April, May, November and December. Counts varied from 7 595 CFU/g wool in December to 1 162 CFU/g wool in July. More moulds were isolated at 25C than at 37C throughout the experimental period. Yeast numbers were lowest in late July, early August, and from October to December. The highest count of 108 662 CFU/g wool occurred in mid May, the lowest in October (3 051 CFU/g wool).

1.1.2 Incubation temperature 37C

At 37C mould counts were highest during April, May and June (924-4 474 CFU/g wool) but fell to zero in July. During the remainder of the experimental period the counts rose again, but to a relatively lower level (maximum 780 CFU/g wool in August).

Yeasts developing at 37C showed greatest numbers during April and May and also in August when a very high count of 633 190 CFU/g wool for one sample was recorded. For most of the experimental period counts at 37C were low. No yeasts were recorded in July, October, November and December at this temperature.

1.1.3 Average fleece count

The average counts for moulds and yeasts able to grow at 25C over the whole experimental period were 3 643 CFU/g wool and 28 923 CFU/g wool respectively (Table 4). The average fleece count at 37C was 825 CFU/g wool for moulds and 11 262 CFU/g wool for yeasts.

Table 4: Variation in average mould and yeast populations of wool samples with time

Date of sampling (1974)	No. of sheep	Average CFU/g wool at 25C		Average CFU/g wool at 37C	
		Moulds	Yeasts	Moulds	Yeasts
29/ 4	5	4 408	52 716	924	3 764
14/ 5	8	5 689	108 662	4 474	37 212
28/ 5	6	2 950	17 747	1 286	3 268
11/ 6	6	2 538	73 154	1 103	7
25/ 6	5	2 180	9 284	448	44
9/ 7	5	1 162	24 788	0	0
23/ 7	5	2 580	4 334	0	0
6/ 8	6	3 488	4 445	780	13
20/ 8	6	3 330	62 863	448	105 531
17/ 9	4	4 212	5 894	575	130
15/10	4	3795 ~~1 854~~	3 051	248	0
13/11	4	5 370	5 808	168	0
10/12	4	7 595	3 248	265	0
Average count (all samples)		~~3 643~~ 4 159	28 923	825	11 537

The variations of mould counts at individual sampling dates was widest in samples taken on 13.11.74 for moulds growing at 25C. The counts varied from 860 CFU/g wool to 9 670 CFU/g wool. At 37C the widest range of counts was of those obtained from samples taken on 14.5.74 The widest range of yeast counts at 25C (190 CFU/g wool to 183 280 CFU/g wool) was obtained from samples taken on 14.5.74 and from samples taken on 20.8.74 and incubated at 37C the counts ranged from 0 CFU/g wool to 633 190 CFU/g wool. The smallest range of mould counts at 25C (40 CFU/g wool to 2 000CFU/g wool) was obtained from samples taken on 9.7.74 and at 37C, counts of from 40 CFU/g wool to 480 CFU/g wool were obtained from samples taken on 10.12.74. For yeasts the smallest range was obtained from samples taken on 6.8.74, the counts being 3 420 CFU/g wool to 4 780 CFU/g wool at 25C. At 37C the smallest range of yeast counts was 0 CFU/g wool to 30 CFU/g wool from samples taken on 11.6.74.

1.2 Variation of fleece count with macroenvironmental conditions

1.2.1 At sampling time

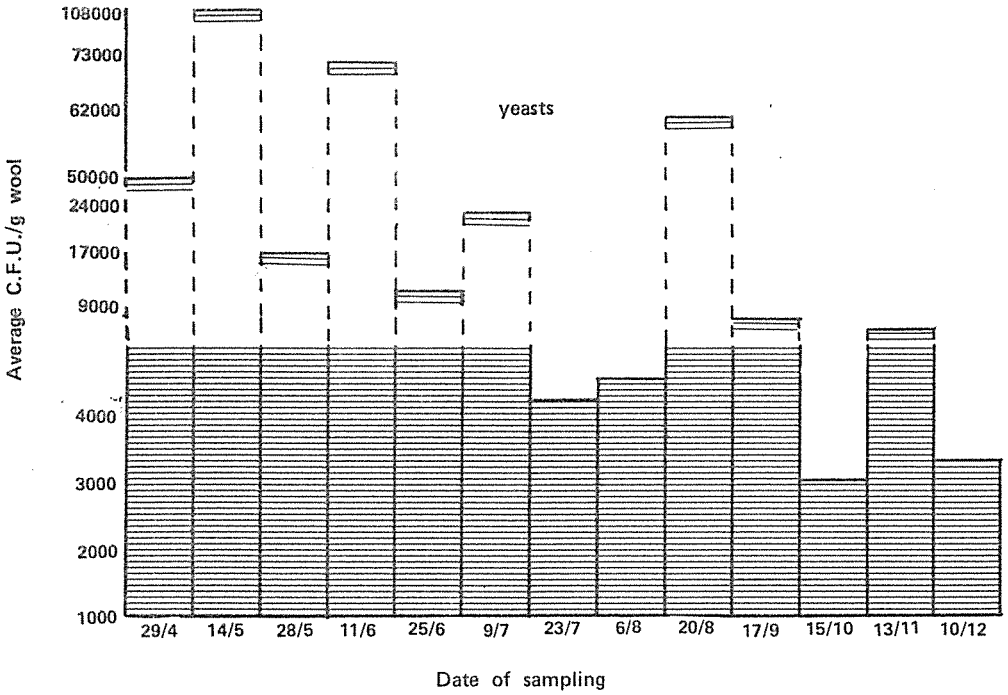
The counts of moulds and yeasts at each sampling period are recorded in Fig 2-1 and Fig 2-2, together with rainfall and temperature information. Both rainfall and temperature levels are averaged over 3 days (day of sampling and the 2 preceeding days). There was no apparent correlation between mould or yeast counts and either rainfall or temperature over the 3 days preceeding sampling.

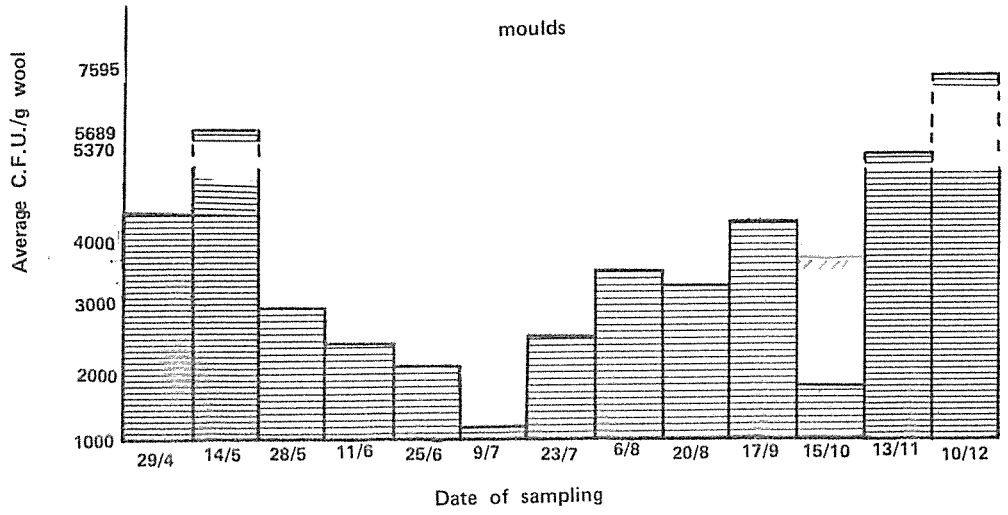
1.2.2 Monthly averages

Average monthly counts of moulds and yeasts are illustrated in Fig 3-1 and Fig 3-2, together with the monthly average rainfall and temperature. Again, there appeared to be no consistent variations in fungal counts with rainfall, but, in general, higher counts of moulds able to grow at 25C were obtained when rainfall was low (i.e. below 4mm). When the rainfall was very high (July), the mould count was lowest. At 25C, the mould count declined with falling temperature from April to July and then increased steadily with increasing temperature from August to Decemoer except in October when the count was low. There was no consistent difference in yeast counts at 25C.

Mould counts at 37C showed a steady decline over the whole experimental period (except a zero count in July) and showed no relation to environmental temperature. The yeast counts recorded also seemed to have no relation with the rainfall and temperature. No yeasts were demonstrated in July, October, November and December when samples were incubated at 37C.

Fig 2-1: Variation in average mould and yeast populations of wool samples with macroenvironmental conditions at time of sampling (25C)





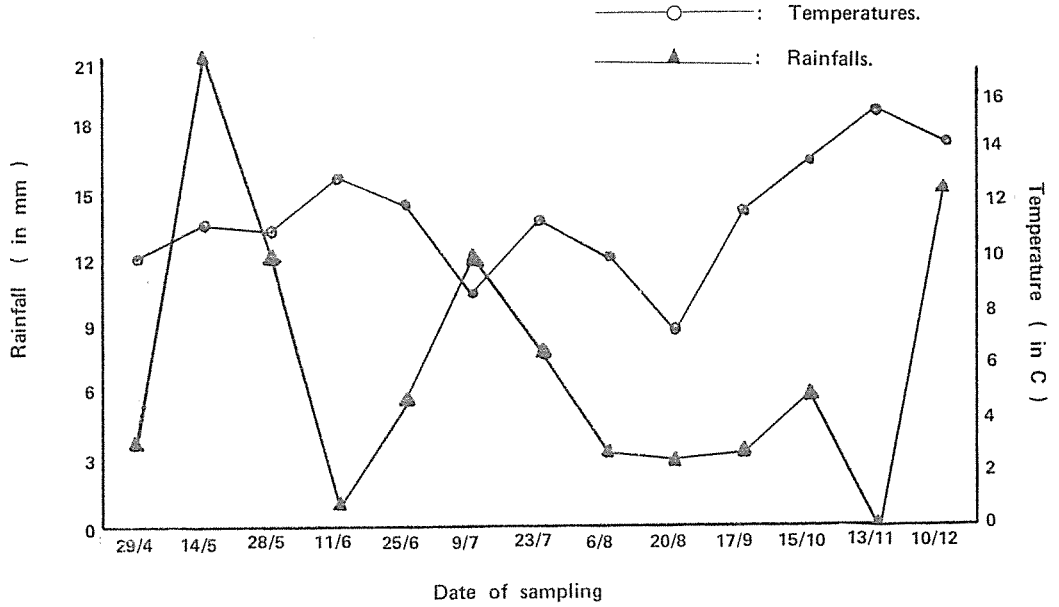
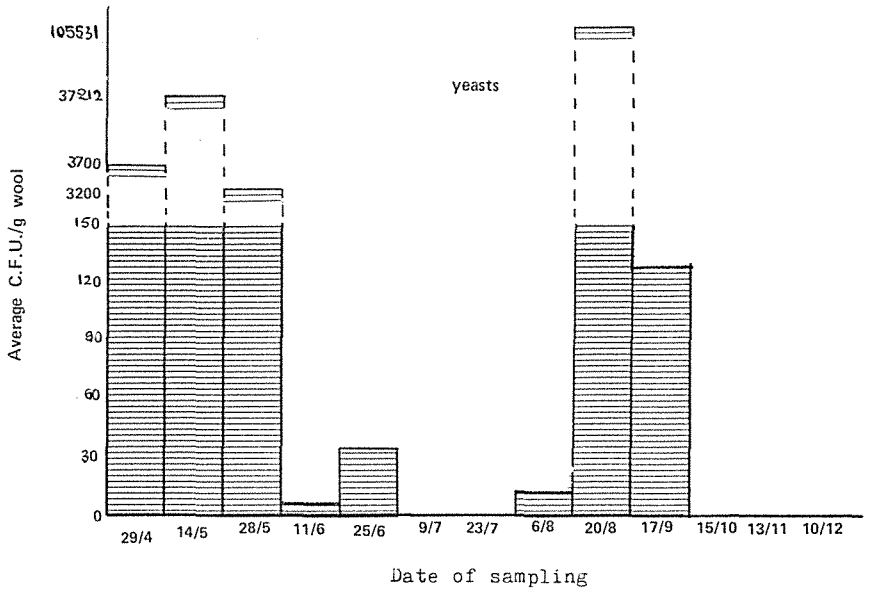
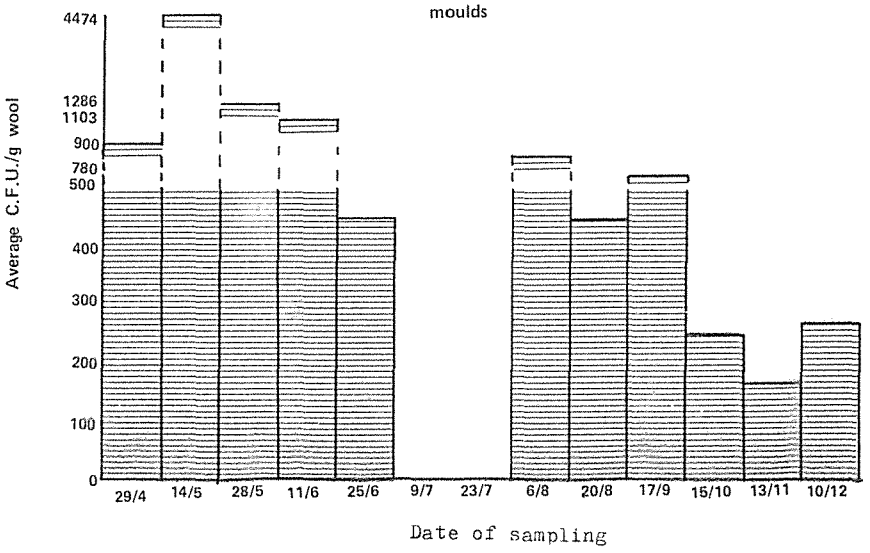


Fig 2-2: Variation in average mould and yeast populations of wool sample with macroenvironmental conditions at time of sampling (37C)





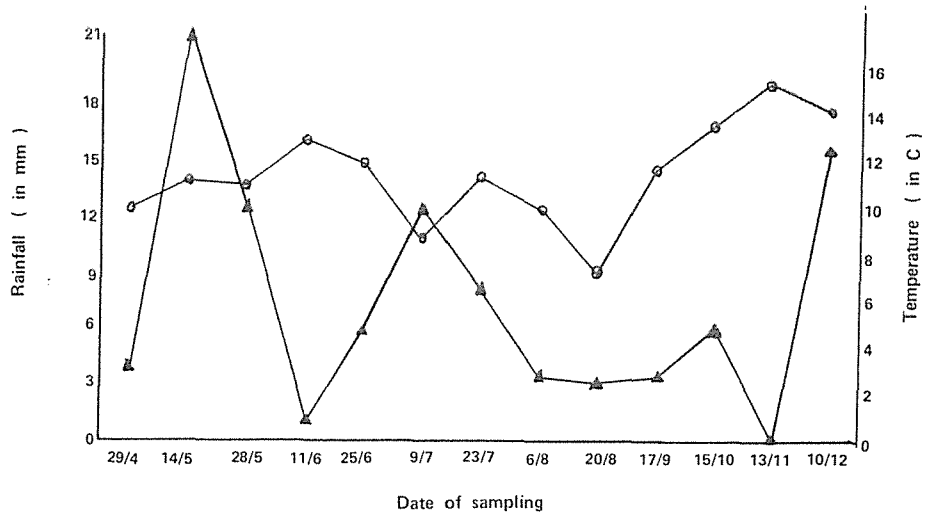
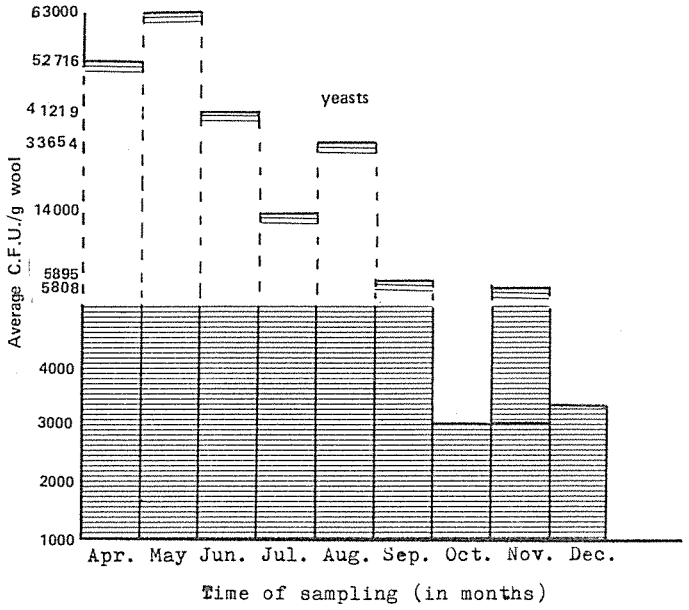
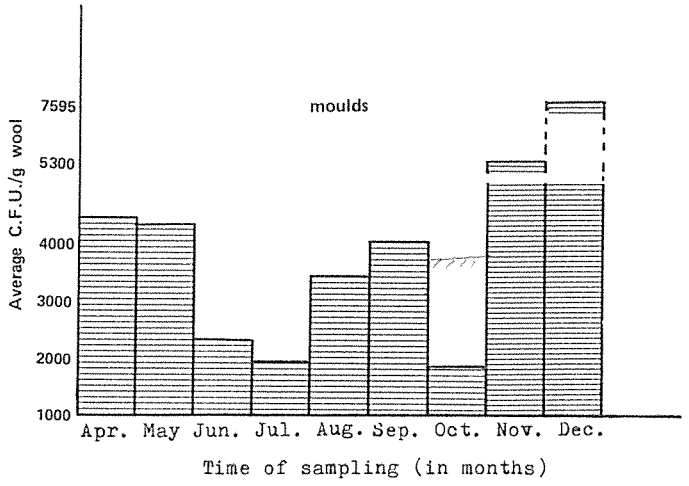


Fig 3-1: Variation of monthly average populations of moulds and yeasts with the macroenvironmental conditions (25C)





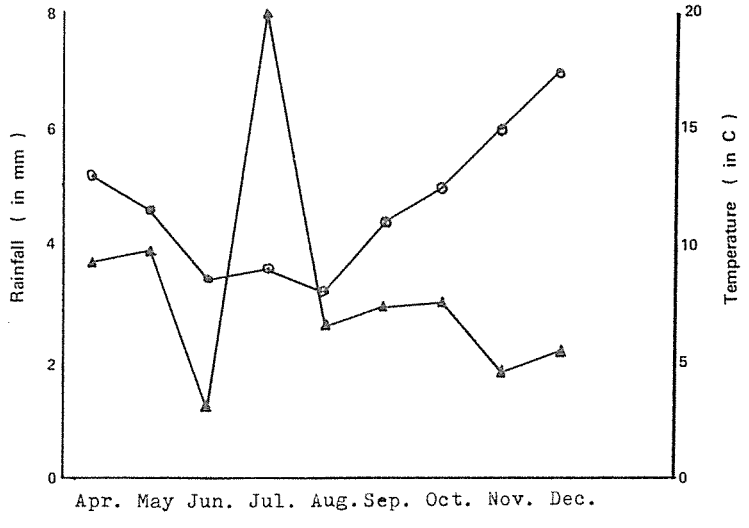
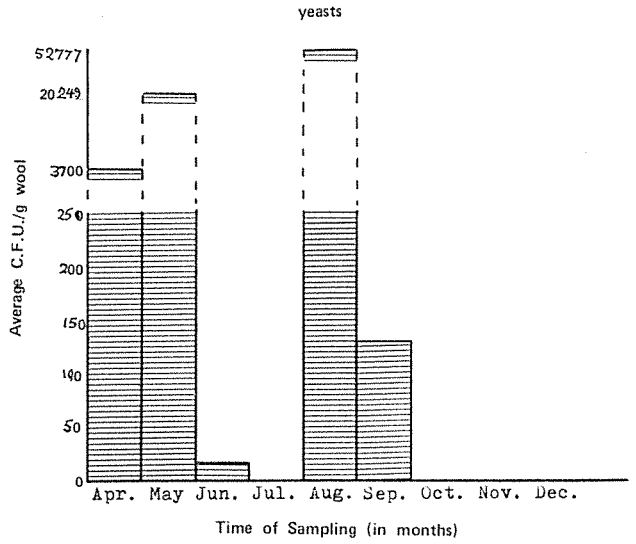
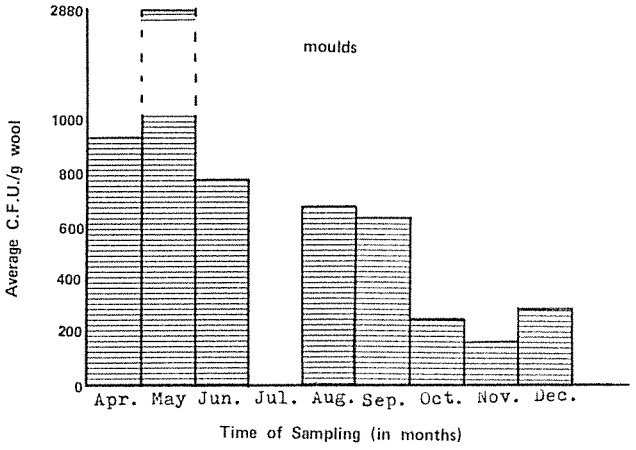
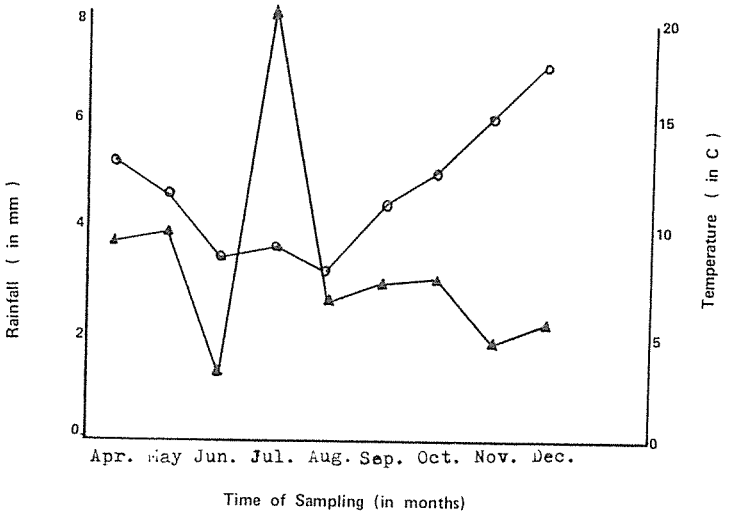


Fig 3-2: Variation of monthly average populations of moulds and yeasts with the macroenvironmental conditions (37C)







1.3 Variation in total population of wools with different pretreatment of the fleece

1.3.1 Untreated sheep

During the experimental period from April to December the average total mould counts recorded from the washings of untreated wools incubated at 25C varied between 1 300 and 5 000 CFU/g wool (with a mean of 3 551 CFU/g wool) (Fig 4f).

The numbers of yeasts obtained at 25C varied between 3 000 and 14 000 CFU/g wool (with a mean of 6 450 CFU/g wool) (Fig 5f). Lower counts were recorded towards the end of the experimental period.

There were fewer fungal colonies (0-1 700 CFU/g wool) developing at 37C (Fig 6f). In the case of yeasts developing at 37C the only positive samples were obtained in May (6 weeks post treatment: 1 760 CFU/g wool) and June (10 weeks post treatment: 10 CFU/g wool) (Fig 7f).

