AN ECOLOGICAL STUDY OF FUNGI IN CALLUNA-HEATHLAND SOILS.

by

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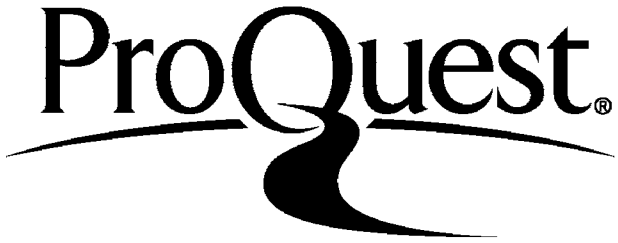

ABSTRACT.

"An Ecological Study of the Fungi in Calluna-heathland Soils".

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The fungal floras of several Calluna-heathland soils distributed over England and Wales were investigated by the soil plate method. Many species of fungi (including Trichoderma viride, Mucor ramannianus, Beauveria bassiana, Penicillium adametzi, P. namyslowskii, P. nigricans, Trichobotrys sp., Absidia orchidis, Mortierella isabellina, Pullularia pullulans, and Zygorrhynchus vuilleminii) were found to be of more or less constant occurrence in these soils. Representatives of all major classes of fungi, excepting Oomycetes, were isolated.

The numbers of species of fungi decreased with increasing depth, although certain species (Mucor ramannianus, Beauveria bassiana and Trichobotrys sp.) were most commonly isolated from the sub-surface, leached horizons. Evidence suggested that the illuviated horizons checked the downward distribution of spore-producing species.

The fungus flora of one small area of heathland soil was investigated in greater detail, on a seasonal basis, by

several recently described methods of isolation including the immersion tube and a new slide-trap method. The latter two methods, designed to isolate only those fungi actively growing in the soil, yielded closely agreeing results; many of the species of fungi shown to be of constant occurrence by the plating method were isolated also as active mycelia. Many of the species isolated, however, varied according to the method employed.

Microscopical examination by the Rossi-Cholodny slide technique revealed the growth habit of many of the species isolated and also the presence of many species which were not isolated - largely humus-inhabiting, dark-coloured hyphomycetes.

Whilst no seasonal variation in the occurrence of fungi was demonstrated by the plating method, marked variation was demonstrated by the immersion tube method, In particular the isolation of Trichoderma viride was closely related to the prevailing temperature.

The use of several methods of isolation, in conjunction with investigations of specific habitats and microscopical examination, provided a useful means of approach to the problems of soil fungal ecology.

CONTENTS.

PART I

HISTORICAL REVIEW AND INTRODUCTION.

HISTORICAL REVIEW.

"The soil population is not a beneficial organisation labouring with singleness of purpose to the end that nutrient elements shall be made available for plants, but a wholly independent population, nutritionally fiercely competitive within itself" (Norman, 1946)

With this statement most soil microbiologists will agree. Unfortunately the findings presented by investigators over the last seventy years have not always been in close agreement. Disharmony exists not only in the soil population. The reason for this disharmony is partly due to the fact that progress in soil mycology - as indeed in any experimental science - is, to a large extent, dependent upon methods; soil mycology is a subject which can be studied and understood only by the use of a variety of tools, for so many variable factors are involved that each method or technique evolved is applicable only to certain specific problems, and the application of different tools to widely differing tasks does not always provide a sound basis for comparative analysis of the results so obtained. In consequence of this all findings need to be qualified and modified with great care. Such caution has not always been observed.

After seven decades of intensive research by a large body of workers the results obtained remain largely of an heuristic nature - posing new problems whilst answering the old only in part - and a survey of the past seventy years' work can be divided, on this basis, into four periods.

The first period (1886 - 1915) was a passive one, in which the presence of fungi in the soil was simply accepted. Then, secondly, followed a period of doubt and ~~un~~certainty (1916 - 1928) in which the question was posed: do fungi actually live in the soil? This query was answered, but gave rise to the problems of the third phase (1928 - 1938): In what habit, and in what relationship to other organisms, and to one another, do fungi live in the soil? These questions were partly answered by ingenious direct microscopic techniques which, however, were limited in their uses and automatically the further inquiry followed: which species of fungi live in the soil? And what are the factors which determine the mutual relationships of fungi and other organisms living in the soil? Such are the two questions which remain largely unanswered at the present time. They will probably remain unanswered for a long time to come, for these questions are not restricted to specific groups of micro-organisms, but the whole life

of the soil, as well as the mechanics and physical nature of the soil itself: close co-operation between a variety of branches of the sciences is necessary for their understanding. Nevertheless, these considerations of the future do not come within the scope of the present review, in which a more detailed study of the four periods is to be presented.