1.3.2 Shorn sheep

The total counts of fungi recorded from shorn sheep are presented in Figs 4e, 6e, 7e (only one sheep was available at each sampling period). The counts obtained at both 25C and 37C fluctuated with the time of sampling. Numbers at 25C varied between 1 400 and 5 000 CFU/g wool for moulds (with a mean of 2 663 CFU/g wool) the higher counts being obtained during the first few weeks after treatment and 1 900 and 112 000 CFU/g wool for yeasts (with a mean of 21 346 CFU/g wool) (Fig 5e). At 37C mould counts varied between 0 and 5 000 CFU/g wool (Fig 6e) and yeast counts between 80 and 14 072 CFU/g wool (Fig 7e).

1.3.3 Benlate - plunge pretreated sheep

The average counts of moulds and yeasts are shown in Figs 4c, 5c, 6c, 7c, in relation to time of sampling. Both mould and yeast counts at 25C decreased with time to 12 weeks but then increased towards the end of the sampling period. Mould counts at 37C showed a similar pattern (Fig 6c). Yeast numbers at 37C were considerably reduced at 8 weeks post treatment, they were absent by 12 weeks and did not appear again until the final sampling at 16 weeks (Fig 7c).

1.3.4 Diazospray - plunge pretreated sheep

Only six samples were available from sheep pretreated with 'Diazospray' by plunging. The total mould counts declined from the initial 7 000 to 2 000 CFU/g wool at 8 weeks post treatment but at 16 weeks after treatment, increased to 4 600 CFU/g wool at 25C (Fig 4d).

The yeast count at 25C showed little change 8 weeks after treatment (115 000 CFU/g wool) but fell to 4 000 CFU/g wool at 16 weeks (Fig 5d). At 37C both mould and yeast numbers decreased appreciably (Figs 5d, 7d).

1.3.5 a Diazotas shower pretreated sheep

Mould and yeast counts of sheep shower-dipped in 0.4% diazinon are shown in Figs 4b, 5b, 6b and 7b, in relation to time. At 2 weeks post treatment, the mould counts at 25C was 57 000 CFU/g wool (Fig 4b). The count fell to 3 500 CFU/g wool at 6 weeks post treatment and remained about this level until week 30 post treatment when it rose to 5 700 CFU/g wool and by week 34 was over 10 000 CFU/g wool. The high yeast count of 63 250 CFU/g wool at 2 weeks post treatment was followed by a decline to 2 745 CFU/g wool at 14 weeks post treatment and then became irregular over the remaining experimental period (Fig 5b). At 37C the mould and yeast counts fell to zero at 14 weeks but mould counts subsequently rose again (Figs 6b, 7b).

1.3.5 b Diazotas spray pre treated sheep

At 25C mould counts of fleece which had been diazotas sprayed in 0.4% diazinon, varied between 2 500 and 6 500 CFU/g wool (with a mean of 3 539 CFU/g wool) (Fig 4a). The highest count was recorded 4 weeks post treatment. Yeast counts declined from 15 000 CFU/g wool to 1 600 CFU/g wool at 16 weeks.

The mould counts at 37C were 1 675 CFU/g wool at 4 weeks post treatment, falling to zero at 12 weeks post treatment and increasing again at 16 weeks post treatment (685 CFU/g wool).

Fig 4 - 7: Variation of mould and yeast populations in treated and untreated fleece in relation to time, at incubation temperatures 25C and 37C.

Treatment groups:

Legend: a) Diazotas spray pretreated fleece
b) Diazotas shower pretreated fleece
c) Benlate-plunge pretreated fleece
d) Diazospray-plunge pretreated fleece
e) Shorn fleece
f) Untreated fleece

Fig 4: Mould populations (25C)

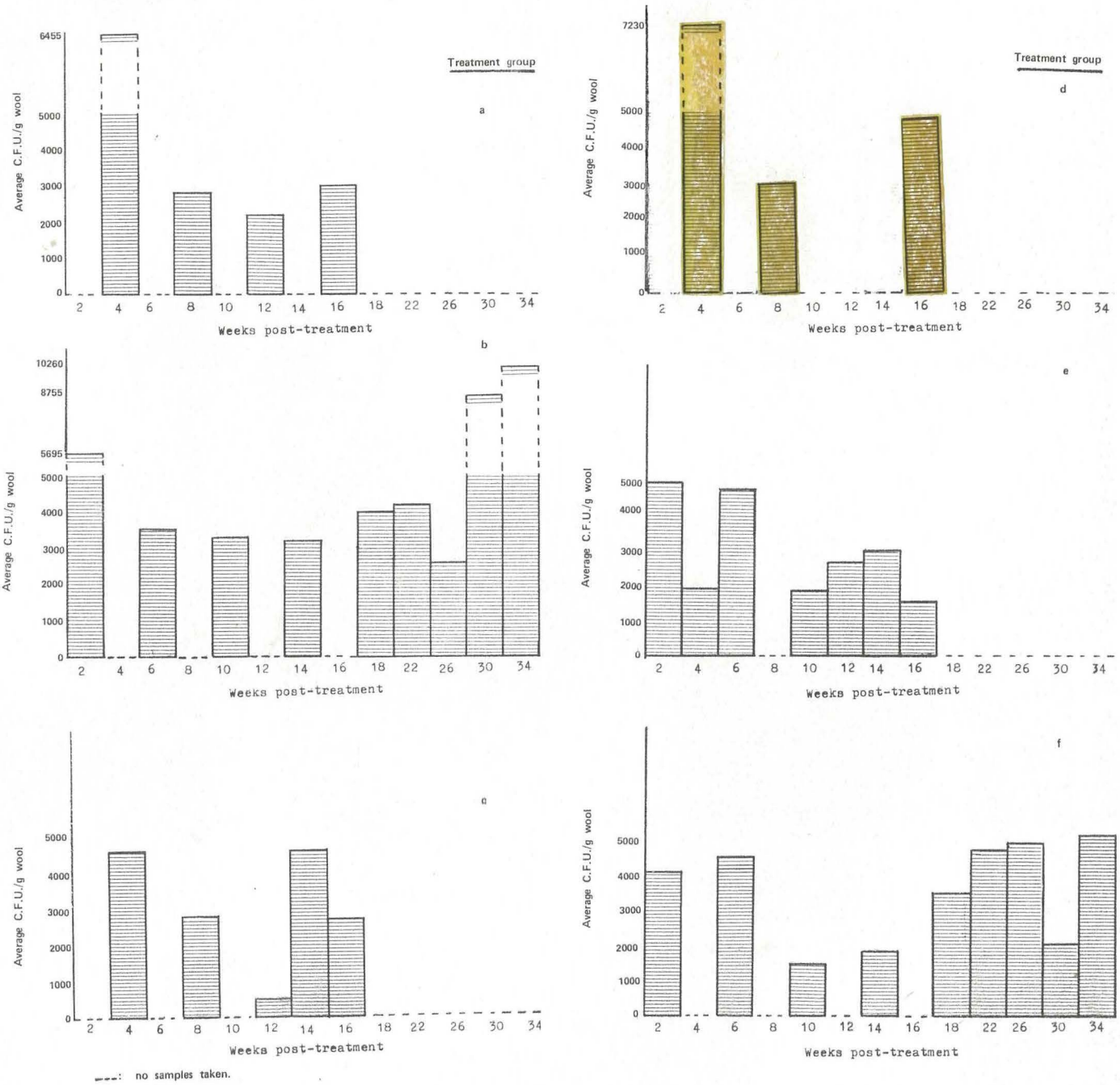


Fig 5: Yeast populations (25C)

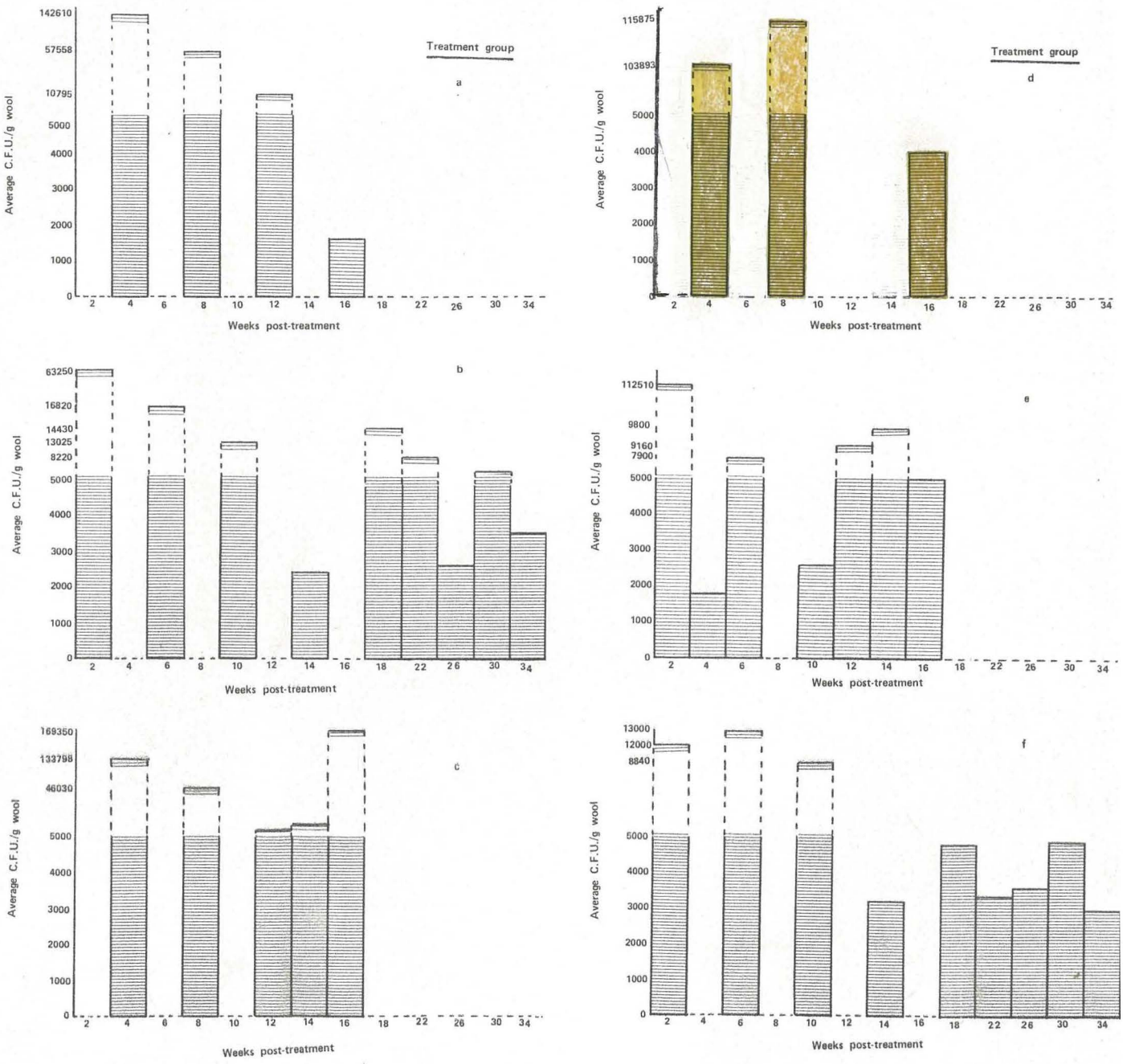


Fig 6: Mould populations (37C)

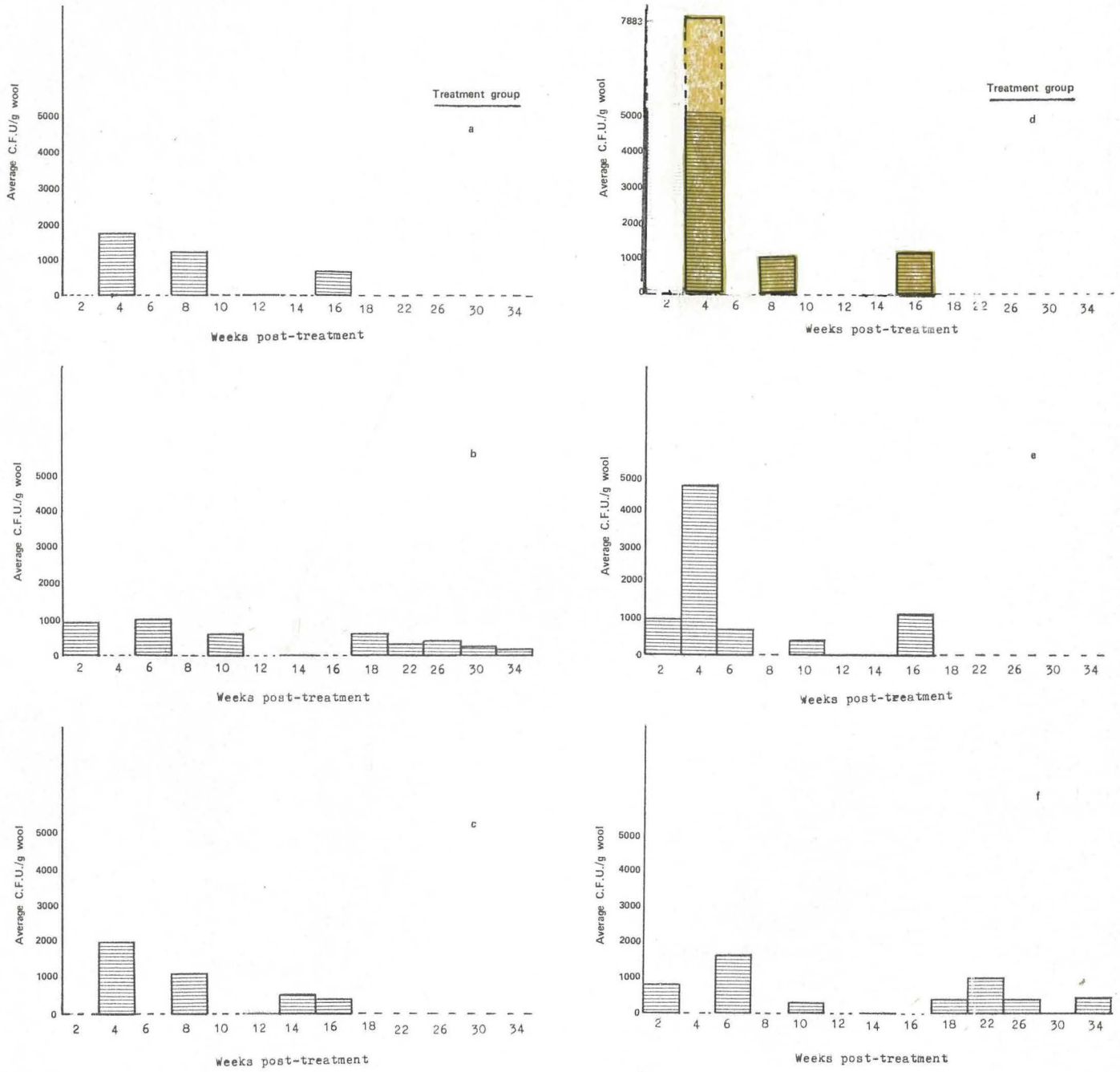
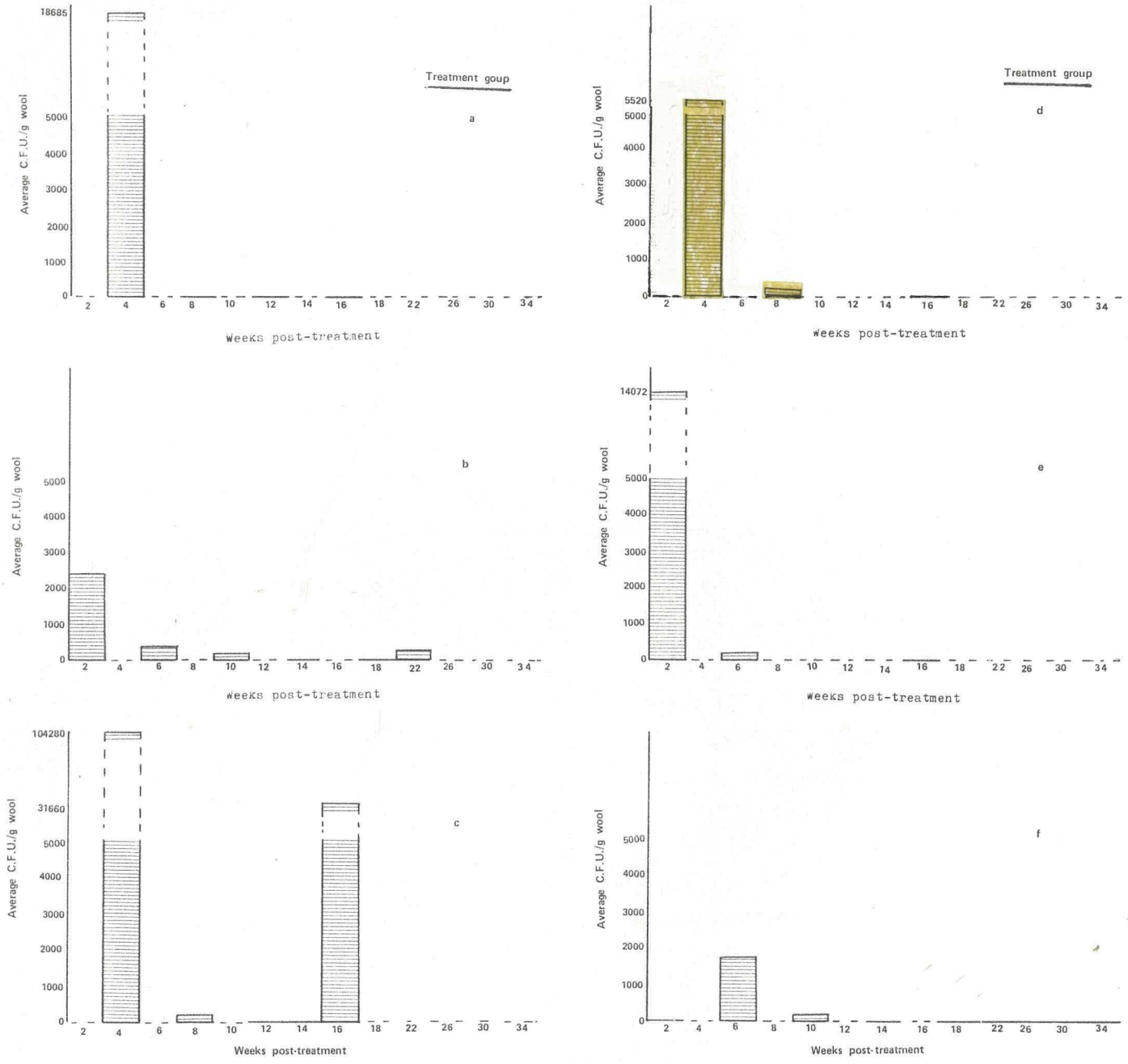


Fig 7: Yeast populations(37C)



1.3.6 Average overall populations of individual treatments

At 25C, the average mould counts of all treatments over the whole experimental period individually showed little difference and varied between 3 000 and 5 200 CFU/g wool. The average yeast counts, however, fluctuated markedly, with the highest count (91 258 CFU/g wool) shown for wool samples collected from Diazospray pretreated sheep and the lowest count (6 450 CFU/g wool) for untreated samples (Table 5).

The counts for both moulds and yeasts at 37C were lower than those recorded at 25C. The mould counts at 37C varied between 460 and 4 500 CFU/g wool while the yeast counts varied between 300 (0.04% diazinon) and 46 000 CFU/g wool (Benlate plunged).

When compared with the overall average fleece counts established previously (Results 1.1.3), shorn and Benlate treated sheep had low mould counts at 25C and Diazotas treated sheep high counts in both showered and sprayed groups. Yeasts at 25C showed lower counts in the untreated and Diazotas showered groups and higher counts after Benlate plunging, Diazotas spraying and Diazospray plunge treatments. At 37C, shorn, Diazotas sprayed and Benlate plunged sheep had higher-than-average mould counts. All groups except Benlate plunged sheep showed lower-than-average yeast counts at 37C.

Table 5 : Overall average populations, individual treatments

Treatment of sheep	Average CFU/g wool at 25C Moulds	25C Yeasts	37C Moulds	37C Yeasts
Untreated	3 336	6 450	603	885
Shorn	2 663	21 354	1 636	6 037
Benlate plunge	3 059	72 571	1 018	45 317
Diazospray plunge	3 539	53 391	899	4 671
Diazotas shower	5 047	14 567	467	310
Diazotas spray	5 162	91 258	4 456	4 146
Overall average fleece counts (Results 1.1.3)	~~3 643~~ 4 159	28 923	825	52 262

2. The Frequency of Fungal Species in Total Samples

Samples used for this study were obtained from the shoulders and legs of the experimental sheep. As there was little difference in the species isolated from the 2 sites, both sites are combined for average counts in the compilation of Tables 6 and 7. The species listed in these tables are arranged in their frequency of isolation by the dilution plating technique.

2.1 Comparison of the dilution plate and brush techniques at 25C

The frequency with which each fungal species was demonstrated by the dilution plating (Methods 5.1) and brush techniques (Methods 5.2) is compared in Table 6. There were 60 species obtained by dilution plating and 55 species obtained by the brush technique. The most predominant species isolated by both techniques were Alternaria alternata, Cladosporium cladosporioides, Fusarium culmorum and Phoma species. Sordaria fimicola, Rhodotorula rubra and Torulopsis candida were also very frequently isolated by dilution plating but not by the brush technique. Cladosporium herbarum, Trichoderma viride, Amaurascus sp, Chrysosporium sp, Penicillium lilacinum, Penicillium oxalicum, Ulocladium sp. and Trichosporon sp, were only isolated by dilution plating. In contrast Chrysosporium pannorum, Chrysosporium merdarium, Cylindrocarpon sp, Gliomastix inflata and Sterile brown mycelium were only isolated by the brush technique.

2.2 Comparison of the dilution plate and brush techniques at 37C

The number of species isolated by both techniques at 37C was lower than those isolated at 25C. Penicillium sp was the predominant species isolated by dilution plating but not by the brush technique. Alternaria alternata, which was frequently isolated by dilution plating, was never isolated by the brush technique. In general, fewer species (25) were isolated by the brush technique than by dilution plating (34). There were 5 species (Mortierella sp, Penicillium commune, Penicillium lilacinum, Penicillium janthinellum and Trichoderma viride) which were isolated by the brush technique but not by dilution plating (Table 7).

Table 6: Relative frequency* of fungal species isolated by Dilution Plating and Brush Techniques at 25C

	Frequency of demonstration			
	Dilution Plates (136 samples)		Brush Plates (106 samples)	
	No. samples positive	Frequency grading	No. samples positive	Frequency grading
Torulopsis candida (Saito) Lodder	132	4	30	3
Alternaria alternata (Fr.) Keissl.	121	4	68	4
Rhodotorula rubra (Demme) Lodder	117	4	15	2
Phoma spp.	112	4	50	4
Cladosporium cladosporioides (Fr.) de Vries	90	4	25	3
Fusarium culmorum Sacc.	78	4	39	3
Sordaria fimicola (Rob) Ces.de Not.	48	4	5	1
Mucor racemosus Fr.	36	3	23	3
Penicillium sp.	23	3	27	3
Aspergillus fumigatus Fr.	20	3	19	3
Penicillium nigricans Bain.	18	3	12	2
Torulopsis sp.	17	3	-	-
Cladosporium herbarum (Pers.) Link ex Gray	14	2	-	-
Pithomyces chartarum (Berk. & Curt.) Ellis	11	2	8	2
Aspergillus terreus Thom	10	2	7	2
Trichoderma glaucum Abbott	10	2	5	1
Sterile grey mycelium	10	2	10	2
Epicoccum purpurascens Ehrenb. ex Schlects	9	2	8	2
Fusarium lateritium Nees	9	2	4	1
Cephalosporium roseo-griseum Saksena	8	2	3	1
Paecilomyces marquendii (Massee) Hughes	7	2	1	1
Pseudoeurotium zonatum van Beyma	7	2	1	1
viride Pers. ex. Fr.	7	2	-	-

Table 6 cont'd

Fusarium oxysporum Schlects	6	2	10	2
Pencillium islandicum Sopp.	6	2	1	1
Alternaria citri Ellis & Pierce	5	1	1	1
Fusarium semitectum Berk.& Rav.	5	1	3	1
Penicillium expansum Link ex Gray	5	1	6	2
Aspergillus niger van Tiegham	4	1	1	1
A. ornatus Raper, Fennel & Tresner	4	1	8	2
Beauveria bassiana (Bals.) Vuill.	4	1	14	2
Penicillium canescens Sopp.	3	1	3	1
Penicillium purpurascens (Sopp.) Raper & Thom	3	1	2	1
Alternaria brassicola (Schw.) Wiltshire	2	1	2	1
Geotrichum candidum Link ex. Pers.	2	1	2	1
Paecilomyces variotii Bainier	2	1	5	1
Pencillium citrinum Thom	2	1	3	1
P. commune Thom	2	1	2	1
P. italicum Wehmer	2	1	1	1
P. janthinellum Biourge	2	1	2	1
P. notatum Westling	2	1	3	1
P. oxalicum Currie and Thom	2	1	-	-
Westerdykella sp.	2	1	-	-
Peyronellaea glomerata (Corda) Goidanich	2	1	4	1
Gliocladium roseum Bainier	2	1	2	1
Amaurascus sp.	1	1	=	=
Alternaria cheiranthi (Lib.) Bolle	1	1	4	1
Aspergillus flavus Link ex. Fr.	1	1	1	1
Aureobasidium pullulans Arnaud	1	1	3	1
Beauveria globulifera Speg.	1	1	2	1
Chrysosporium sp.	1	1	-	-
Dreschlera hawaiiensis (Bugnicourt) Subram.& Main ex Ellis	1	1	1	1
Fusarium solani (Mart) Sacc.	1	1	2	1

Table 6 cont'd

Fusarium sulphureum Schlecht	1	1	1	1
Humicola sp.	1	1	1	1
Mortierella sp.	1	1	1	1
Penicillium lilacinum Thom	1	1	-	-
Ulocladium sp.	1	1	-	-
Trichosporon sp.	1	1	-	-
Sterile black mycelium	1	1	1	1
Chrysosporium pannorum (Link) Hughes	-	-	1	1
C. merdarium (Link) Carm.	-	-	2	1
Cylindrocarpon sp.	-	-	1	1
Gliomastix inflata Dickinson	-	-	1	1
Sterile brown mycelium	-	-	2	1

* Frequency grading

		No. samples positive
1	Very occasional	1 - 5
2	Occasional	6 - 15
3	Frequent	16 - 45
4	Very frequent	46 and above

Table 7 Relative frequency of fungal species isolated by Dilution Plating and Brush Techniques at 37C

	Frequency of demonstration			
	Dilution Plates (136 samples)		Brush Plates (106 samples)	
	No. samples positive	Frequency grading	No. samples positive	Frequency grading
Penicillium sp.	50	4	5	1
A. alternata	19	3	-	-
A. fumigatus	43	3	11	2
S. fimicola	38	3	13	2
R. rubra	27	3	1	1
T. candida	33	3	5	1
P. nigricans	12	2	6	2
Westerdykella sp.	11	2	1	1
Chrysosporium sp.	10	2	-	-
Sterile grey mycelium	10	2	-	-
Torulopsis sp.	8	2	2	1
A. cheranthi	7	2	4	1
M. racemosus	7	2	5	1
P. marquendii	7	2	-	-
Trichosporon sp.	7	2	2	1
A. citri	6	2	-	-
P. islandicum	4	1	2	1
A. flavus	3	1	4	1
F. culmorum	3	1	-	-
G. roseum	3	1	-	-
P. canescens	3	1	1	1
A. ornatus	2	1	9	2
P. expansum	2	1	6	2
P. variotii	2	1	-	-
P. notatum	2	1	-	-
P. oxalicum	2	1	-	-
A. niger	1	1	1	1
Chaetomium globosum Kunze	1	1	1	1

Table 7 cont'd

A. pullulans	1	1	–	–
Byssochlamys fulva Olliver & Smith	1	1	–	–
G. candidum	1	1	–	–
P. italicum	1	1	1	1
P. purpurascens	1	1	2	1
Sterile black mycelium	1	1	–	–
Sterile brown mycelium	1	1	–	–
Mortierella sp.	–	–	2	1
P. commune	–	–	1	1
P. janthinellum	–	–	1	1
P. lilacinum	–	–	1	1
T. viride	–	–	2	1

3. Variation In The Mycoflora Of Wool Fibres Along Their Length.

3.1 Microscopical examination using impression technique

3.1.1 Morphological forms distinguished by impression technique

The spore types recognized in wool impressions on agar are illustrated in Fig 8. The most readily identified spores were those of Alternaria (Fig 8a), Epicoccum (Fig8b), Helminthosporium (Fig 8c, Plate 3), Cladosporium (Fig 8d, Plate 4), Pithomyces (Fig 8e), Dreschlera (Fig 8h) and Curvularia (Fig 8i). Phoma-type pycnidiospores were identified by their size and shape and their clustered arrangement in a blue staining background as is found in pycnidiospores freshly discharged from a pycnidium (Fig 8j). Many one celled brown spores were also observed. These were sometimes found to be present in groups of seven to eight and were therefore tentatively identified as ascospores (Fig. 8k, Plate 5). Their appearance also resembled the spores of an Ascomycete later isolated in culture from these samples . Torula-type spores were also present, each of four or more brown cells in chains (Fig 8g). Multicellular, dark, chlamydospores with irregular septations were identified as the chlamydospores of Peyronellaea (Fig 8l). Masses of pale green conidia were observed on one or two occasions (Fig 8m) and tentatively assigned to the genera Penicillium or Aspergillus. As a species of Penicillium was frequently isolated from these samples in culture it seems probable that the spores illustrated were those of this genus. Paecilomyces was recognized on the basis of its undetached conidia on characteristic conidiophores and was the only example of intact conidiophores being observed on the wool (Fig 8n). Unicelluler budding yeast cells were also seen (Fig 8o). Dictyosporium was identified by its unique spore morphology, appearing as if irregular chain lengths of dark coloured cells had piled into one plane. There were six to seven unidentified spore types, the most frequent being a unicellular, rough walled, light brown spore .

Hyphae were also observed in the wool impression blocks. In a few cases it seemed that the hyphae had been actively growing in the fleece and appeared as masses of filaments showing intact branching. These are referred to as 'active' hyphae (Plate 6). More frequently observed were short filaments which had probably been picked up intact and did not seem to be actively growing in the wool (Plate 7). These are referred to as 'passive' hyphae .

Fig 8: Fungal spore types recognised on wool by the impression technique

Legend:

a) Alternaria

b) Epicoccum

c) Helminthosporium

d) Cladosporium

e) Pithomyces

f) Fusarium

g) Keratinomyces

h) Dreschlera

i) Curvularia

j) Phoma

k) Ascospores

l) Peyronellaea

m) Penicillium

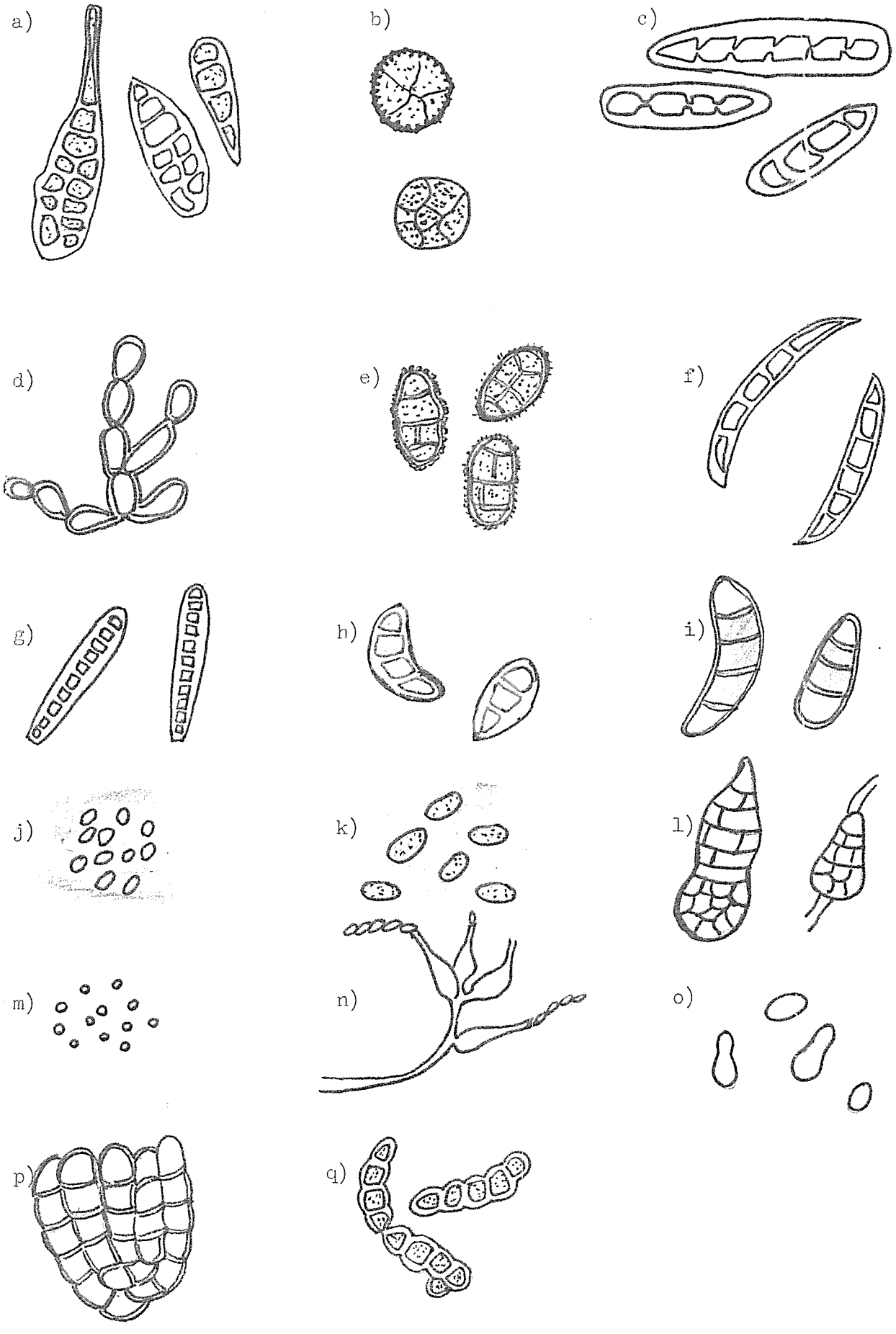
n) Paecilomyces

o) Yeast cells

p) Dictyosporium

q) Torula

Fig 8 :



Plates 3 – 7: Some spore and hyphal types demonstrated on wool impressed agar

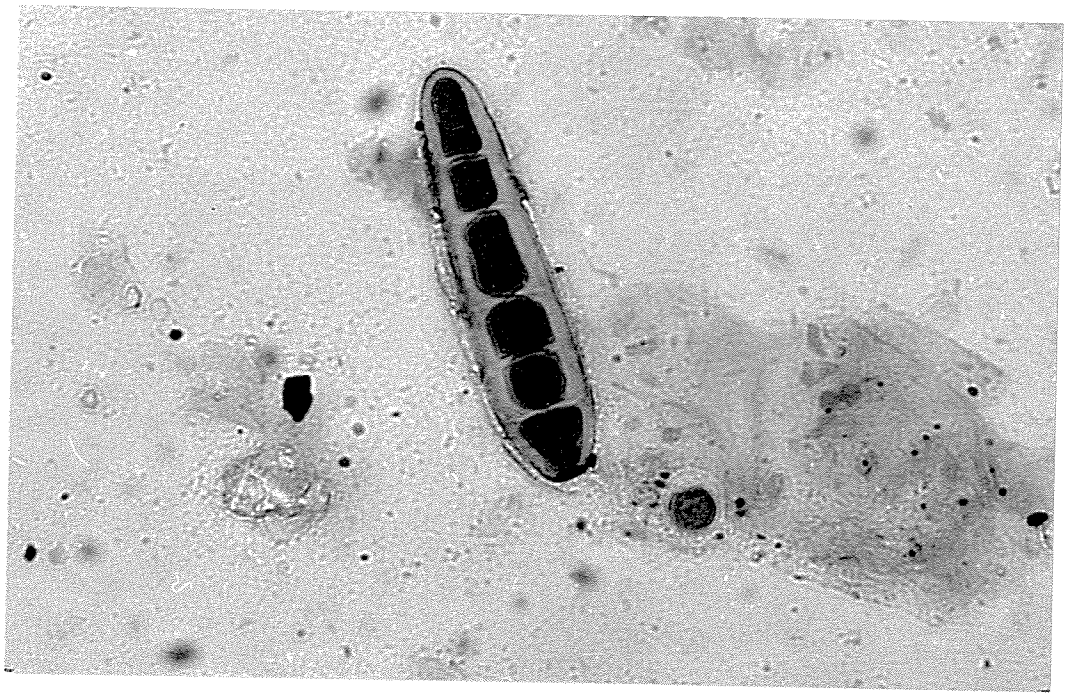


Plate 3: Helminthosporium (400x)

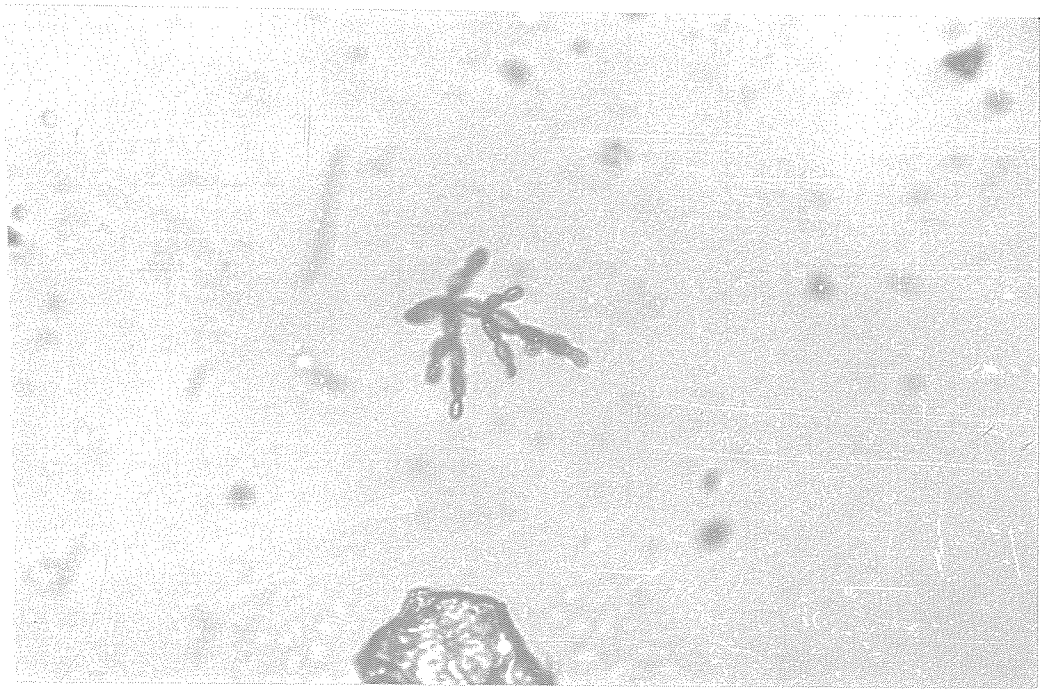


Plate 4: Cladosporium (400x)

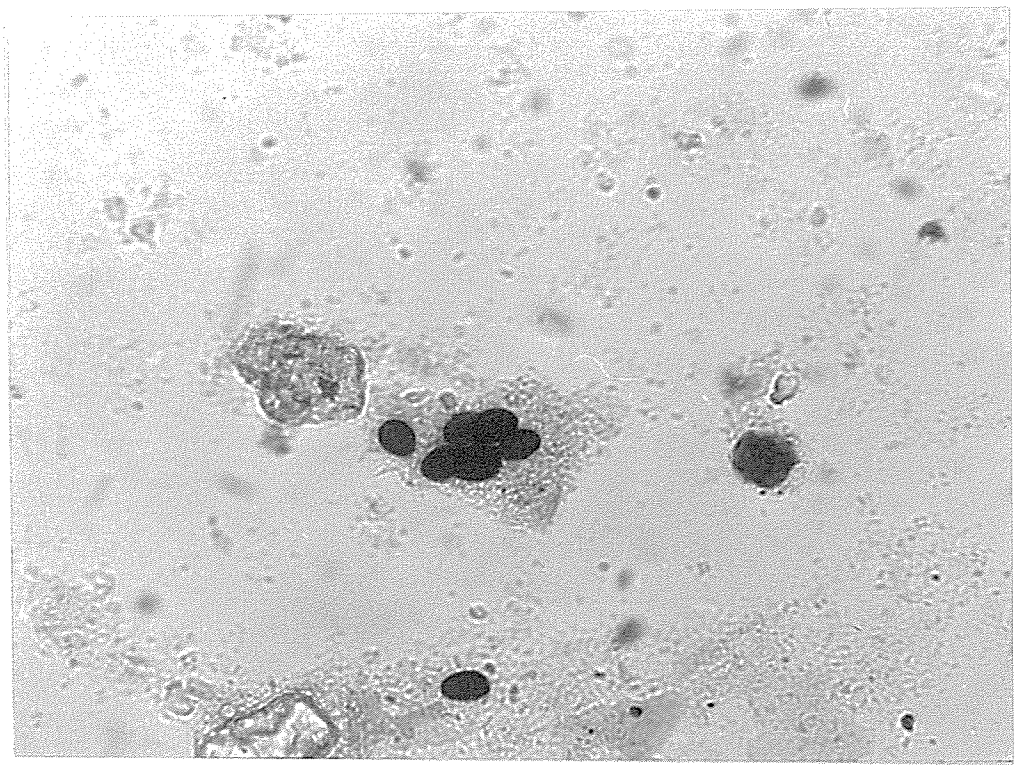


Plate 5 : Ascospores (400x)

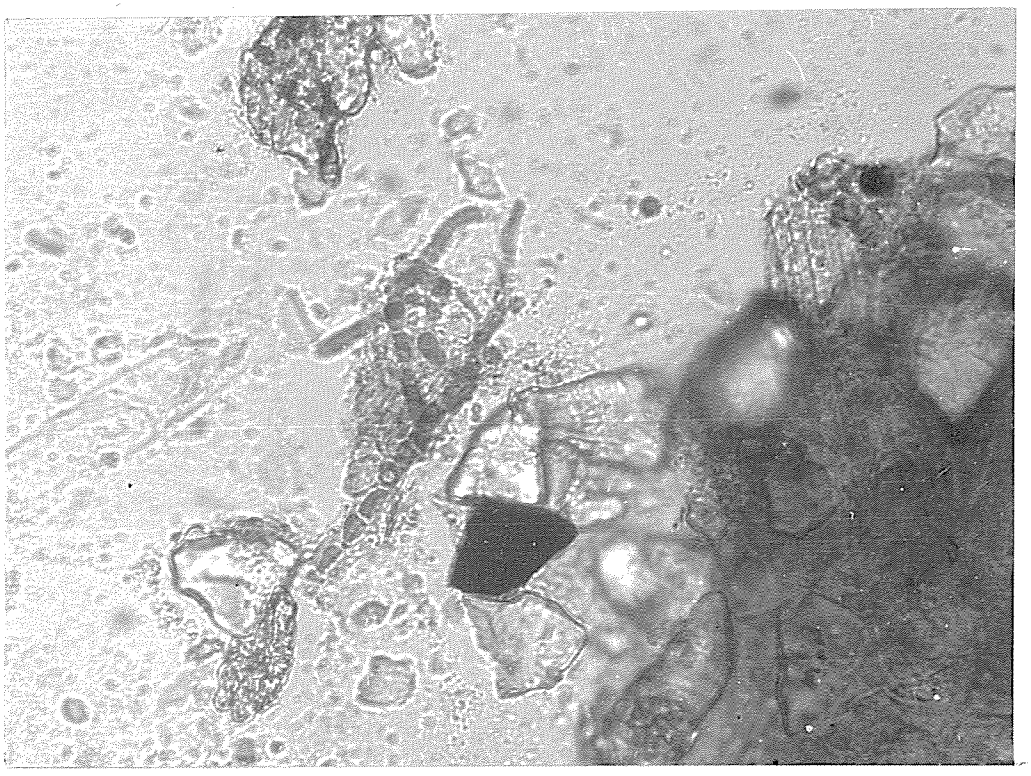


Plate 6: 'Active' hyphae (400x)

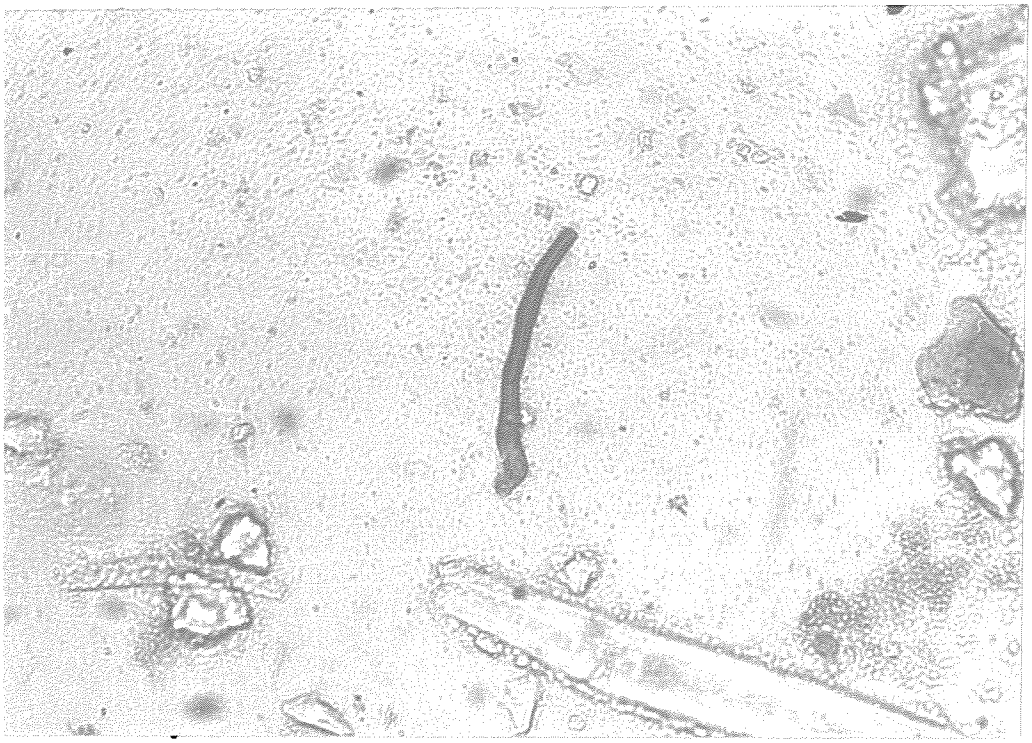


Plate 7: 'Passive' hyphae (400x)

3.1.2 Overall frequency of fungal types in 48 samples examined

In the 48 specimens examined microscopically by the impression technique the 17 spore and 2 hyphal types described above could be distinguished in varying numbers (Table 8). The most common were the spores of Alternaria, Phoma, Pithomyces, Epicoccum, Fusarium and Helminthosporium, but unidentified spores and ascospores were also very numerous. There was no difference between shoulder and leg samples in the types of fungi demonstrated. Alternaria spores were observed in 87.5% of the specimens, Phoma spores and ascospores in 70.8% and 77.1% respectively. Over 50% of spore types were demonstrated in less than 10 of the 48 specimens each. Paecilomyces was observed in 2 specimens and in both appeared to be growing actively. On four occasions individual and budding cells of yeast were observed.