1886 - 1915.

It is generally accepted that the study of soil fungi was founded in 1886 by Adametz,* who isolated 11 species of fungi, described and named them. Nitinsky * (1902) presented a similar study. Oudemans and Koning (1902) studied the fungi occurring on decaying vegetation of woodland soil from which they isolated 45 species, most of which they described and named: their work is worthy of recognition for it represents the first serious attempt to study these soil forms and their results, methods and observations bear favourable comparison with many which have since been presented. Van iterson * (1904) studied some of fungi capable of decomposing cellulose, and in the same year Hiltner introduced the term 'rhizosphere' to describe that part of the soil influenced by the presence of plant roots.

During this period several studies were made of specific groups of soil fungi: Butler (1907) presented

his treatise on the genus Pythium, many species of which he isolated from soil by a baiting method, which at the present day remains in general use; Hagem * (1908, 1910) isolated and described species of Mucorales from several soil types in Norway and emphasised the importante of using monospore cultures in their study, and Lendner (1908) presented a similar study of Mucorales from soils of Switzerland.

Other work worthy of mention includes that of Rivas * (1910) who was probably the first investigator to collect samples by inserting a sterile tube directly into the soil; Goddard's (1913) investigation of the capacity of soil fungi to assimilate free nitrogen and C.N. Jensen's (1912) extensive taxonomic study of fungi isolated from arable soils of N. America. In this country Dale (1912, 1914) examined the fungi occurring in sandy, peaty and other soil types and described many of them, and Hall (1908) presented one of the earliest accounts of fungi and bacteria in soils of differing reaction.

The studies of these early years were largely taxonomic, and partly physiological, in nature. The method of isolation, with few exceptions, was that employed in bacteriological studies, i.e., the dilution plate.

* Quoted from C.N. Jensen (1912). Original papers not available.

(1916 - 1928).

Do fungi produce mycelium in the soil? Waksman (1916) was the first investigator to ask this question. It was, perhaps, due to Waksman that the 'mass isolaters' of the early period were awakened to the fact that their work, important as it was (and remains today), was by no means an end in itself. Waksman (1916) wrote "The question is not how many numbers of types of fungi can be found in the soil, but what organisms lead an active life in the soil. To what depth are these organisms found to produce mycelium in the soil? And finally, do all or at least most of the organisms isolated from the soil actually produce mycelium in the soil? In the same paper Waksman described a method by which fungi occurring in the soil as active mycelia could be isolated; this method depended on the production of visible mycelium within a specified period of time. As a control Waksman demonstrated that the species which he was investigating could not produce a visible mycelium in 24 hours, if they originated from spores. In the following year Waksman (1917) suggested that there was a characteristic fungus flora of the soil.

Conn (1918) introduced a method for the direct microscopical study of the soil microflora, by staining soil smears. He concluded from his examinations that.... "mould hyphae occur so seldom in the soils studied that for all practical purposes they may be considered absent....

it should be pointed out that the species found most abundantly by Waksman are common dust fungi and their general occurrence therefore from soils of different parts of N. America and Europe does not prove that soil has a characteristic fungus flora." Four years later Conn (1922) presented a slightly modified method and admitted to the frequent occurrence of fungal mycelium in soil preparations.