Hyphae were found in 96% of specimens examined microscopically but only 8.0% were of the 'active' type.

3.1.3 Distribution of spore types along the length of the fibres.

The results of the microscopic examination of wool for fungi in relation to distance along the fibres are illustrated in Figs. 9-1 and 9-2. The section along the fibre is indicated by the numerals 1-6, (Methods 7.1.1), number 6 being the apical section. In general, the spores seemed to be most numerous in the middle portions (Sections 4 and 5) of the fibres with a decrease towards the outside of the fleece. There were relatively few spores in sections closest to the skin. Fusarium, Phoma and Dreschlera spores increased in number along the fibres towards the apical section. Spores of Dictyosporium were found only in the tips of one sample; Curvularia, Cladosporium and Paecilomyces only in the middle sections.

3.1.4 Fungal types in relation to time of sampling

Samples collected in May contained the greatest number of spores (Fig 10). The spores of Alternaria, Phoma, Pithomyces, Helminthosporum, Dreschlera, Torula, and ascospores and unidentified spores were observed in wools collected at each of the sampling periods. Paecilomyces, Curvularia, Keratinomyces, Yeast cells, Cladosporium, Dictyosporium and Pencillium each appeared only in one of the sampling periods. Peyronellaea chlamydospores were observed in 2 sampling periods, Epicoccum and Fusarium in three. Again, the general distribution pattern of spores along the fibres was apparent.

Table 8 Overall presence of spore types and hyphae in 48 wool samples

Spore type	Total Positive	Frequency %
Alternaria	42	87.5
Ascospores	37	77.1
Phoma	34	70.8
Pithomyces	23	47.9
Epicoccum	17	35.4
Fusarium	11	22.9
Helminthosporium	10	20.8
Dreschlera	9	18.8
Torula	6	12.5
Keratinomyces	5	10.4
Yeast cells	4	8.33
Penicillium	3	6.25
Peyronellaea	2	4.17
Paecilomyces	2	4.17
Cladosporium	1	2.08
Curvularia	1	2.08
Dictyosporium	1	2.08
'Passive' hyphae	42	87.5
'Active' hyphae	4	8.33
Unidentified spores	43	89.6

Fig 9-1: Distribution of the more common spore types along the length of the fibres.

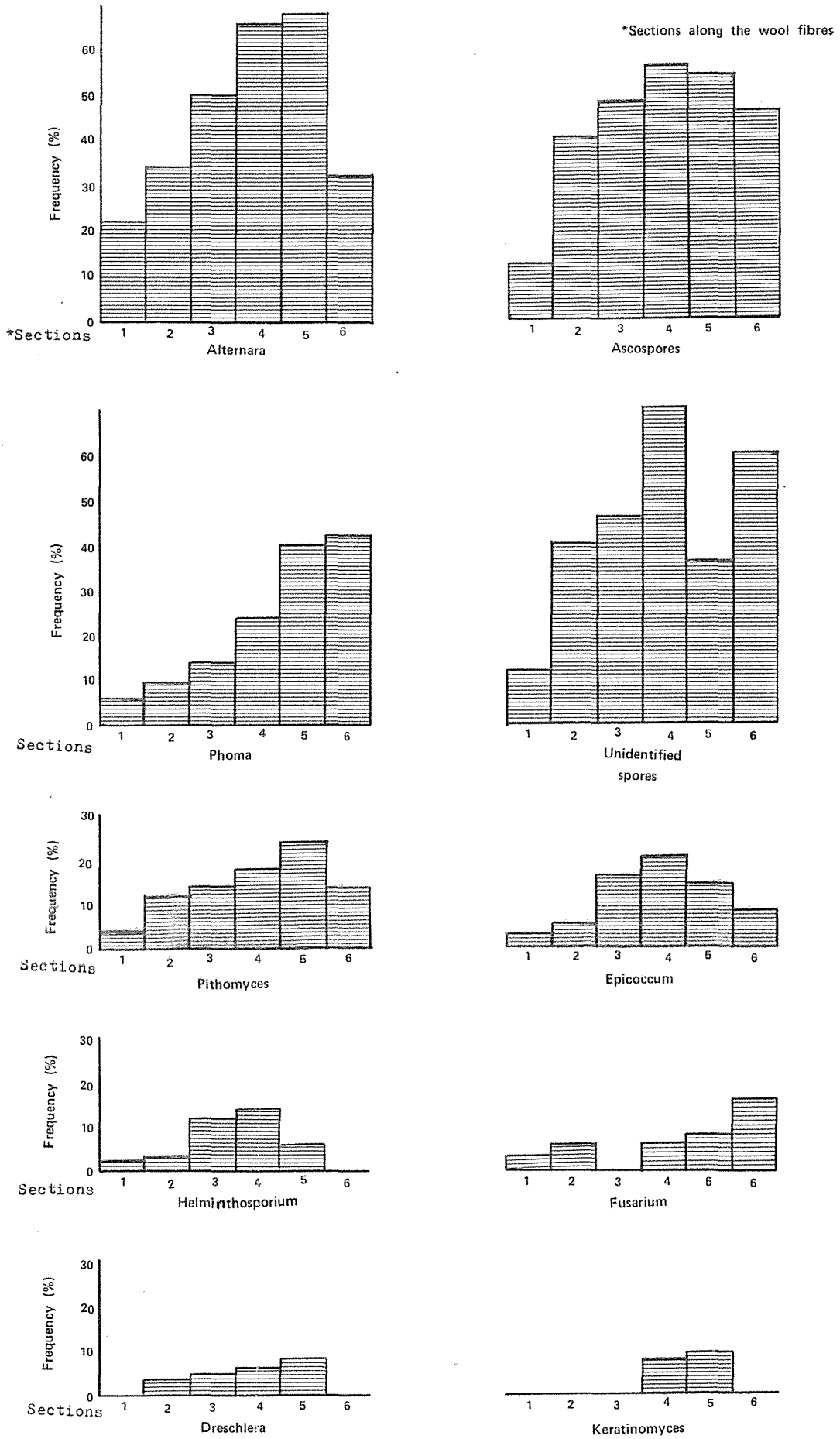


Fig 9-2: Distribution of less frequent spore types along the length of the fibres

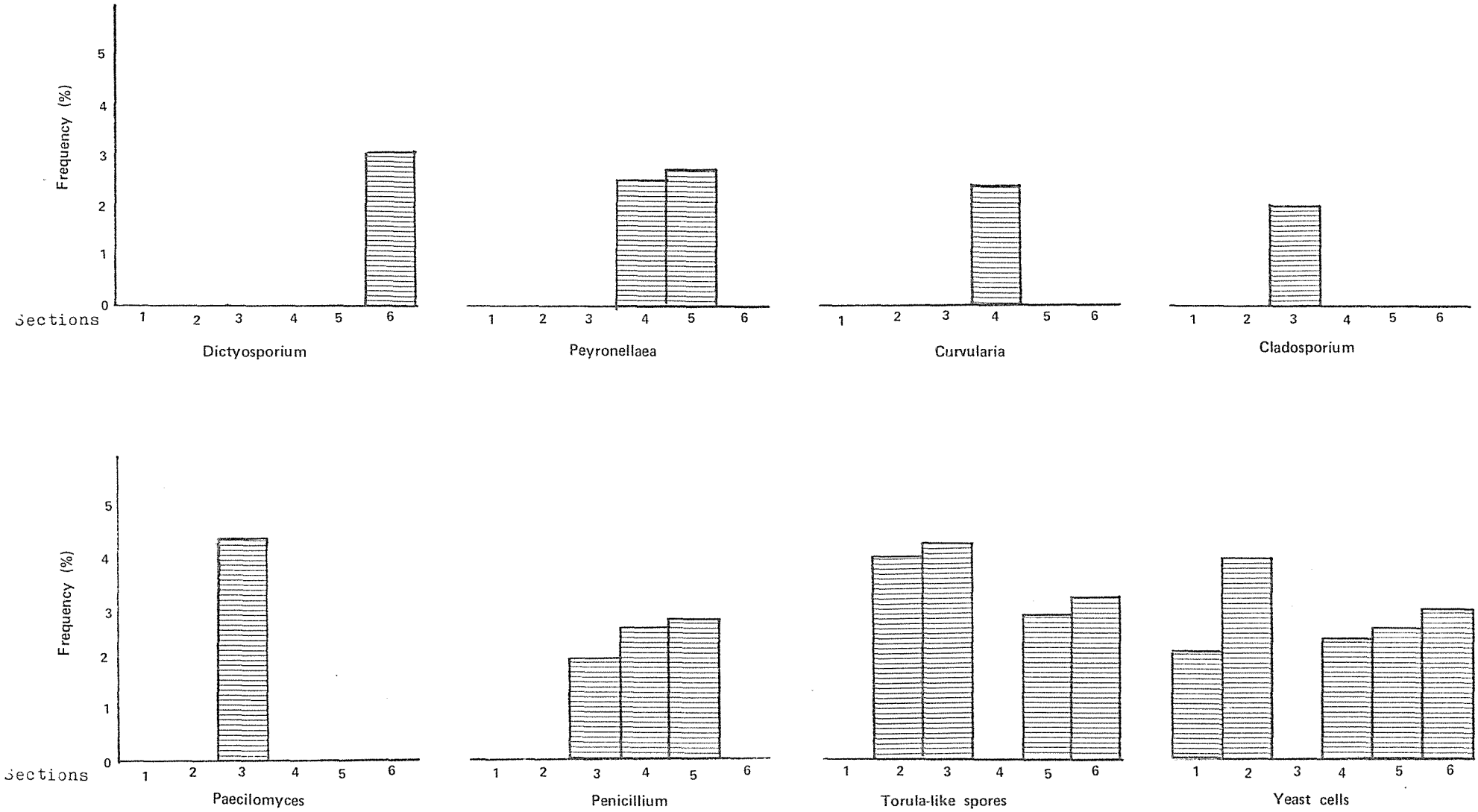


Fig 10a - 10c Distribution of fungal spore types along the length of the fibres in relation to time of sampling

Fig 10 a

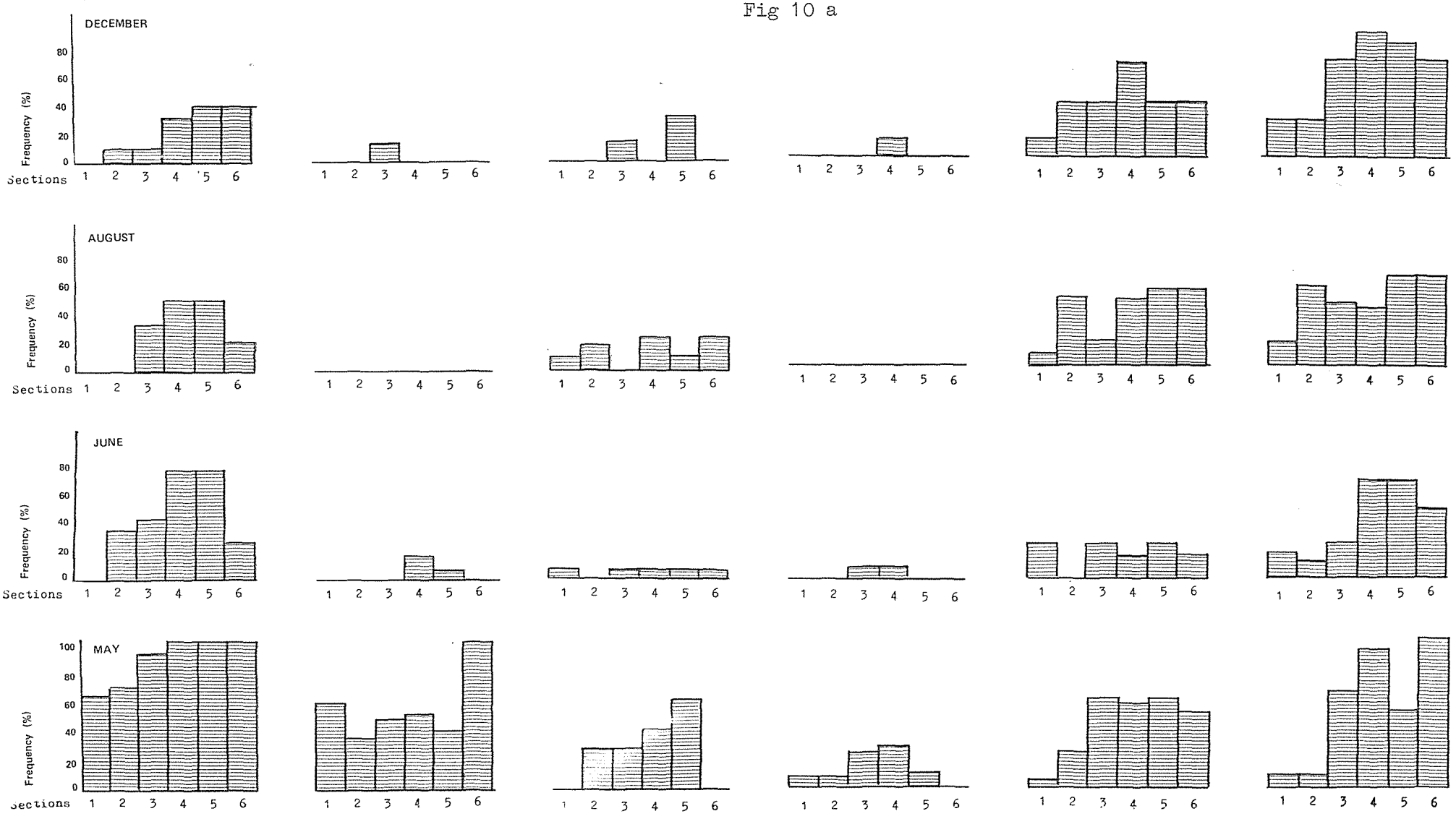

DECEMBER
AUGUST
JUNE
MAY
Frequency (%)
Sections
1 2 3 4 5 6
Alternaria
Epicoccum
Pithomyces
Helminthosporium
Ascospores
Unidentified spores

Fig 10 b

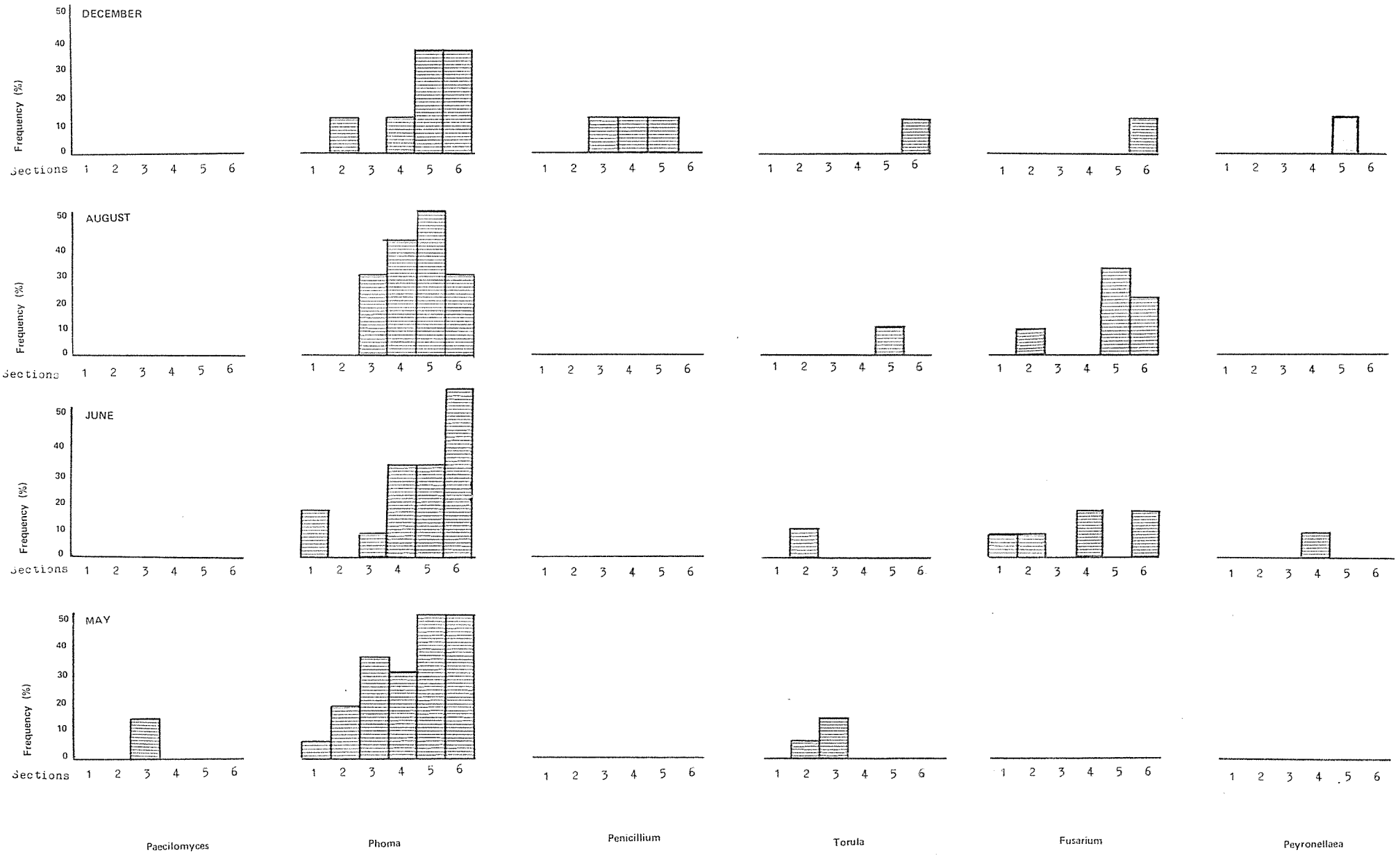
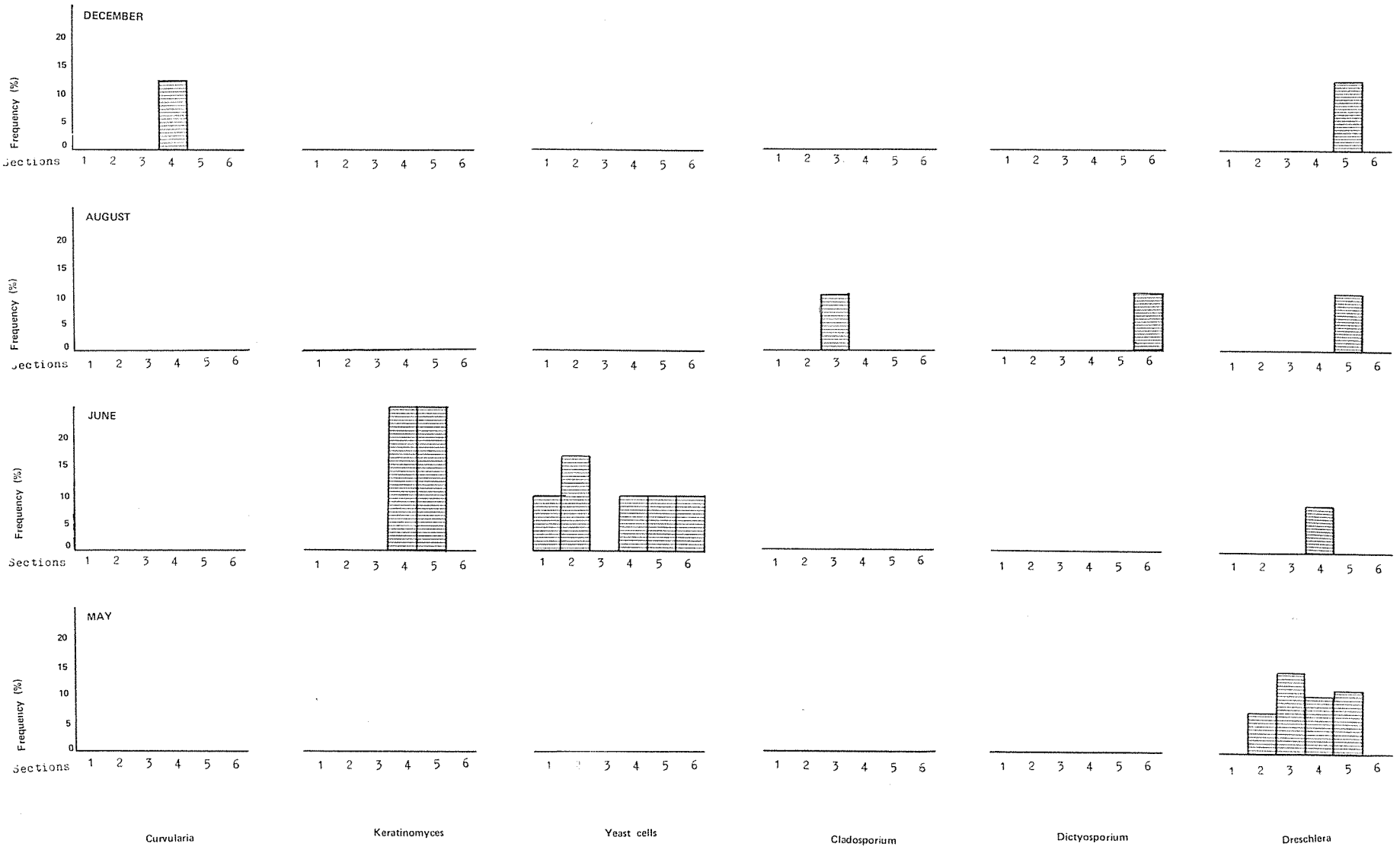

DECEMBER
AUGUST
JUNE
MAY
Frequency (%)
0
10
20
30
40
50
Sections 1 2 3 4 5 6
Paecilomyces
Phoma
Penicillium
Torula
Fusarium
Peyronellaea

Fig 10 c


DECEMBER
AUGUST
JUNE
MAY
Frequency (%)
0
5
10
15
20
Sections
1 2 3 4 5 6
Curvularia
Keratinomyces
Yeast cells
Cladosporium
Dictyosporium
Dreschlera

3.1.5 Presence of species in relation to pretreatment of fleece

The total frequency of spore types in relation to pretreatment of the fleece is illustrated in Table 9 . Wool from sheep that had earlier been shorn had fewer fungal spores compared with wool of unshorn sheep. Alternaria spores were present in all the wool samples of this series and its presence did not seem to be affected at all by the fungicide or insecticide used. The spores of Fusarium and Dreschlera were observed in all chemically treated fleece but not in untreated wool. The chemically pretreated wools had the greater variety of spore types compared to untreated wools as most of the more rarely observed spores occured only in chemically pretreated fleece.