General taxonomic and physiological studies continued throughout these years: fungi of cultivated and uncultivated soils were considered to be essentially similar by Werkenthin (1916), whilst Swift (1929) found some variation between soil types; investigations were made on the effect of soil acidity on numbers of fungi (Waksman, 1924), and on the influence of environmental factors on soil fungi, (Coleman 1917); the ammonifying powers and ability of soil fungi to decompose cellulose were investigated by Waksman (1916), Abbott (1923) and Paine (1927). The fungal floras of virgin soils were examined by Werkenthin (1916) Abbott (1923), Brown and Benton (1927) and Paine (1927)

It was Waksman (1916) who expressed the opinion that the dilution plate method was not satisfactory for the estimation of numbers of soil fungi, and later (1921) he suggested a new method employing the use of lower dilutions and an acidified medium which would reduce the numbers of bacteria occurring on plates. An attempt was made to

standardise the dilution plate method by Brierley et al (1927)

Brierly (1927), in an adress to the Quekitt Microscopical Club, concluded that he could.... "see no reason why almost any fungus able to live saprophytically might not sooner or later be isolated from soil existing as a casual or permanent inhabitant".... the occurrence of a fungus being.... "a matter of chance or determined purely by local conditions". These comments may be attributed to the dilution plate method, which was employed in the majority of studies of soil fungi; a method showing no distinction between active and inactive components of the flora. It was left to Thom (1927) to appeal for a more objective approach to the study of soil fungi, although his appeal for a distinction between those fungi which are contributors to profitable fertility and those which are unprofitable boarders, was perhaps premature at a time when so little was known of the factors affecting biological equilibrium. In the subsequent years, however, methods were to be described which permitted considerable progress to be made in the direct study of the soil population.

1928 - 1937.

As the work of Waksman dominated the thoughts and actions of investigators in the previous thirteen years, so the researches of Rossi and Cholodny were to guide and

stimulate many workers in the ensuing decade. Chesters (1949) describes Rossi's technique as... "one of those simple methods of biological experimentation which acts as the trigger to set all sorts of reactions in motion"

Rossi (1928) pressed clean microscope slides against a freshly exposed soil surface and then fixed and stained the preparations to display bacteria, fungi and other organisms in their positions relative to one another and to soil particles. Cholodny (1930), without knowledge of Rossi's work, produced a technique essentially similar, but involving a period of incubation of from one to three weeks during which time the slide or cover slip was left buried in the soil, thus allowing micro-organisms in the soil to grow over the slide surface.

The Rossi-Cholodny slide technique was employed by many workers in a variety of studies: Conn (1932) applied the technique to study the relationships existing between bacteria, fungi and actinomycetes in soils contained in glass tumblers and maintained under controlled conditions; the technique was used by Demeter and Mossel (1933) and by H.L. Jensen (1934) who, using it in conjunction with the plating method recommended their use together as each compensated for the disadvantages of the other;

H. Jensen (1935) employed the Rossi-Cholodny technique as a quantitative index of the growth of fungi in the soil

under the influence of different kinds of organic matter; Ziemiecka (1935) actually smeared the slides with various organic substances, observed the succession of developing micro-organisms, and noted that moulds alone were responsible for many decomposition processes in acid soils; the method was employed by Starkey (1937 and 1938) in an investigation of the rhizosphere flora.

Rossi et al (1936) presented a survey in English of the soil impression and soil crushing methods, and summarised their work to that date.

The problem of active and inactive components of the soil fungus flora was investigated also by McLennan (1928) who attempted to separate the mycelial and spore fractions by treating soil to desiccation under reduced pressure. She claimed that this treatment destroyed the mycelial components, whilst leaving the spores unaffected, and concluded from her investigation that fungi exist in the soil almost exclusively in the active mycelial condition.

Kubiena (1932, 1935) examined the soil microflora directly by means of a system of direct vertical illumination, whilst employing high microscopic magnifications. His results are of very great interest although his method is limited in application by the technical difficulties involved.

During this decade a great number of papers were

presented on a variety of topics concerned with soil fungi; the majority of these investigations employed the bacteriological method of study despite repeated criticisms of this method both as a means of estimating numbers of fungi, and as a method which tends to favour the isolation of those fungi having a high sporing capacity. Many of these studies were of fungal species occurring in relation to gross habitat factors and some were even without reference to the habitat. Le Clerg and Smith (1928) examined fungi in Colorado soils; Bayliss-Elliott (1930) concluded from a survey of the fungi from the Dovey Salt Marshes that there was very little difference in the flora under different types of vegetation, whilst Jensen (1931), investigating the fungus flora of Danish soils, reported a variation of flora between soil types; Morrow (1932) investigated fungi of pine forest soils; Ma (1932, 1933) reported marked seasonal variation innumbers of fungi in the soil and showed that the fungus flora of the oriental world was similar to that of the occidental; Bisby, James and Timonin (1933) supported Jensen's (1931) finding of definite differences in the fungus flora of distinct soil types; the fungi of Punjab soils were investigated by Chauhuri and Sachar (1934) and by Singh (1937), both reports include lists of typical soil fungi; the latter worker was unable to detect fluctuations of numbers of fungi over the seasons, cf. Ma (1932), and concluded that...."the method