'Active' hyphae were found to occur only in wool that had been treated with Diazinon applied by spraying and Benlate. 'Passive' hyphae were present in untreated wools and the wools from all treatment groups.

3.2 Cultural studies of impressed agar

3.2.1 Overall frequency of fungi in the 48 samples examined

There were 20 species of fungi isolated in culture from the 48 specimens of wool impressed onto agar but 2 of these were sterile mycelia. The species isolated are shown on Table 10.

The predominant isolate was Alternaria alternata which appeared in 91.7% of the samples cultured. The next most common isolates were Fusarium culmorum and Torulopsis candida with 62.5% and 52.1% respectively. The remaining species shown in Table 10 occurred in less than 10 samples each, with Aspergillus fumigatus and Botrytis species appearing from only one sample each.

3.2.2 Overall frequency of fungi in relation to distance along the fibre

The distribution pattern of the cultured fungi along the wool fibres is illustrated in Plates 8 and 9. Less fungal growth was observed in the basal sections 1 and 2 but the growth was denser in the middle and apical parts. This distribution pattern is similar to spore distribution established by microscopy, but there were again a few exceptions to this basic pattern (Figs 11-1 11-2), Cephalosporium roseo-griseum and Trichoderma viride being most frequent in the basal sections.

Table 9 : The frequency of demonstration of fungal spores and hyphae in samples from 24 sheep, in relation to pretreatment

Pretreatment	Untreated	Shorn	Frequency of spore types in total sheep sampled: Benlate plunge	Diazospray plunge	Diazotas shower	Diazotas spray
No. sheep sampled;	2	2	6	6	2	6
Alternaria	2	2	6	6	2	6
Epicoccum	1	1	3	3	0	4
Pithomyces	1	1	4	4	1	4
Helminthosporium	0	0	2	3	0	3
Fusarium	0	0	3	3	1	2
Dreschlera	0	0	2	1	1	2
Phoma	2	1	6	6	2	5
Ascospores	2	2	5	5	2	6
Paecilomyces	0	0	1	0	0	0
Keratinomyces	0	0	1	0	0	2
Peyronellaea	1	0	0	0	0	1
Yeast cells	0	0	1	1	0	1
Dictyosporium	0	0	1	0	0	0
Curvularia	0	0	0	0	1	0
Cladosporium	0	0	0	0	0	1
Torula	0	0	0	3	1	2
Pencillium	1	0	0	0	2	0
'Active' hyphae	0	0	2	0	0	2
'Passive' hyphae	2	2	4	6	1	4
Total no. of types	8	6	14	11	10	15

Table 10: Overall frequency of cultured species from 48 samples

Fungal species	No. Positive	Frequency (%)
Alternaria alternata	44	91.7
Aspergillus fumigatus	1	2.08
Botrytis sp	1	2.08
Cephalosporium roseogriseum	2	4.17
Chrysosporium sp	4	8.33
Cladosporium cladosporioides	4	8.33
Epicoccum nigrum	3	6.25
Fusarium culmorum	30	62.5
Mucor racemosus	9	18.8
Paecilomyces marquendii	3	6.25
Penicillium sp	7	14.6
Phoma spp	22	41.7
Pithomyces chartarum	2	4.17
Sordaria fimicola	10	39.6
Sterile brown mycelium	1	2.08
Sterile grey mycelium	3	6.25
Trichoderma glaucum	3	6.25
Trichoderma viride	2	4.17
Rhodotorula rubra	16	33.3
Torulopsis candida	25	52.1

Plates 8 & 9: Variation of the mycoflora along the length of wool fibres as demonstrated by the Impression Technique

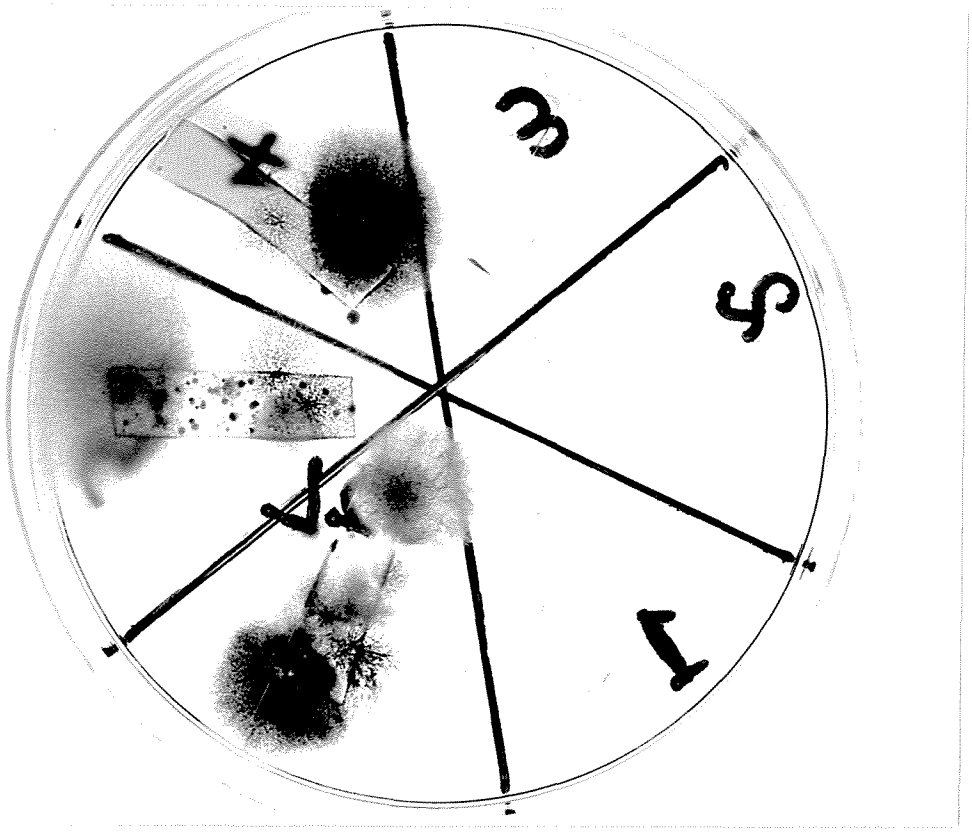


Plate 8

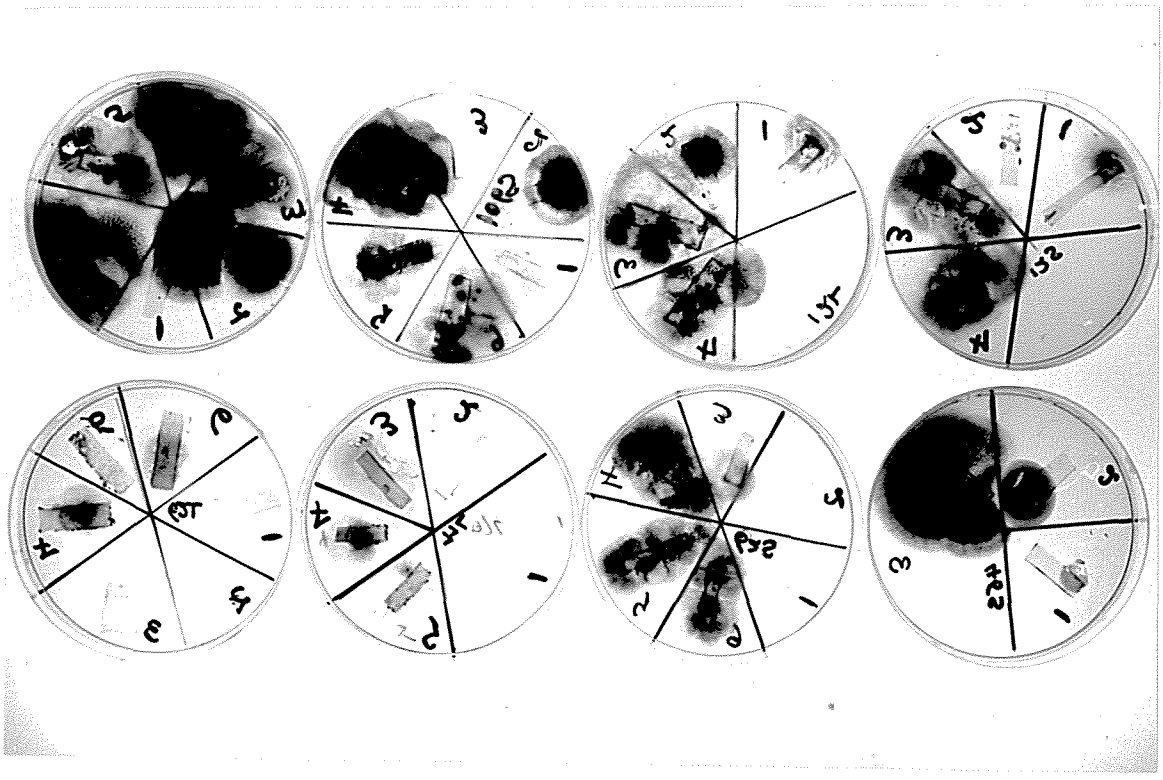
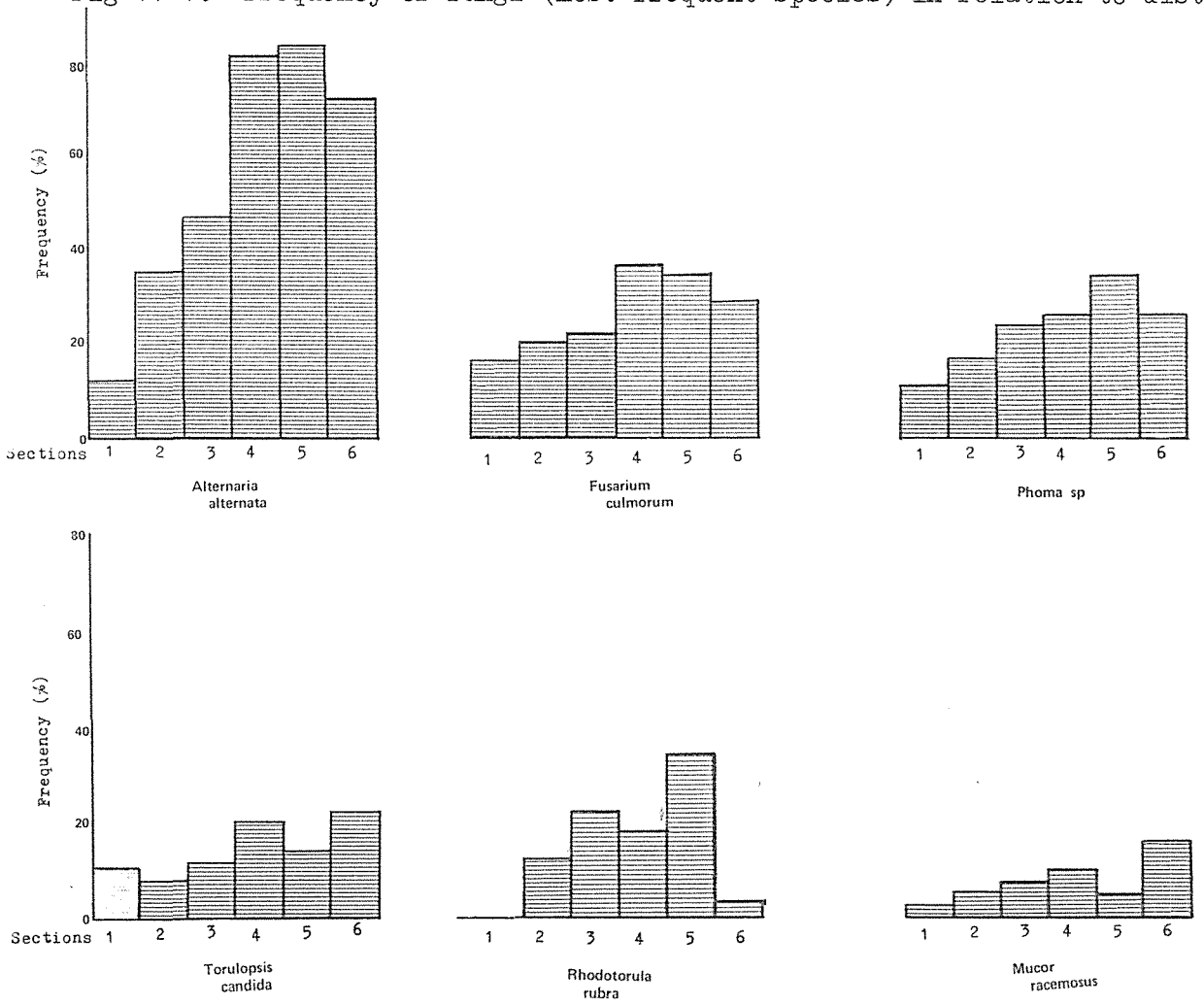


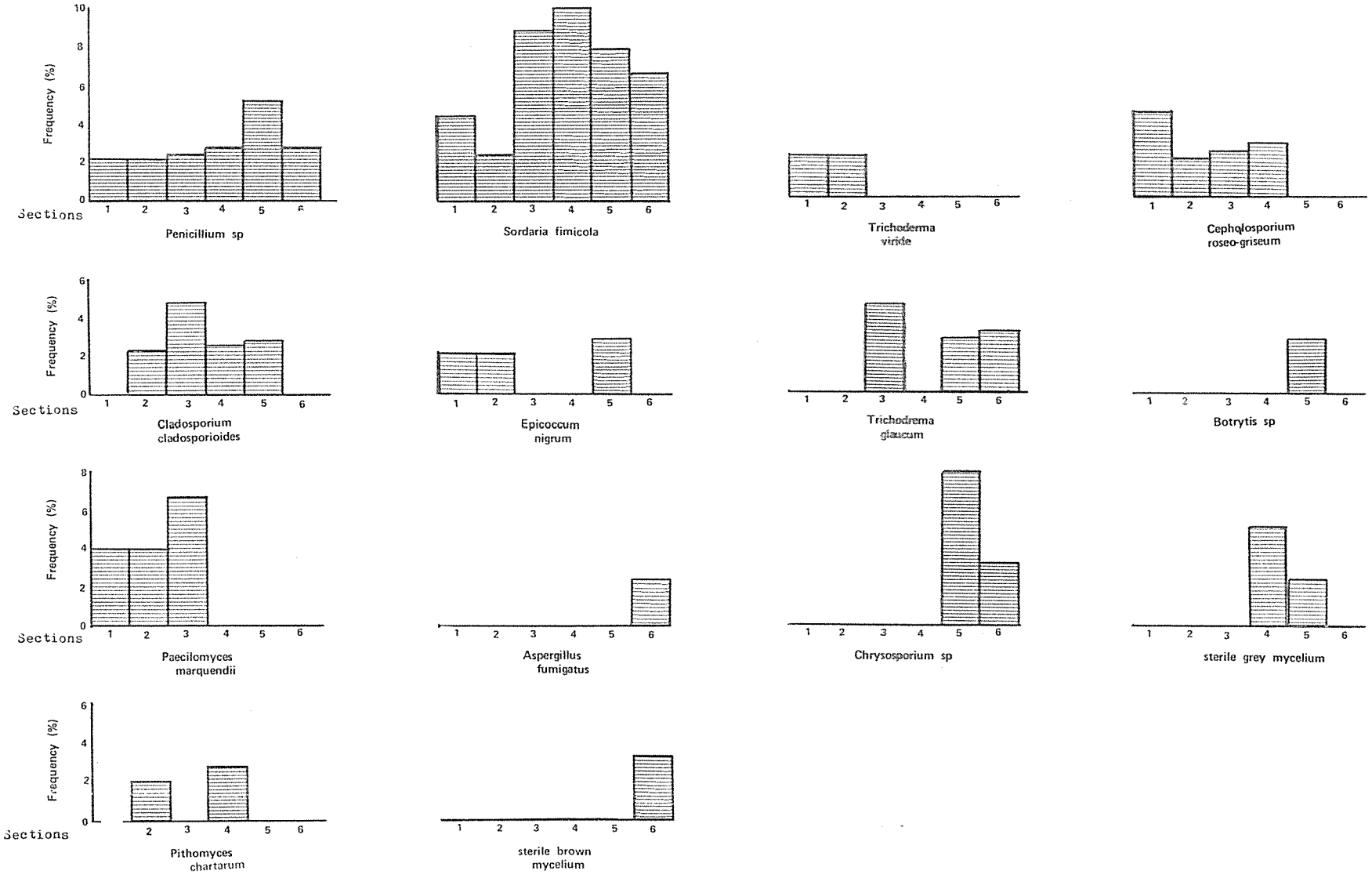
Plate 9

Fig 11-1: Frequency* of fungi (most frequent species) in relation to distance along fibre



* % presence in relevant section in 48 samples examined

Fig 11-2: Frequency of fungi (less frequent species) in relation to distance along fibre



3.2.3 Frequency of cultured fungi in relation to time of sampling

Generally, the numbers of species in wools collected in May, June, August and December fluctuated considerably, but the August samples had the greatest variety isolated and also the highest overall frequency of fungi (Figs. 12 a-d). The most commonly isolated species were Alternaria alternata, Fusarium culmorum, Phoma spp and the 2 yeasts Rhodotorula rubra and Torulopsis candida, and these were present in samples from all 4 periods. In contrast, Mucor racemosus, Penicillium sp, Sordaria fimicola, Cladosporium cladosporioides, Epicoccum nigrum, Paecilomyces marquendii, and Chrysosporium sp were isolated from samples collected in only 2 or 3 of the sampling periods and the remainder occurred only in one sampling period.

3.2.4 Presence of species in relation to pretreatment of fleece

Culturally, Alternaria alternata appeared to be the predominant species present in wool and its occurrence was not influenced by the fungicide and insecticides used (Table 11). Fleeece samples obtained from sheep that were previously shorn showed a lower overall frequency of species. Alternaria alternata, Phoma spp and the 2 yeasts occurred in both treated and untreated fleeces. The sterile brown mycelium was isolated only from wool pretreated by tip spraying with diazinon. The greatest variety of fungi was obtained from wools previously treated by 'Diazospray' spraying. Untreated wool had the least variety.

3.3 Comparison of microscopical and cultural results

A total of 26 genera were demonstrated by the microscopical and cultural techniques (Table 12). The spores of 55.6% of the cultured species were observed by direct microscopy. These species included Alternaria, Fusarium, Sordaria, Epicoccum, Paecilomyces and Cladosporium. The remaining 44.4% of the species cultured had not been observed by direct microscopy. There were 7 species observed by microscopy which were not isolated in culture. These were Helminthosporium Dreschlera, Torula, Peyronellaea, Keratinomyces, Dictyosporium and Curvularia.

3.4 Microscopic examination of sheep skin with Potassium Hydroxide

Direct microscopic examination of sheep skin samples from leg and shoulder specimens obtained from 24 sheep revealed that no fungal fragments or spores were present in the epidermal and dermal layers. Only on one occasion were Alternaria and Epicoccum spores observed but these were attached to the remaining wool external to the epidermis of the skin.

Figs 12a - 12 D : Frequency of Fungi (cultured) along the length of the fibres in relation to time of sampling

Fig 12 a.

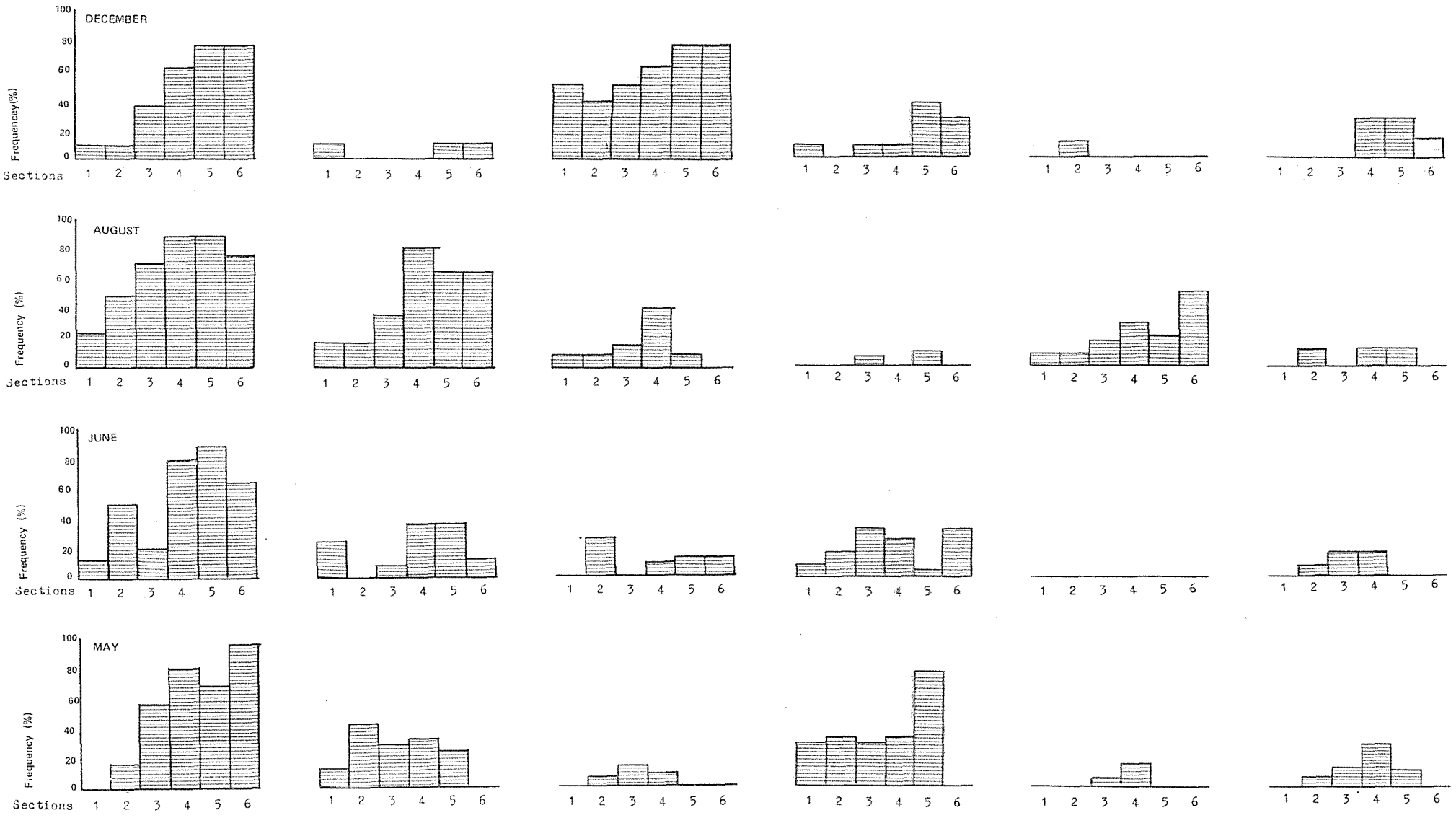

DECEMBER
AUGUST
JUNE
MAY
Frequency(%)
Frequency (%)
Sections
1 2 3 4 5 6
0 20 40 60 80 100
Alternaria alternata
Fusarium culmorum
Phoma sp
Torulopsis candida
Mucor racemosus
Rhodotorula rubra