employed was not suitable for its exhibition"; Bisby, James and Timonin (1935) studied the fungi from soil profiles in Manitoba and found no striking difference in the fungi from different horizons, although their evidence seemed to indicate that certain types of fungi occurred in certain types of soil; Johann (1935) was able to differentiate three associations of Mucorineae occurring fairly regularly in woodland soils; Killian and Feher (1935) isolated many species of fungi from dry Sahara soils, which had previously been supposed sterile; Sabet (1935) was the first to present a list of Egyptian soil fungi; Timonin (1935) investigated the fungus flora of profiles from virgin soils and reported periodicity of numbers in surface samples only. This body of work yielded many contradictory results, and a few discoveries of interest and importance, but, in relation to its mass, added little to an understanding of the relations existing between the soil and its contained microflora.

The influence of higher plants on soil micro-organisms attracted the attention of several workers. Already preliminary observations had indicated that micro-organisms occur in greater abundance in soil about plant roots than in the 'open' soil. Starkey (1929 a.b.c.), in the first of an illuminating series of studies, confirmed these observations, but found the influence of roots to be greater on bacteria

than on fungi, and greater on some groups of bacteria than others. He found the activity of the root zone population to be higher than that of the general soil microflora, and showed the influence of roots to vary with the age of the plants. Starkey suggested that root excretions were of importance in determining the relations which exist between higher plants and micro-organisms. Starkey (1931) later, reasserted his earlier findings and demonstrated a gradual increase in the abundance of micro-organisms in passing from the soil towards the root surface. Sabinin and Minina (1930) investigating the microflora of sandy desert soils found them to be more or less sterile except in proximity to roots of higher plants. Thom and Humfield (1932) confirmed Starkey's findings and reported the epidermal cells and cortical parenchyma of apparently healthy plants to be penetrated by fungal hyphae. Further, they showed that the soil reaction in the root zone was less acid than that of the open soil. This latter observation was confirmed by Jahn (1934). That the root zone flora is not necessarily beneficial to the higher plant was shown by Simmonds and Ledingham (1937) who classed 50% of their total identified fungal isolates from wheat roots as pathogenic.

Before concluding the survey of this decade, 1928 - 1937, some note must be made of a rather more specialised type of investigation which, nevertheless, has general and far-

reaching effects on the study of the relationships of soil micro-organisms. Henry (1931) demonstrated the inhibitive action of the general soil microflora on the development of two pathogenic soil fungi (Fusarium Graminearum and Helminthosporium Sativum), and showed the fungal component of the soil microflora to be the most effective both in suppressing the development of these pathogens and in inhibiting their sporulation in the soil. Henry (1932) also found the sppressive action of the soil microflora to vary with temperature. One of the commonest and most widespread of soil fungi, Trichoderma viride was shown by Weindling (1932) to parasitise, in culture, several other common soil fungi, and he suggested the possibility of its use in controlling pathogenic soil organisms. In later investigations Weindling (1934 a.b.c.) isolated the 'lethal principle' from Trichoderma and studied the factors influencing its production. He further demonstrated that other common soil fungi possessed similar, though less vilulent, powers to Trichoderma. The results of researches by Ludwig and Henry (1943) Warcup (1952) and Mollison (1953) may be conveniently included here, as these investigators have shown that practically sterilised soils are rapidly colonised by Trichoderma, and its prolonged dominance in these soils is perhaps attributable to the production of its 'lethal factor', which Weindling (1941) has termed Gliotoxin. Waksman (1937 a.b.c.) reviewed the work on associative and antagonistic effects of soil micro-organisms.

1938 - 1953.