Fig 12 b

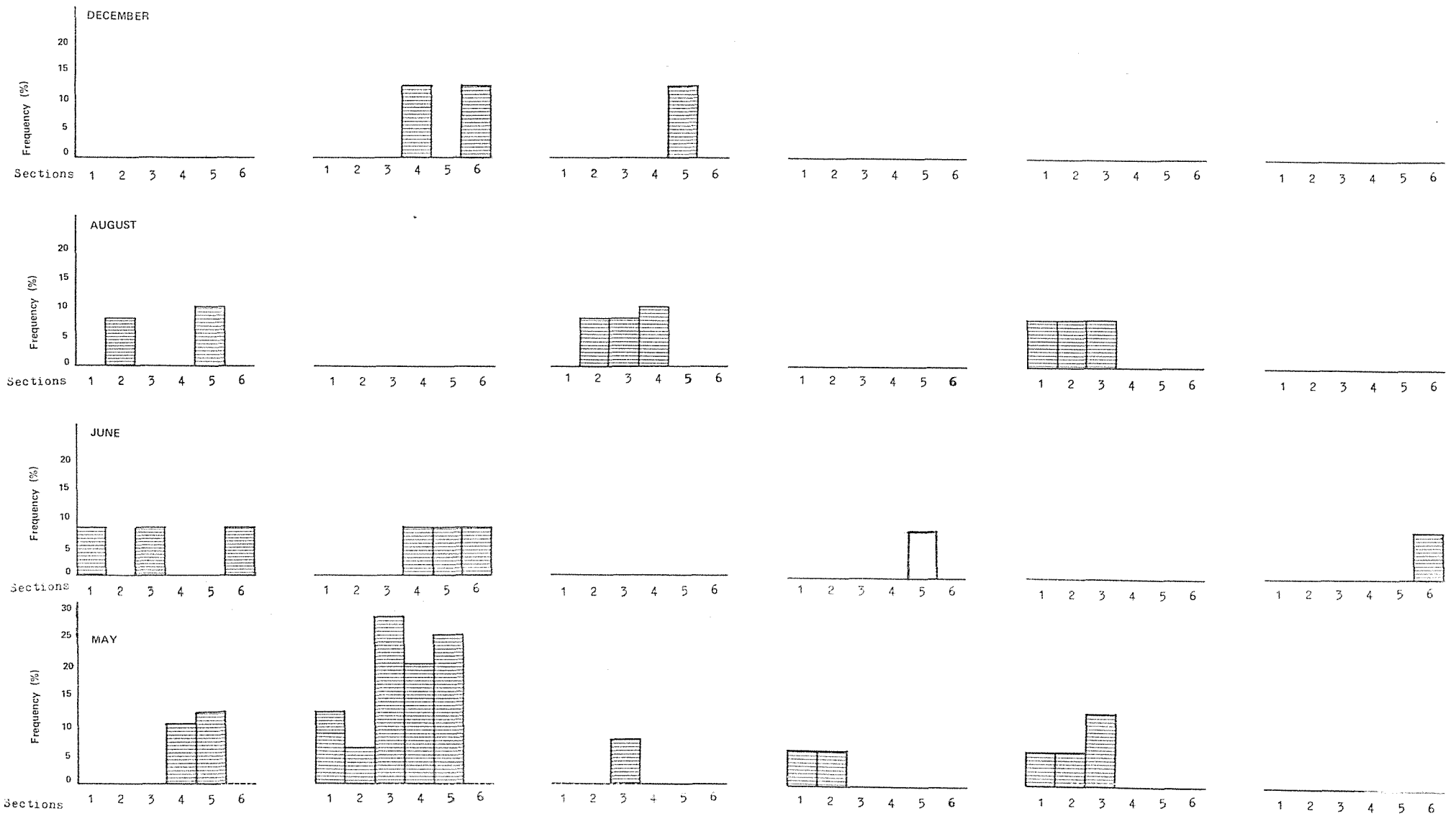

DECEMBER
AUGUST
JUNE
MAY
Frequency (%)
Sections
1 2 3 4 5 6
Penicillium sp
Sordaria fimicola
Cladosporium cladosporioides
Epicoccum nigrum
Paecilomyces marquendii
Aspergillus fumigatus

Fig 12 c

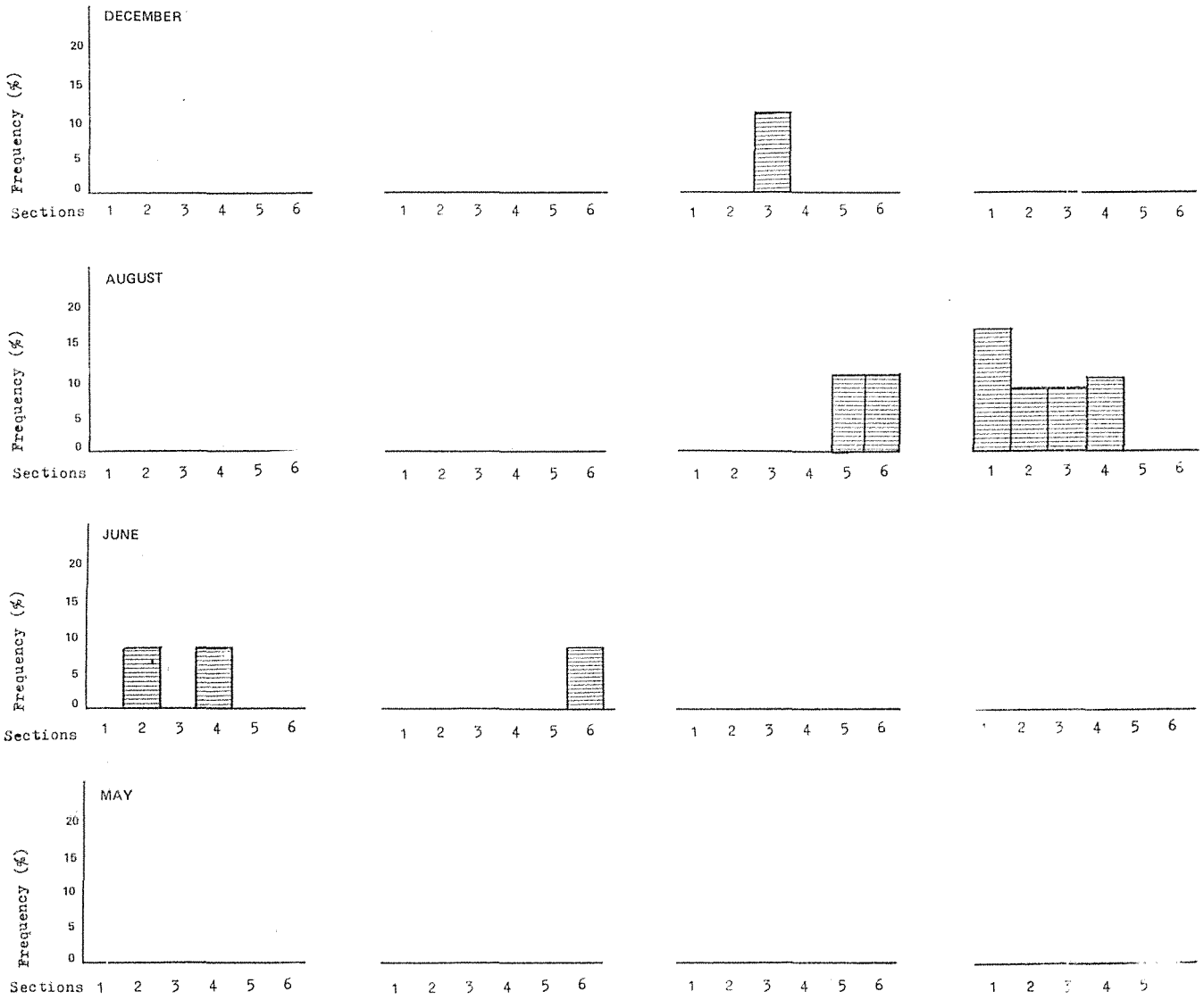

DECEMBER
AUGUST
JUNE
MAY
Frequency (%)
0
5
10
15
20
Sections 1 2 3 4 5 6
Pithomyces chartarum
Sterile brown mycelium
Trichoderma glaucum
Cephalosporium sp.

Fig 12 d

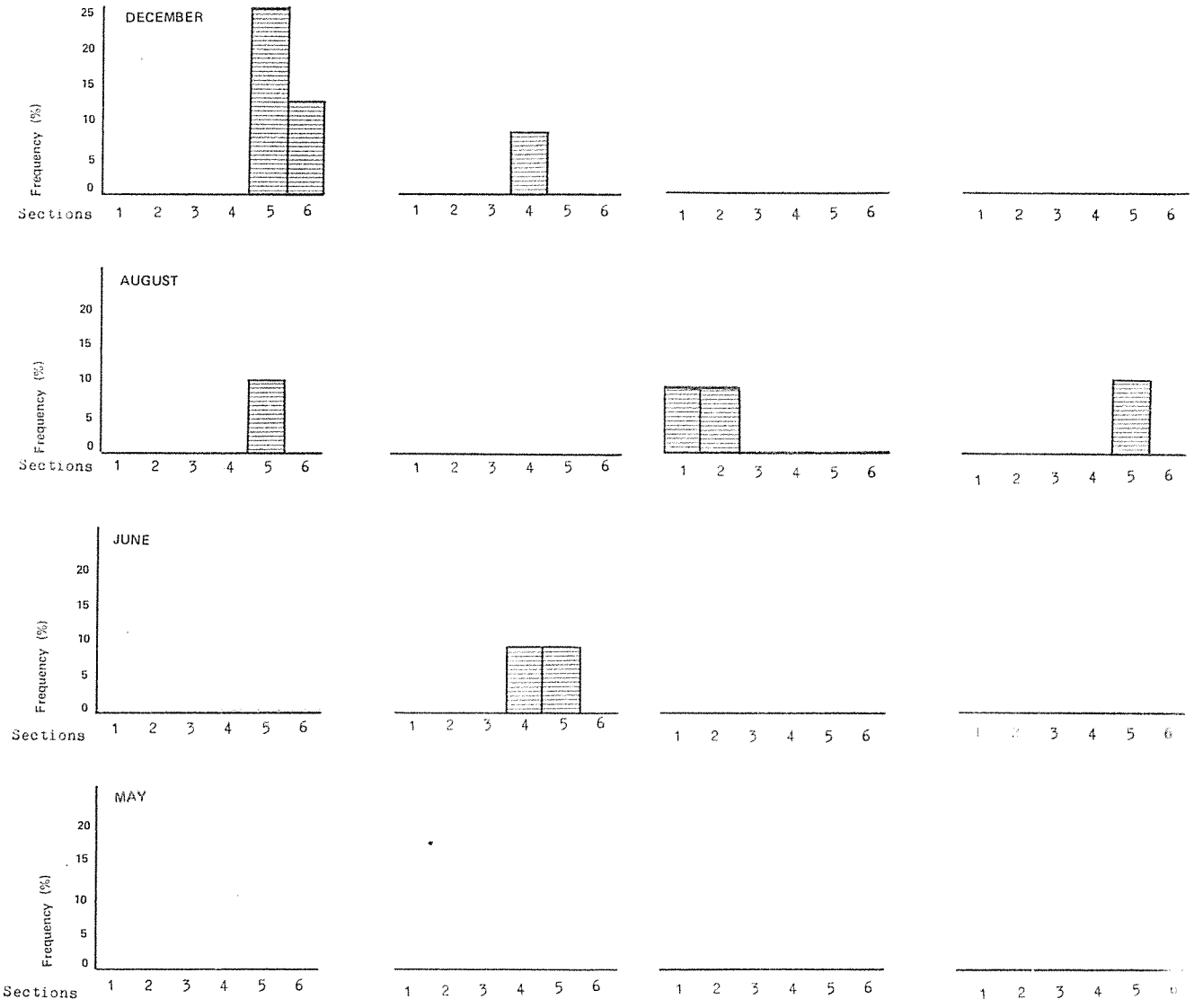

DECEMBER
AUGUST
JUNE
MAY
Frequency (%)
Sections
1 2 3 4 5 6
Chrysosporium sp.
sterile grey mycelium
Trichoderma viride
Botrytis sp.

Table 11: The frequency of demonstration of species in samples from 24 sheep in relation to pretreatment

Pretreatment	Untreated	Shorn	Frequency of species in total sheep sampled: Benlate plunge	Diazospray plunge	Diazotas shower	Diazotas spray
No. sheep sampled:	2	2	6	6	2	6
Alternaria alternata	2	2	6	6	2	6
Fusarium culmorum	1	2	4	6	1	5
Phoma spp	2	1	1	4	2	4
Mucor racemosus	0	1	2	0	1	2
Penicillium sp	0	1	1	2	0	3
Sordaria fimicola	1	0	1	2	1	4
Chrysosporium sp	0	0	1	0	0	0
Cladosporium cladosporioides	0	0	1	0	1	2
Epicoccum nigrum	0	1	1	1	0	0
Paecilomyces marquendii	0	0	2	0	0	0
Trichoderma glaucum	1	0	0	0	0	1
T. viride	0	1	0	1	0	0
Pithomyces chartarum	0	0	0	1	0	1
Cephalosporium sp	0	1	0	0	0	1
Aspergillus fumigatus	0	0	0	1	0	0
Sterile brown mycellium	0	0	0	0	0	1
Sterile grey mycellium	1	0	1	0	0	1
Botrytis sp	0	0	0	1	0	0
Rhodotorula rubra	2	0	3	3	1	2
Torulopsis candida	1	1	4	4	1	3
Total No of species	8	9	13	12	8	14

Table 12: Comparison of Microscopical and Cultural result

No. of species demonstrated by	
both microscopy and culture	10
No. of additional species demonstrated by	
microscopy only	7
culture only	9
Total species	26

4. Mycoflora of the Air

4.1 Monthly incidence of fungal spores in the air

The fungal spores present in the air over the paddock in which the experimental sheep were grazed were estimated using a Casella slit sampler (Methods 3.3). During the experimental period from April to December the air was sampled on 8 occasions and on each occasion no fungi were isolated at 37C incubation temperature. Therefore, only the average count of 2 plates incubated at 25C at each sampling period is recorded (Table 13). The count was highest in December with an average of 95 colonies/2000 l air. Counts during the preceeding 8 months varied little, between 14 colonies/2000 l air being recorded in November and 7 colonies/2000 l air in April (Table 13).

4.2 Incidence of individual fungi in the air

The monthly incidence of fungal species are listed in Table 13. The most frecuent spores were those of Cladosporium herbarum. This species was isolated more often in December and November (79 spores/2000 l and 10 spores/2000 l respectively), but it was not present in April. It was the high count of C. herbarum in December which was responsible for the high total count recorded in this month. There was also a rise of Fusarium spores in December (12 spores/2000 l air). The remaining species encountered showed no increase at this time and were present only infrecuently over the whole of the sampling period. Aspergillus fumigatus was present only during the winter months.

Table 13: Monthly incidence of fungal species in the air (No. colonies/2000 l air) (av 2 Plates)

	April	May	June	July	Aug	Sept	Nov	Dec	Total	%
Alternaria alternata	1	1	1	0	1	0	1	2	7	4.4
Aspergillus fumigatus	0	0	1	1	1	0	0	0	5	3.1
Aspergillus flavus	1	0	0	0	0	0	0	0	1	0.6
Cladosporium herbarum	0	3	2	1	1	1	10	79	97	61.6
Dreschlera hawaiiensis	1	0	0	0	0	0	0	0	1	.6
Fusarium culmorum	1	1	0	0	1	1	0	12	16	10.0
Fusarium lateritium	1	0	0	0	0	0	0	0	1	0.6
Fusarium oxysporum	1	0	0	0	0	0	0	0	1	0.6
Mucor racemosus	0	0	0	0	0	1	0	0	1	0.6
Penicillium frequentans	0	0	0	1	0	1	0	0	2	1.3
Pencillium nigricans	0	0	2	2	2	0	0	1	7	4.4
Penicillium sp	0	2	3	2	1	0	1	0	9	5.6
Phoma spp	1	0	1	0	0	2	0	1	5	3.1
Pithomyces chartarum	0	2	0	0	0	0	0	0	2	1.3
Sordaria fimicola	0	1	0	0	0	0	0	0	1	0.6
Rhodotorula rubra	0	0	0	1	0	0	1	0	2	1.3
Torulopsis candida	0	0	0	0	0	1	1	0	2	1.3
Total colonies	7	12	10	8	7	7	14	95	160	100.0

5. Degradation Of Wool By Selected Fungi

5.1 Assessment of degree of degradation

After incubation in wool-containing media, many of the fungal species selected (Methods 8.1) had colonised the wool and broken down the intact structure of the fibres, resulting in liberation of cortical cells into the medium. With some species at the shorter incubation period, this was noted only at the ends of the fibres (Plate 10). As such damage could be due to factors not associated with fungal growth, degradation was assessed as the liberation of cortical cells from the central parts of the fibres. Such degradation could be assessed as mild, i.e. a few cells liberated (Plate 11), moderate (Plate 12) and strong, i.e. many cells liberated (Plate 2). Other samples showed no degradation (Plate 13) nor did control cultures (Plate 14).

5.2 Degradation in wool-agar medium

5.2.1 2 weeks culture

When grown on propylene oxide sterilized wool in an agar medium for 2 weeks, 6 of the fungi selected (*Alternaria cheranthi*, *Fusarium oxysporum*, *Fusarium semitectum*, *Phoma* sp, *Trichoderma viride* and *Trichophyton mentagrophytes*) showed some ability to breakdown the wool. On autoclaved wool, a.n additional 5 species (*Aspergillus flavus*, *Beauveria bassiana*, *Cladosporium cladosporioides*, *Epicoccum purpurascens* and *Trichoderma glaucum*) showed ability to breakdown the wool (Table 14).

5.2.2 4 weeks culture

At 4 weeks incubation period 11 of the isolates had degraded both types of wools. Of the species which did not degrade wool at 2 weeks, *Peyronellaea glomerata* had degraded autoclaved wool at 4 weeks, and *Epicoccum nigrum*, *Pithomyces chartarum* and *Sordaria fimicola* had degraded both propylene oxide sterilised and autoclaved wools. *Aspergillus niger*, *Aureobasidium pullulans*, *Paecilomyces marquendii*, *Penicillium canescens* , *Pseudoeurotium zonatum* and the control cultures showed no degradation of wools at either period. There was no further change after 4 weeks incubation (Table 14).

5.3 Degradation in wool-water medium

5.3.1 2 weeks culture

Of the 20 isolates tested only 3 (*A. niger*, *P. canescens*, *Pseudoeurotium zonatum*) failed to attack either of the wool substrates

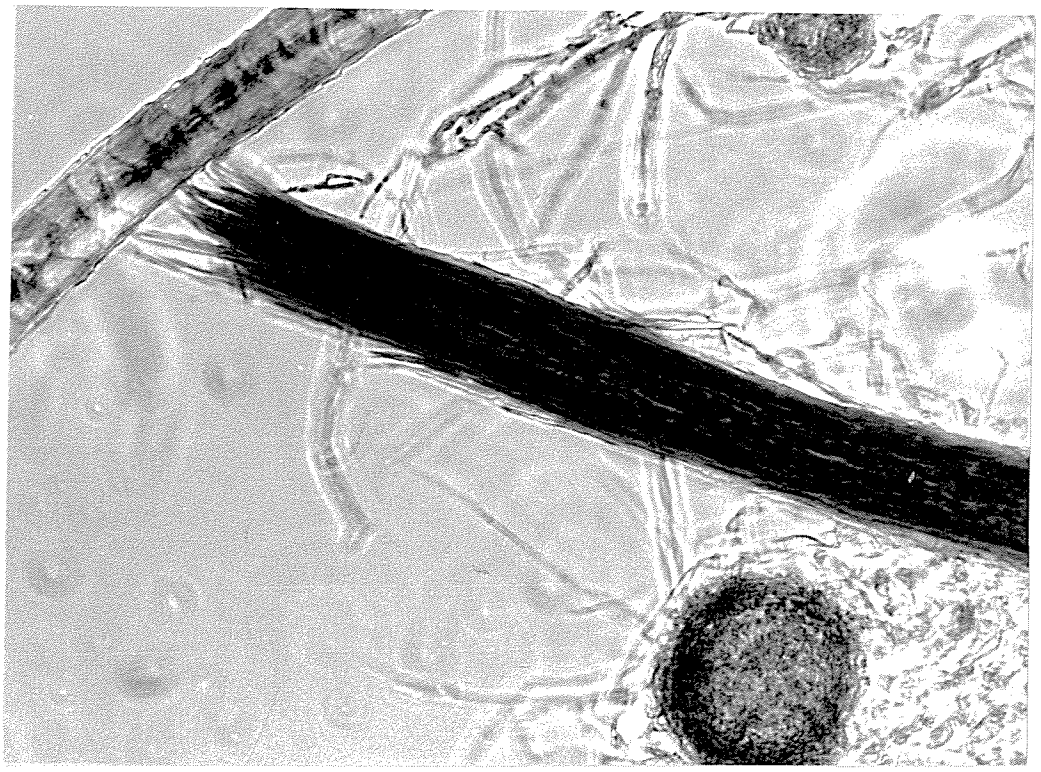


Plate 10: Degradation of wool at the end of fibre as demonstrated by Phoma sp. in wool-yeast extract medium at 2 weeks incubation period.

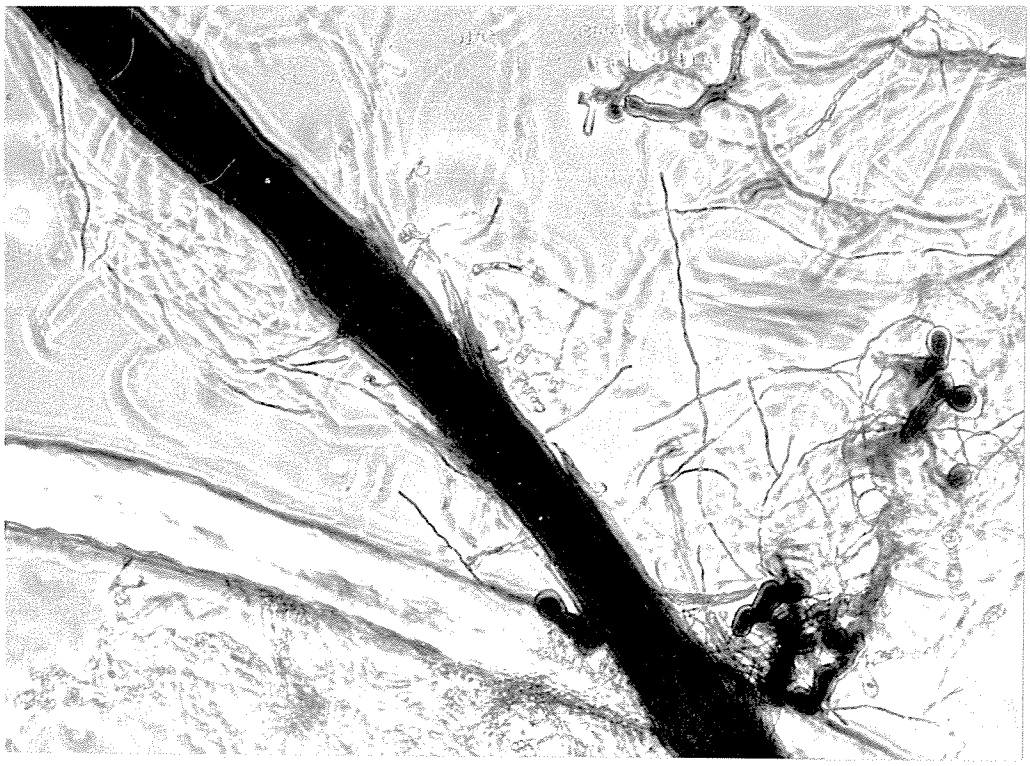


Plate 11: Mild degradation of wool fibre as demonstrated by Aureobasidium pullulans in wool-yeast extract medium at 4 weeks incubation period.