The decade, 1928 - 1937, was a fruitful one, and a considerable part of its achievement was due to the development of the direct observation techniques; but in such a study as soil mycology no method so far designed is beyond criticism, for no single method can take into account the multiplicity of variable factors which are involved and altered by the method itself. Garrett (1952) wrote... "in direct observation methods one sees what one cannot identify, and in the plate count method one identifies what one cannot see". The implication of this statement is clearly the need of further means: methods enabling the isolation of fungi which can either at the same time be observed, or are known to be actively growing within the soil complex.

Perhaps it may seem that throughout this review too great an importance has been accorded to the development and use of methods. Methods are, admittedly, only a means to an end. But the fact that the means must be found before the end can be achieved is indisputable, as is the fact that the study of soil fungi has been controlled and limited throughout by the slow development and adoption of techniques. This need for further methods of study has not been neglected in recent times: during the past fifteen years the number of tools for the isolation and observation of soil fungi has steadily increased, and a brief account of some is given below.

Of the selective isolation methods Yarwood's (1938) use of carrot discs for the isolation of Thielaviopsis basicola and Meredith's (1940) method for the isolation of Phycomycetes deserve mention. By using a selective means of isolation, in which mycelial activity in the soil is a necessary factor, Meredith was able to demonstrate a marked seasonal fluctuation in the occurrence of soil Pythia. The immersion tube method (Chesters, 1940) and the slide traps method (La Touche, 1948) provide valuable means of isolating fungi which are actively growing in the soil; both of these methods are discussed at greater length in the experimental section of this thesis, and no comment is included here except to note that the isolation of fungi by means of the immersion tube necessitates the growth of hyphae from the soil into the tube by way of capillary inlets. Chesters (1948) and Nicot and Chevaugeon (1949) consider that competition at the capillary orifice may restrict the penetration of certain species of fungi. It was partly in order to eliminate this localised control of penetration that Thornton (1952) devised the screened immersion plate method, wherein a thin film of agar medium contained in a shallow box is buried in the soil. One face of this box is perforated by holes of sufficient size to reduce the competition factor between penetrant hyphae to a negligible amount. Thornton also indicated that the immersion plate had the additional advantage that penetrant hyphae were not subjected to partially anaerobic

conditions as in the immersion tube. Chesters (1948) described a means of separating the organic matters from the mineral fraction of soil and isolating its fungus content. In the soil plate method, Warcup (1950) reduced the degree of dispersion of soil particles, and so minimised the advantage given to heavily sporing fungi by the dilution plate method. Warcup (1951) demonstrated the possibility of determining the relative frequency of fungal colonies which develop from spores and from pre-existing mycelium by staining soil plates after a short period of incubation. A method of selectively isolating Ascomycetes by subjecting soil to short periods of steaming before plating was also described by Warcup (1951). A technique for observing the microflora on roots in situ was described by Linford (1940): this technique enables the microscopical examination of roots growing in soil contained in transparent "root observation boxes". Jones and Mollison (1948) modified Conn's soil smear technique (1918) to provide a means of quantitative study of the soil microflora.

Studies of a rather more general ecological and physiological nature were less frequent than in earlier years: Campbell (1948) investigated the distribution of Mucorales in a number of soils; Kursanoff and Stiklyar (1938) compared the microflora of soils of Moscow and Batum, and suggested the occurrence of physiological races of soil fungi; Vandecayeve and Baker (1938) concluded that the microflora

of two soil types was governed predominantly by the inherent soil characteristics; Newman and Norman (1941), investigating the activity of the microflora in various horizons of several soils confirmed the conclusion of Vandecayeve and Baker, stating that... "the soil population is directly a characteristic of its immediate environment and perhaps not so flexible as has been supposed". Later (1943) they showed the subsurface population to be less flexible than the surface population and that it tended to be relatively more stable and uniform in character; Jeffries et al (1953) although their work was primarily a quest for antibiotic-producing fungi, demonstrated that acid heath soils contained a more or less constant fungus flora; Stenton (1953) investigated the soil fungi of Wicken Fen and was able to determine differences between two sites of a quantitative nature only.

In all probability the reduced number of papers concerned with ecological distribution and relationships of soil fungi represents a 'breathing-space' rather than a decline of interest. For in recent years a few ecological studies of a more detailed nature have been presented, and together with the current investigations of antibiotic production in the soil (with all its ecological implications) and the appeals of Garrett (1952) for a closer approximation to the methods of higher plant ecology and of Chesters (1948) for detailed investigations of the microhabitat, these may well represent the beginning of a refreshed and more objective period of study.