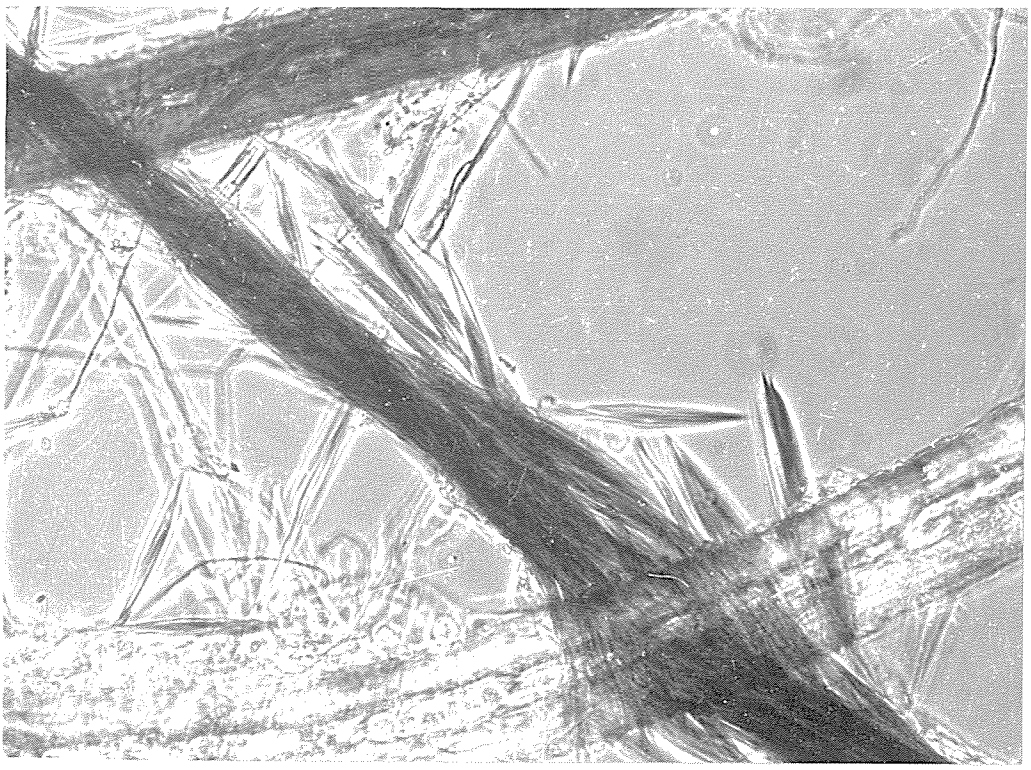


Plate 12: Moderate degradation of wool fibres as demonstrated by Fusarium semitectum in wool-yeast extract medium at 4 weeks incubation pericd.

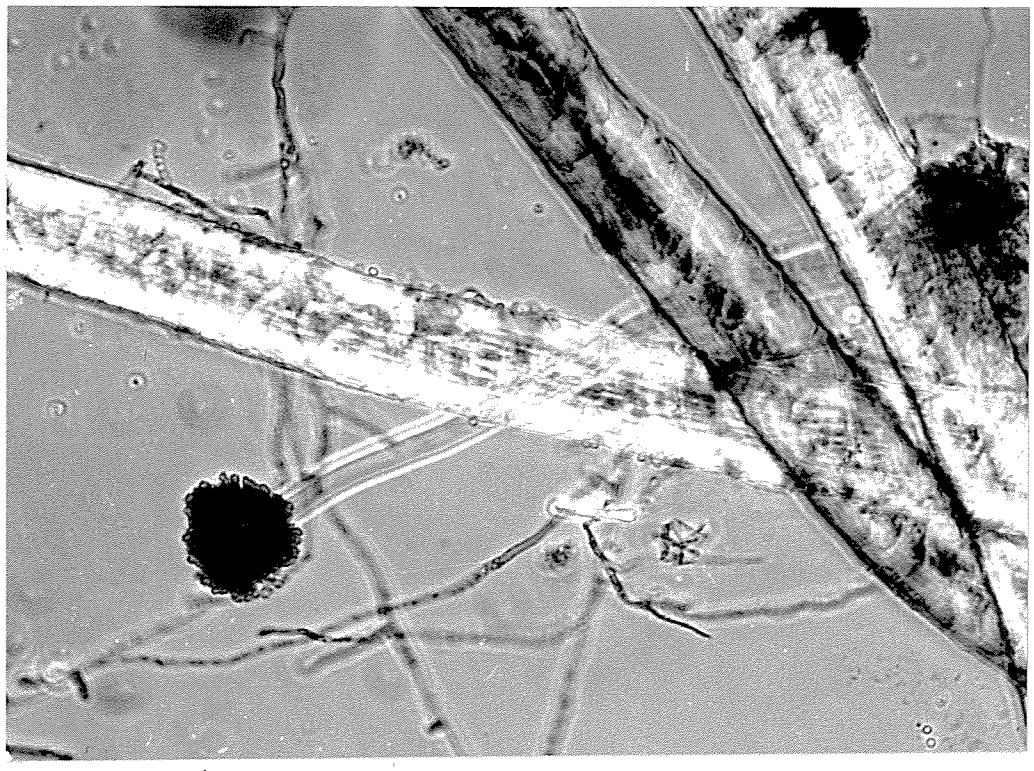


Plate 13: Negative degradation of wool fibres as demonstrated by Aspergillus niger in wool yeast extract medium at 4 weeks incubation

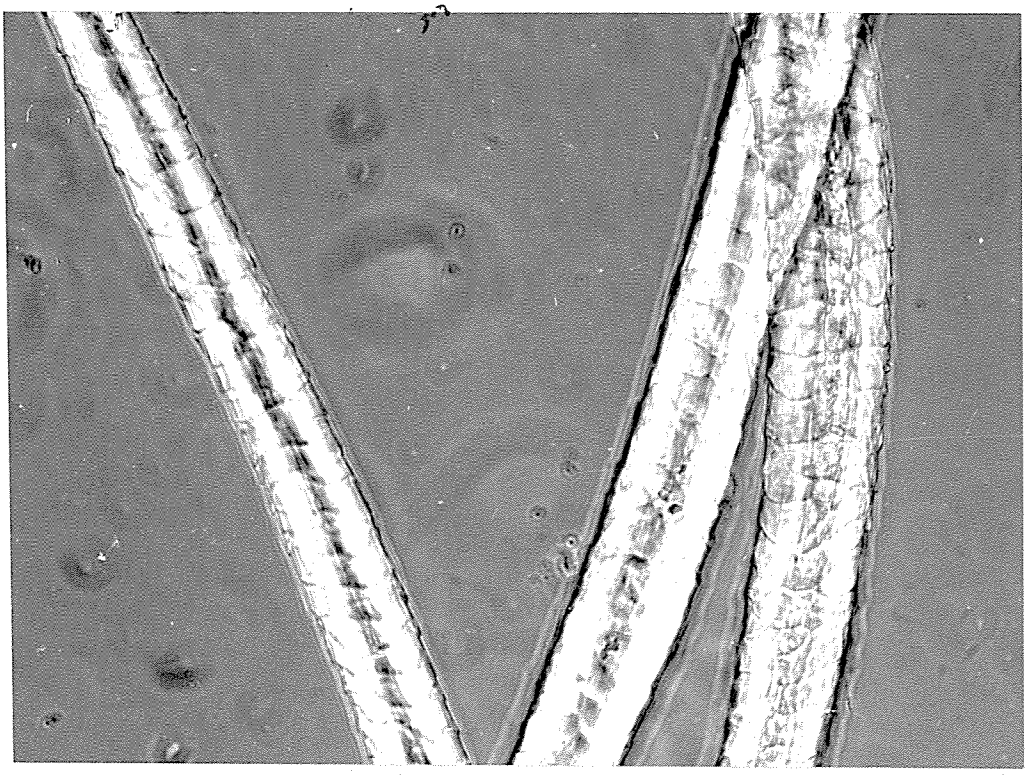


Plate 14: Negative degradation of wool fibres as demonstrated in control culture (wool-yeast extract medium)

Table 14 Degradation of wool by fungi in wool-agar medium*

Substrate #	Incubation period 2 weeks P	2 weeks A	4 weeks P	4 weeks A
Aspergillus flavus	-	+	-	+
Aspergillus niger	-	-	-	-
Aureobasidium pullulans	-	-	-	-
Alternaria cheranthi	+	+	++	+
Beauveria bassiana	-	+	-	+
Cladosporium cladosporioides	-	+	-	+
Epicoccum nigrum	-	-	+	+
Epicoccum purpurascens	-	+	+	+
Fusarium oxysporum	+	++	+	++
Fusarium semitectum	+	+	+	++
Paecilomyces marquendii	-	-	-	-
Pencillium canescens	-	-	-	-
Peyronellaea glomerata	-	-	-	+
Phoma species	+	+	+	+
Pithomyces chartarum	-	-	+	+
Pseudoeurotium zonatum	-	-	-	-
Sordaria fimicola	-	-	+	+
Trichoderma glaucum	-	+	+	+
Trichoderma viride	+	+	+	+
Trichophyton mentagrophytes	++	++	+++	+++
Control	-	-	-	-

* Degradation assessed as extent of cortical cell release on a scale from

\+ (a few cells liberated)to

+++ (many cells liberated and

\- (no free cortical cells detected)

Substrate: P = propylene oxide sterilised wool

A = autoclaved wool

in water culture after 2 weeks incubation (Table 15) but A. flavus, A. pullulans, C. cladosporioides, E. nigrum, E. purpurascens, and P. marquendii attacked only the autoclaved wool. The remaining 11 species had degraded both the autoclaved and propylene oxide sterilised wool.

5.3.2 4 weeks cultures

At 4 weeks incubation period the 4 previously undegraded samples remained so, including the control. P. marquendii and A. flavus which attacked only autoclaved wools at 2 weeks, were found to have degraded both autoclaved and propylene oxide sterilised wool at 4 weeks. There were no further changes after 4 weeks.

5.4 Degradation in wool-yeast extract medium

5.4.1 2 weeks cultures

Table 16 shows that after 2 weeks incubation at room temperature, 15 of the isolates disintegrated autoclaved and/or propylene oxide sterilised wool in wool-yeast extract culture medium. A. flavus and C. cladosporioides degraded only autoclaved wools. The remaining 5 isolates showed no ability to breakdown either autoclaved or propylene oxide sterilised wool within 2 weeks.

5.4.2 4 weeks cultures

Of the 5 previously negative cultures, 2 (A. pullulans and P. zonatum) degraded both autoclaved and propylene oxide sterilised wool at 4 weeks. C. cladosporioides which had degraded only autoclaved wool at 2 weeks degraded both types of wool at 4 weeks. A. niger, P. marquendii, P. canescens and the control showed no attack of the wool after 4 weeks incubation.

5.5 The uptake of lactophenol cotton blue by wool fibres during colonisation

In the series of 168 wool samples examined to determine the ability of fungi to degrade wool, records were made of the uptake of lactophenol cotton blue by the wool fibres following incubation with the test fungi.

Uptake of the stain occurred with actual degradation of the wool fibres in 123 (73.2%) of the total samples (e.g. Plate 2). A further 32 (19%) showed positive uptake but no degradation but the staining of the samples was light and irregular in distribution. (Plate 13).

Table 15: Degradation of wool by fungi in wool-water medium

Substrate	Incubation period: 2 weeks P	2 weeks A	4 weeks P	4 weeks A
A. flavus	–	+	+	+
A. niger	–	–	–	–
A.pullulans	–	+	–	+
A. cheranthi	+	+	++	+
B. bassiana	+	+	+	++
C. cladosporioides	–	+	–	+
E. nigrum	–	++	+	++
E. purpurascens	–	++	+	++
F. oxysporum	+	+	+	+
F. semitectum	+	+	+	+
P. marquendii	–	+	+	+
P. canescens	–	–	–	–
P. glomerata	+	+	++	+
Phoma sp.	++	++	++	++
P. chartarum	++	++	++	++
P. zonatum	–	–	–	–
S. fimicola	+	+	+	+
T. glaucum	+	+	++	+
T. viride	+	+	+	+
T. mentagrophytes	++	++	+++	+++
Control	–	–	–	–

Table 16: Degradation of wool by fungi in wool-yeast extract medium

Substrate	Incubation period: 2 weeks P	2 weeks A	4 weeks P	4 weeks A
A. flavus	–	+	+	+
A. niger	–	–	–	–
A. pullulans	–	–	+	+
A. cheranthi	+	++	++	++
B. bassiana	+	+	++	+
C. cladosporioides	–	+	+	+
E. nigrum	+	+	++	+
E. purpurascens	+	+	++	+
F. oxysporum	++	++	++	++
F. semitectum	++	++	+++	+++
P. marquendii	–	–	–	–
P. canescens	–	–	–	–
P. glomerata	+	+	+	++
Phoma sp.	+	+	+	+
P. chartarum	+	+	++	+
P. zonatum	–	–	+	+
S. fimicola	+	+	++	+
T. glaucum	+	+	+	+
T. viride	++	+	++	++
T. mentagrophytes	++	+	++	++
Control	–	–	–	–

There were 13 samples which showed no uptake of the stain and no degradation (Table 17).

Of the 8 control samples individual fibres in 50% of the cultures showed uptake of stain but this was not as consistant as in degraded samples. No difference was observed between the frequency of uptake of stain by wool fibres in wool-yeast extract medium and wool-water medium.

Table 17: The uptake of stain by wool fibres after the growth of fungi

	Wool-yeast extract medium	Wool-water medium	Total
No. samples taken	84	84	168
No. samples showing positive stain uptake	76	76	155
No. samples showing positive degradation	62	61	123
No. samples showing positive stain uptake + negative degradation	17	15	32
No. samples showing negative stain uptake + negative degradation	5	8	13
No. control cultures	4	4	8
No. controls showing positive stain uptake	2	2	4

6. Additional Studies

6.1 Colonisation of human hair by the selected fungi in yeast-extract medium

All the fungi tested (Methods 9.1) were found to grow in hair-yeast extract medium but only T. mentagrophytes showed positive internal colonisation of the hair. Although A. flavus and P. glomerata did not colonise the hair, free cortical cells were detected in 4 week cultures (Table 18).

The hairs colonised by T. mentagrophytes were densely stained by lactophenol cotton blue. The hairs in cultures of some of the other species showed positive stain uptake also, but the staining of the hair was less dense in these cases.

6.2 Ability of fungi to grow at skin temperature

Of the 20 selected species, 12 were found to be unable to grow at 37C (Methods 9.2). Only 8 species (A. flavus, A. niger, Alternaria cherathi, Aureobasidium pullulans, P. marquendii, P. canescens, S. fimicola and T. mentagrophytes) were positive for growth at this temperature. S. fimicola, A. flavus and A. niger showed extensive growth, S. fimicola occupying the whole of the surface area of the plate after 7 days. (Table 19).

6.3 The effect of wool fat on selected fungi

The growth of 10 of the 20 species tested was inhibited by wool fat (Methods 9.3). A. niger showed the highest inhibition while A. cheranthi was the least inhibited species. The wool fat had no effect on F. semitectum and S. fimicola (Table 20). The growth of the remaining 8 species was stimulated. The physical appearance and pigmentation of the colonies of many of the selected species was also affected but no account is taken of this in the present section.

6.4 Proteolytic activity of the selected fungi

Of the 20 species tested (Methods 9.4) Pseudoeurotium zonatum possessed the highest proteolytic activity (2.857) followed by Cladosporium cladosporioides and Trichophyton mentagrophytes. Alternaria cheranthi, Aureobasidium pullulans and E. nigrum showed negative proteolytic activity. The proteolytic activity of the remaining species varied between 1.000 and 1.200 (Table 21). The activity is illustrated in Plate 15.

Table 18: Colonisation of human hair in yeast-extract medium

Fungal species	Growth		Colonization		Stain uptake	
	2 wks	4wks	2 wks	4 wks*	2 wks	4 wks*
Alternaria cheranthi	+	+	–	–	+	–
Aspergillus flavus	+	+	–	–*	–	+
Aspergillus niger	+	+	–	–	–	–
Aureobasidium pullulans	+	+	–	–	–	–
Beauveriabassiana	+	+	–	–	–	–
Cladosporium cladosporioides	+	+	–	–	–	+
Epicoccum nigrum	+	+	–	–	–	–
Epicoccum purpurascens	+	+	–	–	–	–
Fusarium oxysporum	+	+	–	–	–	–
Fusarium semitectum	+	+	–	–	–	–
Paecilomyces marquendii	+	+	–	–	–	–
Pencillium canescens	–	+	–	–	–	–
Peyronellaea glomerata	+	+	–	–*	+	–
Phoma sp.	+	+	–	–	–	–
Pithomyces chartarum	+	+	–	–	–	–
Pseudoeurotium zonatum	+	+	–	–	–	–
Sordaria fimicola	+	+	–	–	–	–
Trichoderma glaucum	+	+	–	–	–	–
Trichoderma viride	+	+	–	–	–	–
Trichophyton mentagrophytes	+	+	+	+	+	+

* free cortical cells detected

Table 19: Growth of fungi at 37C

Species	Growth *
A. cheranthi	0.005
A. flavus	0.842
A. niger	0.942
A. pullulans	0.031
B. bassiana	-
C. cladosporioides	-
E. nigrum	-
E. purpurascens	-
F. oxysporum	-
F. semitectum	-
P. marquendii	0.031
P. canescens	0.03
P. glomerata	-
Phoma sp.	-
P. chartarum	-
P. zonatum	-
S. fimicola	1
T. glaucum	-
T. viride	-
T. mentagrophytes	0.093

* $\frac{\text{Area of colony}}{\text{Surface area of agar}}$ (Methods 9.1)

Table 20: Inhibition of fungal growth by wool fat:

Species	Inhibition (%)*
A. cheranthi	4.17
A. flavus	18.5
A. niger	31.4
A. pullulans	+50.0
B. bassiana	9.52
C. cladosporioides	5.56
E. nigrum	+21.1
E. purpurascens	+27.3
F. oxysporum	6.67
F. semitectum	0
P. marquendii	+4.76
P. canescens	+14.3
P. glomerata	+8.70
Phoma sp.	+14.8
P. chartarum	25.0
P. zonatum	14.3
S. fimicola	0
T. glaucum	13.6
T. viride	7.32
T. mentagrophytes	+9.09

* $\frac{Rc-Rf}{Rc}$ X 100 (Methods, 9.3)

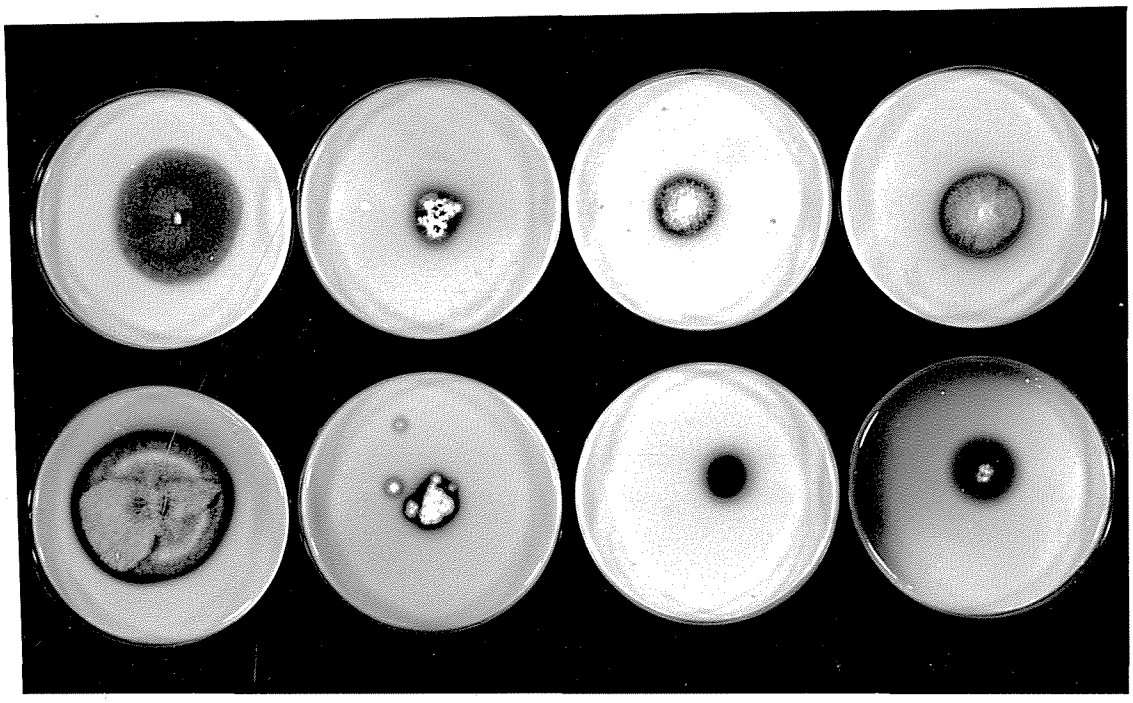
\+ Growth stimulated

Table 21: Proteolytic activity of the selected fungi

Species	Proteolytic activity *
A. cheranthi	0
A. flavus	1.046
A. niger	1.018
A. pullulans	0
B. bassiana	1.079
C. cladosporioides	1.405
E. nigrum	0
E. purpurascens	1.013
F. oxysporum	1.062
F. semitectum	1.052
P. marquendii	1.055
P. canescens	1.034
P. glomerata	1.140
Phoma sp.	1.048
P. chartarum	1.071
P. zonatum	2.857
S. fimicola	1.000
T. glaucum	1.012
T. viride	1.012
T. mentagrophytes	1.360

* (Method 9.4)

Plate 15: Positive proteolytic activities (as shown by clear zone around fungi) as demonstrated by various fungi on gelatin agar. Top row from left : *T. viride*, *B. bassiana*, *D. hawaiiensis* *P. canescens*



Bottom row from left: *F. semitectum*, *T. mentagrophytes* *C. cladosporioides*, *P. zonatum*

DISCUSSION

The enumeration of the fungal population in an environment such as fleece wool is influenced by many factors, including the isolation techniques employed, the viability of the fungal components in the fleece, the media used for isolation, and the incubation temperature. In the present study, two different incubation temperatures (25C and 37C) were used and it has been shown that the population of fungi able to grow at 25C is considerably higher than that of those able to grow at 37C (Table 4). The overall average mould count at 25C, for example, was just over 3 000 CFU/g wool while at 37C it was 825 CFU/g.

Theoretically, as the majority of fungi on the fleece must be introduced from the environment, climatic changes in the environment, and hence the number of fungi within the environment, should lead to changes in the fungal population of the fleece. Factors influencing the counts obtained at individual sampling dates, therefore, could include rainfall and environmental temperature. It has been reported that the number of bacteria in the fleece of Corriedale sheep varies with rainfall (Mulcock & Fraser, 1958). In the case of mycelial fungi it is unlikely that growth occurs within the fleece (to be discussed later). It would seem probable, nevertheless, that as rainfall increases more fungal components are impacted onto the fleece from the air (Gregory, 1973). Furthermore, wet wool may trap fungal fragments from the general environment to a greater extent than dry fleece. In Figs 2-1, 2-2, at both 25C and 37C, some of the average mould and yeast counts were markedly high in samples taken when the rainfall in the preceeding 3 days was at its peak. For example, for the samples taken at 14.5.74, the mould count was 5 689 CFU/g wool at 25C and the yeast count 108 662 CFU/g wool. On this sampling date it was raining and the rain in the preceeding day reached 59.3mm. The average rainfall over the 3 preceeding days was 21mm, the highest recorded during the whole experimental period (Fig 2-1). This hypothesis did not hold true, however, for other samples such as those taken in November, when the rainfall in the preceeding 3 days was zero, so that the fleece wool would have been dry and yet the count for moulds was well above average (5 370 CFU/g).

The activity of microorganisms is contingent upon the presence of moisture and fleece moisture is a primary factor controlling bacterial growth in the fleece (Fraser, 1957). In the case of yeasts, it is possible that multiplication might occur in damp fleece so that counts

could be high. A very high yeast count of 108 662 CFU/g wool was obtained on 14.5.74, when the fleece was very wet. Although high yeast counts were sometimes associated with high rainfall, on other occasions high counts were obtained when the fleece was dry. For example, the yeasts at 37C had a very high count of 633 190 CFU/g wool on 20.8.74 (which influenced the overall average count of 11 537 CFU/g wool, so that all other samples gave below average counts). Although there had been some slight rain before the sampling (the average over the 3 preceeding days being 3.3mm) on the day of sampling it was dry and the count could be expected to be lower.

There was, therefore, no apparent correlation between the fungal population within the fleece and the environmental rainfall immediately preceeding sampling; nor was there any correlation between fungal counts and average monthly rainfall. This latter could be considered a better reflection of the relationship between fungal levels in the environment and rainfall, as it influences the growth of fungi in the general environment and hence their availability for impaction or deposition onto the fleece. It is also possible that heavy rainfall over a period might reduce the fungal population of fleece wool by washing spores and fragments off the wool. This could, perhaps, be demonstrated by the count in July, when the rainfall was high (8.1mm) and the mould count was below average, but, again, this was not consistent during other months.

Thus, because of the multifactorial nature of the relationship between rainfall and the total fungal population of fleece wool, it is impossible to derive any correlation between the two.

Environmental temperature is the next factor to be considered. Again, counts bore no constant relationship to temperatures during the 3 days preceeding sampling. When average monthly temperatures are considered (Figs 3-1, 3-2) some relationship to counts can be seen in the case of moulds growing at 25C. The mould populations at 25C varied with season. The population was generally lower in winter (e.g. 1 900 CFU/g wool in July) and higher in the warmer months (7 595 CFU/g wool in December and 4 500 CFU/g wool in April). These trends were true for most groups of sheep examined. But similar variations were not observed for moulds at 37C nor the yeast populations at both 25C and 37C (Figs 3-1, 3-2). For example mould counts at 37C averaged 265 CFU/g wool (i.e. well below average in December when the highest average monthly temperature (15.7C)

was recorded. Similarly for yeasts at 37C, both very high and very low counts were obtained in August and July respectively when the temperature was lowest.

The length of wool on the animal may be an influential factor in the fleeces ability to retain microorganisms and this could affect the size of the fungal population within the fleece. But the results presented here indicate no major difference between the fleece of untreated and shorn animals. Moulds at 25C from these 2 fleece types seemed to be equally influenced by the average monthly environmental temperatures rather than by fleece length. Mould counts at 37C and yeast counts at both temperatures also failed to show any correlation with fleece length.

Other factors influencing counts could include sheep to sheep and sheep to vegetation contact, but these factors were not able to be studied.

The treatment of stock with insecticides to control ectoparasites is a routine task on all sheep farms in New Zealand. Thus it seemed reasonable to look at the effect of some of the commonly used insecticides on the mycoflora of fleece. The fungicide Benlate was also included in these comparisons. The persistence of the insecticides on the fleece depends on a number of factors including the method of application and the amount of grease in the fleece (Charleston (1976); personal communication). If the chemical has an effect on the mycoflora, therefore, a low viable fungal count might be expected in the earlier samples taken, when the concentration of the chemical is highest, and then a gradual increase in the count should occur as post-treatment time increases. The results shown in Figs 4 - 7 did not support this possibility. For example, 2 weeks after treatment the mould count (at 25C) of samples taken from sheep which were pretreated by Diazotas shower were higher (5 695 CFU/g wool) than that of samples taken at 6 weeks (3 580 CFU/g wool) and 10 weeks (3 345 CFU/g wool) post treatment (Fig 4). Similarly, the yeast count (63 250 CFU/g wool) at 25C of 2 weeks post-treatment samples was shown to be higher than the rest of the samples (Fig 5). The counts varied little from those of untreated animals and seemed more related to environmental temperature than to exposure to any particular treatment. The fungal counts of Benlate-treated fleeces also showed no major differences to those of untreated fleeces, nor to those of Diazinon treated fleece. After dipping, the fleece wool must remain wet for some time and this might increase the chances of more fungi being trapped (as was discussed earlier), thus tending to increase fungal counts in the earlier samples.

When considered over the whole experimental period, the shorn samples had a somewhat below average count for moulds at 25C (Table 5). The mould counts of Diazotas shower and Diazotas spray treated fleece had higher than average counts and average counts of samples of other treatments such as Benlate and Diazospray were close to the average of 3-643 CFU/g wool. The mould counts at 37C and the yeast counts of the individual treatments at both 25C and 37C did not produce similar patterns.

It would seem, therefore, that these chemicals have little or no consistent effect on the total mycoflora of the fleece.

The dilution plating technique used in this study provided information on the total fungal population of fleece wools. To determine the actual distribution pattern of fungi along the length of the fleece it was necessary to use a method such as the impression technique. The mycoflora at the tips of the fibres could be expected to differ from that in the middle and basal sections of the fleece. The microclimatic relative humidity in the tip and middle of the fibres is reported by Fraser (1957) to be considerably influenced by rainfall. Only pronounced changes of macroclimate lead to a change at the basal level. Under moderate weather conditions a positive relative humidity and moisture gradient exists from base to tip of the fibres (Fraser, 1957). On wetting of the fleece the gradient markedly increases. Extreme wetting causes a temporary disruption of the gradient owing to the abundant moisture in the basal and middle sections. Thus these factors might influence the distribution of fungi within the fleece.

In the present study it has been repeatedly shown that the fungal components observed by both microscopy and culture in the impression technique were generally more concentrated in the middle sections of the fibres and that there were fewer fungi in the apical and basal parts of the fleece. Alternaria spores for example, were shown to be concentrated in the 3rd, 4th and 5th sections of the fibres. A lower incidence of fungi in the apical parts of the fleece is to be expected, as the tip is the most exposed part of the fibre and is influenced by extraneous factors, such as rainfall, wind and handling of the animals, to a greater degree than deeper parts.

The fleece section nearest to the skin was also shown to have a markedly lower incidence of fungi. The factor involved here could be the 'trapping' effect of the dense felt of fibres in the median parts of the fleece for spores falling onto the fleece from outside and being washed

down from the tip by rainfall. Furthermore, as the fleece is constantly growing outwards, the basal sections would tend to be relatively clear of spores. As the median part of the fleece might thus act as a trap for spores, it might be expected that larger spores would be more readily caught amongst the fibres in the median parts, but smaller spores might penetrate deeper into the fleece. Such an hypothesis is not supported by the present findings as over the whole number of samples examined both large and small spore types were in the basal parts of fibres in similar numbers, although in culture small-spored types predominated (Figs 9-1, 9-2, 11-1, 11-2).

As was demonstrated in the studies concerning the total fungal population, the impression technique again showed a preponderance of the major fungal types in the warmer months (May), with a decline in numbers in June and August. Alternaria spores, for example, were frequently observed along the fibres in May but showed a decline over June and August (Fig 10). The distribution of the less common types observed by microscopy was, however, scattered, and some spores e.g. Cladosporium and Curvularia were seen only in one sample each. The cultural technique did not support the general seasonal pattern of species, however, as even the most common types e.g. Alternaria showed a fairly uniform distribution over the four months examined (Fig 12). The presence of fungi within the fleece, therefore, presumably varies with the abundance of the fungi in the general environment.

Insecticides and fungicides have been shown to have little or no effect on the total fungal population in the fleece, but the results of the impression technique suggested that these chemicals can, to a certain extent, affect the actual variety of fungi present. Fewer fungal species were isolated or seen in the untreated and shorn samples and most of the fungi recorded in Tables 9 and 11 were observed or isolated from treated samples. Such a result might indicate that treatment in general makes the fleece more prone to trapping spores and hyphae. There was no difference in this respect between Benlate and Diazinon treated animals. Nor was there any difference between shorn and untreated animals although it might be expected that the fleece of shorn sheep would trap fewer spores.

The distribution of fungi in fleece wools has not been investigated previously. Only Peyronellaea has previously been reported to be carried in the tips of the fleece of many sheep (Mulcock, 1965; Austwick, 1975) and Mulcock also reported that pycnidia are commonly formed here. No

pycnidia were ever observed within the fleece in the present study but the chlamydospores of this fungus were occasionally found (Fig. 10b).

The technique involved in the study of fungal distribution within the fleece environment was an adaptation of the leaf impression technique often used in plant pathology (Leben, 1961). As some fungal species which were isolated by cultural techniques were not seen microscopically and vice versa, it is suggested that both methods should be used in the technique. The two methods together improved the overall picture of the types of fungi present. Furthermore, microscopy can inform us of the variety of fungi at different levels but cannot differentiate between viable and dead spores or hyphae. Hyphae were observed by microscopy and were grouped as 'passive' (Plate 6) and 'Active' (Plate 7) but it is impossible to determine with certainty that the 'active'hyphae were viable and the 'passive' hyphae were not. The grouping is thus arbitary. During the experimental period there was only two samples in which actively growing conidiophores bearing conidia were seen microscopically on wool-impressed agar and the fungus was identified as a species of Paecilomyces.

While larger spores such as Helminthosporium, Curvularia, Keratinomyces and Dictyosporium were readily observed by microscopy, none of these were isolated in culture. This could be due to their overgrowth by other species, or their conidia seen on microscopy being non-viable. Some of the species producing smaller spores e.g. Cephalosporium roseogriseum and Chrysosporium species grew in culture but were never distinguished by microscopy.

As many fungi were found to be present in fleece wools, although few apparently showed active growth in vivo, it was considered worthwhile to investigate to what extent fungi could actually grow on wool in vitro. Wool fibres, as described in the introduction, are composed of keratin which is resistant to enzymatic attack. Untreated keratin can be utilized in vitro by relatively few fungi.

In laboratory investigations into the growth of fungi on wool, it is most important to avoid any denaturation of the wool, such as occurs during autoclaving (Mathison, 1964). (Noval & Nickerson, 1959) found satisfactory sterilization of wool with ethylene oxide and could not detect any damage, nor were any substances associated with wool removed. Ethylene oxide has a very low boiling point and is explosive in an air mixture, and therefore cannot be handled satisfactorily in the laboratory at room

temperature without special equipment. Hensen and Snyder (1947) found pea straw apparently remained in its natural condition after being sterilised by the safer propylene oxide. This method was adapted and used to sterilize hair by Griffin (1959), with satisfactory results. Propylene oxide was therefore preferred as sterilant in the present study.

Of the 19 fungi selected for investigations of their ability to breakdown wool in vitro most were shown to disinte grate autoclaved fibres to cortical cells. There were fewer able to degrade propylene oxide sterilised wool (Tables 14, 15, 16), which is in agreement with the findings of other workers (Mcquade, 1964, Mulcock, 1965). The manner in which fungi attack wool differs to that by which they breakdown hair in that hair is attacked by means of boring hyphae, perforating organs and/or cuticle lifting (English, 1963). No free cortical cells can usually be detected in hair cultures, which may be due to the presence of a thicker cuticle around the hair cortex. In the present studies a few cortical cells were liberated in hair cultures by A. flavus and P. glomerata only.

The degradation of wool was studied in three media. Based on the relative abilities of the selected fungi to degrade wool fibres in wool-agar medium (Table 14) it was possible to establish three groups : Those fungi degrading autoclaved wool only (A), those degrading autoclaved and propylene oxide sterilised wool (A + P) and those degrading neither (O) (Table 22).

Table 22: Groupings of fungi according to their ability to degrade wool in wool-agar medium

Group:A	A + P	O
A. flavus	A. cheranthi	A. niger
B. bassiana	E. nigrum	A. pullulans
C. cladosporioides	E. purpurascens	P. marquendii
P. glomerata	F. oxysporum	P. canescens
	F. semitectum	P. zonatum
	P. chartarum	
	Phoma sp.	
	S. fimicola	
	T. glaucum	
	T. viride	
	T. mentagrophytes	

There was a different distribution of species among the three groups in the other two media. More species of fungi showed positive degradation of both types of wool and only A. niger and P. canescens consistantly failed to attack either of the wool samples in all media. A. niger, P. canescens and P. marquendii failed to degrade wool in wool-yeast extract medium.

In general, wools degraded by these fungi were more densely stained with lactophenol cotton blue than the undegraded wools (Table 17). Similar effects have been briefly noted by English (1963) and in somewhat more detail by Pinetti (1966). In the present studies some undegraded wools and some controls showed positive uptake of cotton blue, but the staining was scattered and irregular. Thus the intensity of stain uptake by the wool fibres could be an indication of the degree to which these fibres are altered during fungal colonisation. In the case of control fibres, the slight stain uptake seemed to occur between the cuticle cells and proceeded between cells. This suggests that it is the cementing material rather than the actual keratin which is being altered after exposure to fungi, to allow stain absorption.

The results of these experiments, therefore, indicate that a range of fungi can degrade wool in vitro. It is unlikely that the release of cortical cells from the fibre is an indication of actual digestion of the keratins of wool. Since the cortical cells are held together by cementing material the utilization of this more accessible material could lead to the release of cortical cells into the medium, without any digestion of the keratin having occurred. Such a release of cortical cells has been reported by Mathison (1964) as the first stage in the breakdown of wool by K. ajelloi, which goes on to digest the actual keratin enzymatically.

If the release of cortical cells is due to digestion of the proteinaceous cementing materials around the cortical cells, there might be expected to be a correlation between the general proteolytic activity of the fungus and its ability to degrade wool. This correlation was not apparent in the present studies. High proteolytic activity was observed for A. niger and P. canescens but they were never shown to degrade either types of wool. While A. cheranthi and E. nigrum were able to degrade some types of wool, no proteolytic activity could be detected after their growth on gelatin agar. But it is possible that gelatin as a substrate may not have been appropriate for estimating the proteolytic activity in relation to degradation of wool.

Propylene oxide sterilised wool has been shown to be degraded *in vitro* by some of the fungi isolated from the fleece environment. Although *in vitro* conditions differ from those *in vivo*, it is possible that some of these fungi can degrade fleece wool in its natural condition. Potentially the fleece of sheep provides a habitat suitable for the growth of many microorganisms. It has been demonstrated in the studies reported here that fungi are present within the fleece often in considerable numbers (Tables 6 & 7) but very few are likely to be permanent members of this habitat. Most are probably present as 'transients' having originated elsewhere and then by chance having been deposited on the fleece either as spores or as vegetative hyphae. The availability of nutrients suitable for fungal growth within the fleece must be limited. In contrast to skin, wool is a particularly pure form of keratin (Baxter, 1968) and any nutrients present within the fleece are probably derived from external sources. Some of the transient fungi may grow for a short period because they have been deposited on the fleece together with nutrient materials derived from their old environment. Sources of these transient fungi must include the air, other animals, vegetation and soil.

Sheep stand in the open air throughout the year, and heat, therefore, is generally gained from solar radiation. Under the sun, the tips of the fibre are the most directly exposed parts of the fleece and it has been reported (Mcfarlane *et al*, 1958) that in tropical climates the outer parts of the fleece can reach very high temperatures (e.g. 81C) compared to those in the basal parts, which usually approach the skin temperature of 38C. The temperature gradient in the fleece of sheep in New Zealand is unlikely to be so marked. Of the 19 species selected for special studies, only 7 were found to be able to grow at 37C. Of these *A. niger*, *P. canescens* and *P. marquendii* were shown to be unable to degrade wool. *A. pullulans*, *A. cheranthi*, *S. fimicola* and *A. flavus* could all grow at 37C and were found to be able to degrade wool. Two of these four species (*A. pullulans* and *A. cheranthi*) had low proteolytic activities. *A. flavus* and *S. fimicola* had high proteolytic activities. These two species at least, therefore, should theoretically be able to grow in the natural fleece environment. Proteolytic activity as determined here did not seem a reliable indicator of the ability of these species to grow on wool, thus it is possible that all 4 of these species could grow in wool *in vivo*.

Wool fats have been shown to have different effects on different fungal species (Table 21). In some cases the growth of the fungus was inhibited by wool fat, in others, the growth was stimulated. The growth of A. cheranthi and A. flavus, which degraded wools and grew at 37C, were inhibited by wool fat. The growth of S. fimicola was unaffected and that of A. pullulans was stimulated. Thus under the experimental procedures used here, of the 19 species tested, S. fimicola and A. pullulans would appear to be the 2 most likely to grow within the fleece.

The growth of T. mentagrophytes has been reported to be inhibited by wool fat (Baxter & Trotter, 1969) but in the present work its growth was stimulated. This may be due to the different types of wool used to obtain the fat and also the different strains of fungus. It has also been suggested (Baxter & Trotter, 1969) that the inhibitory effect of the fatty material of keratins is of more consequence to the pathogenic establishment of dermatophytes in vivo, as the fungus will have a small 'innoculum potential' compared to that of its saprophytic phase in soil etc. Similarly, fungi present in fleece wools could show growth inhibition.

Among the species isolated from fleece, many are found in the environment e.g. Penicillium, Alternaria, Cladosporium and Aspergillus. Cladosporium herbarum has been reported to be the most common fungus present in the atmosphere (Gregory, 1973). This fungus has been frequently isolated from the air in New Zealand (Dye & Vernon, 1952). C. herbarum was also the most frequent air isolate in the present study. Many of these air borne fungi are derived from fungal growths on plants and the soil. Stout (1964) reported that a wide range of bacteria, fungi, and yeasts were present in New Zealand pastures but fungal activity was considered to be greatest in pastures which have been subjected to periods of drought sufficient to inhibit growth of bacteria i.e. in late summer. McKenzie (1975) also reported on fungi frequently isolated from New Zealand pastures. Many of the fungi he listed are those present in fleece wools, and include A. fumigatus, P. chartarum, C. herbarum, and Penicillium species. Many of these fungi were most frequently isolated in the summer and autumn.

The presence of abundant decaying materials in autumn would probably explain the large numbers of fungi isolated in this season. Similarly the highest concentrations of the facial eczema fungus in pastures occurs

in late summer and autumn and this can also be considered to be an indicator of general environmental conditions suitable for fungal growth. The higher levels of fungi present in fleece wools at this time of the year is, therefore, probably a reflection of the abundance of fungi in the general environment.

During the entire experimental period, no dermatophytes or other truly keratinophilic fungi were isolated from fleece wool. Although Keratinomyces spores were observed on a few occasions by microscopy, they were never actually cultured. A few Chrysosporium species were isolated in the present study and some species of this genus are keratinophilic. Most of the fungi present in fleece wools, therefore, would seem to be transient and without clinical significance. However, under suitable conditions, many of these transient fungi may be able to infect the host. Such infections are known as opportunistic infections. In humans, a large number of fungi, for example, can be isolated from the external auditory canal, but they are not actively growing in that area (Lea, Schuter & Harrell; 1959). The same is true for animals (Baxter, personal communication). Nevertheless, in the abnormal condition of otitis externa, there is evidence that these saprophytic fungi can grow actively and may even play some part in the aetiology of the condition. Aspergillus species, especially A. niger, A. fumigatus and A. terreus appear to be involved most commonly.

In skin ulcers, fungi, particularly strains of A. niger, F. oxysporum and F. solani have been known to establish themselves for long periods (English, 1972). Other species may be found causing superficial infections on the skin. Cladosporium spp have been associated with Tinea nigra palmaris. A. niger, A. fumigatus and some Fusarium species have been reported to invade glabrous skin as well as causing infections of the toe and finger nails (Rush-Munro, Black & Dingley, 1971). Some normally saprophytic fungi e.g. A. fumigatus have also been known to invade burns or surgical wounds, causing widespread systemic infection and even death (Caro & Doglioti, 1973). Moreover, it has been reported that under certain, as yet unclarified conditions the eye provides an apparently ideal climate for some fungi, resulting in occular infection. Fungi which may be involved include species of Aspergillus, Penicillium, Fusarium, Cephalosporium and Curvularia.

The position of Phoma-like species as oppotunistic fungi is of particular interest as certain of these species have been demonstrated to be capable of growing within fleece wools (Mulcock, 1959). Phoma species have been implicated as rare incitants of Mycetoma (Segretain and Descombes, 1969) and in a few instances Phoma-like species have been associated with lesions on humans or animals (Bakerspigel, 1969). Peyronellaea, a segregate of the genus, is usually a saprophyte on decaying vegetation or a secondary pathogen of plants, but has also been seen in wool fibres which were discoloured by an internal growth of the dark hyphae of this fungus (Mulcock, 1959). The same species has been found to infect the pinnae of the ears of goats and pycnidia were demonstrated in the lesions (Dawson & Lepper 1970). The repeated isolation of Peyronellaea cava and the presence of pycnidia-like structures within host material suggested that the fungus was the aetiological agent of a deer's cutaneous infection (Gordon, Salkin & Stone, 1975). Gorden et al also reported that pycnidia were seen within the hair of a child displaying ringworm-like lesions and this was the first report of this fungus being a human skin pathogen. The somewhat similar Botryodiplodia theobromae has been implicated in nail infections (Restrepo et al, 1976). Thus Phoma-like fungi and many other species which are normally non-pathogenic can cause infections of animals and man if presented with the opportunity.

In addition to the ability of certain of these saprophytic fungi isolated from fleece wools to produce infections, some are also psychrotrophic e.g. Cladosporium spp, Alternaria alternata and Epicoccum purpurascens and can contaminate frozen meats in the freezing works (Baxter & Illston, 1976). Consequently, a knowledge of the mycoflora of fleece wools can be useful in assessing the potential of fungal contamination in the meat works, as once within the slaughtering area, spores may be air dispersed to stored meats in other parts of the works.

There is no indication that the fungi isolated from fleece wools in this study are anything other than harmless saprophytes, but the possibility exists that under abnormal circumstances they may become pathogenic to the sheep. Furthermore, these fungi may be a hazard in the meat industry and especially to personnel who constantly handle sheep and wool, if they are for some reason susceptible to infection. Therefore, knowledge of the types of fungi present on the fleece is necessary so that measures can be taken to reduce the possibility of such accidental infections.

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