Chesters (1948) investigating the fungi of grass turf, by three methods simultaneously employed, was able to obtain information concerning the sporing capacity and the mycelial activity of some of the species present, the relationship existing between these two phases of activity and also to study the substrate relationships of some of these fungi as represented by their occurrence on specific types of organic debris. Warcup (1951) studied the influence of soil type and horizon on the distribution of microfungi in five grassland soils which had been investigated previously from the viewpoint of their higher plant vegetation. Warcup demonstrated the different distribution of fungi in the five soils and was able to divide them into two distinct groups on the basis of their occurrence in acid and alkaline soils. Webley, Eastwood and Gimingham (1952) investigated the development of the soil microflora in relation to plant succession on sand dunes, and the rhizosphere flora associated with the colonising species; they demonstrated the close association of the microflora with the vegetational succession, and suggested that the microflora played some part in the maturation of the habitat and could be included among the biotic factors influencing change in the plant communities.

The work described above has yielded valuable information concerning the fungal flora of the soil, but a review of this subject, however cursory, would be incomplete without

reference to two types of research of a different nature, concerned firstly with the vertical distribution of soil fungi and secondly with the possible influence of antibiotic production as an ecological factor.

In two studies, Burges (1950, 1953) ~~has~~ investigated factors affecting the vertical distribution of fungi in soil. He has shown that gloiospores are rapidly moved downwards through columns of sand by percolating water whilst xerospores are little affected. Burges considers this factor to be an important source of error in many investigations of the vertical distribution of soil fungi. A later investigation concerning the effect of carbon dioxide on the growth of soil fungi, led him to suggest that tolerance to high concentrations of carbon dioxide rather than low concentrations of oxygen, was a determining factor in the vertical distribution of soil fungi.

The high proportion of soil micro-organisms, including fungi, which produce antibiotics, and a variety of other observations, have led to the belief that antagonism between soil saprophytes and root parasites and the ecological relations of the soil microflora generally, are due, in part, to the capacity of certain species and strains of species to produce antibiotic substances. Whilst evidence on this subject remains inconclusive, it tends to support this hypothesis. (Brian, 1945, 1949 a.b., 1951 and Hersayon 1953). Dobbs and Hinson (1953) report the inhibition of

germination of the spores of many soil fungi in their native soils: this widespread fungistasis in soils has been confirmed only in part by Jeffries et al (1954)

Many important aspects of the study of soil fungi have been largely, or completely, omitted in this survey; prominent amongst them is the extensive work concerning the rhizosphere population and its more specialised aspects of root-attacking fungi, and researches relating to the mycorrhizal association, which have been the subject of reviews by Garrett (1938), Burges (1939), Harley (1948) and Katznelson, Lochhead and Timonin (1948). Their application to the present work, however, is limited and their omission therefore perhaps justifiable.

INTRODUCTION.

The present work was undertaken to investigate the variation and constancy of occurrence of soil fungi in association with a specific type of higher plant vegetation (Callunetum); to ascertain the distribution of these fungi in depth, in area, in season and in relation to the root system of the dominant higher plant; a variety of methods were employed to obtain information concerning the activity and substrate relationships of some of these fungi, and at the same time to provide a means of comparing this range of methods.

The numbers of methods available for the study of soil fungi has greatly increased in recent years and, used in conjunction, they provide an opportunity for ecological study which previously has not existed. Whilst the conception of a non-selective, perfect method has receded the view that a greater range of selective methods would be more useful, has taken its place, (Garrett, 1951). That different methods are complimentary rather than mutually exclusive has been demonstrated by the researches of H. L. Jensen (1934) and Chesters (1948)

Natural, undisturbed soils were selected as it was felt that they would provide a medium for the study of a more stable and uniform community of micro-organisms than would agricultural soils which are periodically disarranged and altered in chemical and physical composition.

Also it has frequently been inferred that the ecological distribution of an endemic microflora would be best demonstrated by investigation of virgin soils associated with particular natural communities of vegetation. (Ling-Young 1930, Warcup 1951).

Several attempts at ecological classification of soil fungi (Waksman 1917, Garrett 1946, Burges 1939) have been successful only in part (Harley 1948, Thom and Morrow 1937), perhaps because they emphasised too greatly the substrate relationships. Norman (1946) has commented that... "the soil population is not composed of a collection of prima donnas, but instead consists almost exclusively of organisms having a wide range of activities". A closer approximation to the methods of higher plant ecology in discerning fungal communities, similar to the concept of "soil fungal patterns" suggested by Thornton (1952), may prove more useful, initially, in providing an understanding of the "pattern and process" of plant communities (Watt 1947). Webley et al (1952) have indicated that this form of approach may yield results of considerable interest.

The selection of Calluna Vulgaris Salisb as a marker species in this present investigation has several advantages. The soil types on which Calluna occurs are of similarity in that they are all base-deficient, are between pH 3.5 and pH 6.7, and are not subjected to excessive fluctuations in the humidity of air and soil (Beijerinck 1940)

Calluna very commonly occurs on soils derived from certain geological formations which are well represented in this country. The soils in which Calluna grows are frequently podsolised, and thus conveniently provide a means of comparison of the biological and physical soil horizons. Calluna, although occurring in a variety of plant communities, is often the dominant, and not infrequently the sole higher plant species present over considerable areas of soil.

PART II.

SECTION A: INVESTIGATION OF THE FUNGUS FLORAS OF SOME WIDELY DISTRIBUTED CALLUNA-HEATHLAND SOILS BY A STANDARD PLATING METHOD.

CONTENTS.

1. INTRODUCTION.

The experimental work presented in this section is concerned with the soil fungi occurring in a variety of Calluna-heathland soils, as demonstrated by the soil plate method (Warcup 1950). Whilst supplying only a limited picture of the fungus flora of these soils, the main object of this study is to determine the constancy and variation of fungal species occurring in well separated areas, and to give some account of their vertical distribution in the soil profiles.

2. MATERIALS AND METHODS.

(a) The Soils.

The soils of thirteen distinct heathland communities, distributed widely in England and Wales, were sampled. In all communities Calluna vulgaris Salisb provided the dominant higher plant cover.

A total of 39 profiles was sampled: the soils showed extreme variation in profile structure ranging from humus-rich loams and sands without vertical zonation, to mature podsols.

The soils sampled were located in Surrey, Kent, Dorset, Norfolk, S. Wales, N. Wales and Anglesey. Those of S.E. England were mostly of the podsol type. One of the areas, (Burnt Hill, Chobham Common, Surrey) was selected for a detailed investigation by a variety of methods, over a period of 18 months.

(b) Collection of Soil Samples.

The profiles were exposed by digging a pit, the size of which varied with the depth; usually, these pits were approximately 2 ft. square and 30 in. in depth. One face of the pit was prepared as the profile, and to this a flexible tape measure was pinned so that horizon depths and sampling levels could be recorded accurately. Samples were collected at every inch to a depth of 6 in., and below this at every third inch: where these arbitrary depths did not coincide with noticeable features of the profile extra samples were collected. At the sampling points the surface soil was removed with a sterile spatula and samples were collected by boring into the profile face with a previously sterilised sampling tube (3 in. x ½ in.). Soil samples for the determination of pH were collected in a similar manner at successive 3 in. levels; 4 in. x 1 in. specimen tubes were used.

(c) Isolation of fungi.

The soil plate method was employed throughout for the isolation of fungi. All soil plates were prepared under a sterile hood contained in a small draught-free inoculating room. The majority of the soils collected were plated on the day of collection.

Soil plates were incubated at room temperature and were usually retained for a period of one month, during which time they were periodically examined. Soil plates were only

opened for the final examination and recording of species, except in cases where rapidly growing species were present or where a large number of colonies were developing; in these circumstances colonies were isolated onto slopes of potato-dextrose agar. Often it was possible to detect the presence of rapidly growing species within two days of inoculation and occasionally these could be completely excised from the soil plate. Many of the fungi developing could be recognised in situ and recorded without isolation and the use of a wide-field binocular microscope, as recommended by Warcup (1951), was found to be helpful for scanning the plates; even so it was necessary to isolate into pure culture a great many fungi and in all a little over 2,500 such isolations were made. All species of Penicillium, Mortierella and Mucor were isolated into pure culture before identification could be accomplished.

In the majority of profiles examined soil samples were collected to a depth of 30 in., thus 14 samples were obtained and each of these was plated in triplicate. The three plates from each soil sample were prepared with soil from successively deeper levels in the collecting tube; after one plate was prepared a portion of the soil core in the collecting tube was removed and discarded. Either two or three experiments were prepared from each set of profile samples, a different agar medium being used in each of the experiments. Thus from 13 heaths, 39 profiles were sampled

and 84 sets of soil plate experiments were prepared. The number of profiles sampled from each of the 13 heaths was not constant.

In the preparation of the soil plate experiments a total of seven different media were employed:-

a. Czapek-Dox + 0.5% yeast ext. pH 4.5

b. Czapek-Dox + rose bengal (1:15,000) pH neutral

c. Soil-extra agar, pH 4.5

d. Czapek-Dox + 0.5% yeast ext., pH 4.5 (Glucose as carbon source)

e. Waksman's glucose-peptone medium, pH 4.5

f. Potato-dextrose agar, pH unadjusted.

g. Plain water agar, pH unadjusted.

These seven media were employed only in the initial experiments. The media used most constantly were the first three listed (a, b and c.) Warcup (1950) recommended the use of medium "a" in the preparation of soil plates. The soil extract agar (used by Chesters for routine isolations with immersion tubes) was prepared from Calluna-heathland surface soil (40 gm./l.), containing in addition K_2HPO_4 (0.2 gm.), yeast extract (0.1 gm.) and glucose (1.0 gm.)

It may conveniently be recorded here that the media employed in these soil plate experiments appeared not to affect the species of fungi isolated. The average numbers of species isolated in soil plate experiments by the three media employed most consistently are listed overleaf.

a. Czapek-Dox + yeast extract. 13.7

b. Czapek-Dox + rose bengal. 13.8

c. Soil extract agar. 14.4

That isolations from soil extract agar yielded a higher number of species was probably due to its limited nutrient content, with consequent slow and thin mycelial development, which considerably facilitated the examination and isolation of fungi growing on this medium. The use of rose-bengal restricted, to a limited extent, the growth of some of the more rapidly spreading species, but this advantage was often diminished by the fact that colours produced in colony reverses were not discernible and this necessitated the isolation of certain species (from soil plates into pure culture) which were otherwise immediately identifiable.

(d.) Method of Recording Species.

In each soil plate experiment the triplicate sets of plates at each level were counted as one unit; a fungus occurring on one or more of these plates was given a positive record. No attempt was made to determine the number of colonies of individual species at any level, but in cases where a fungus was very frequent the fact was noted.

(e) Presentation of Results.

Reduction of the data accumulated in these experiments to tabular form has presented some difficulty and inevitably some of the finer points of distribution of species have been lost.

The soil plate experiments from each profile have been grouped into one table, thus one such table may represent the accumulated data of three experiments. The media by which a fungus has been isolated are represented in the tables by an index letter (as displayed in the list of media aforementioned) Since the different media used represent separate experiments the index letters indicate the media on which a fungus has been isolated, and the number of different index letters represent the number of experiments in which the fungus was recorded.

The number of levels of sampling, of necessity, has also been reduced by grouping. As far as possible the sampling levels have been grouped in such a manner as to coincide with the natural horizons of the profiles; these horizons have been indicated with the experimental data.

3. RESULTS.

The locations of the Calluna-heath and areas of Callunetum investigated are listed below together with the numbers of profiles exposed and experiments executed, the pH ranges and geological formations.

Location.	Geological Formation.	pH Range.	No. of Profiles	No. of Expts.
Surrey: Staple Hill, Chobham Common	Bagshot Beds	3.8 - 5.2	5	8
Chobham Crossroads, " "	Barton Beds	3.8 - 4.8	2	4
Burnt Hill, " "	Bagshot Beds	3.6 - 4.9	10	30
Ship Hill, " "	Bagshot Beds	3.6 - 4.8	1	3
Puttenham Common, Puttenham.	Folkestone Beds	3.9 - 5.0	2	3
Blackheath, Nr. Albury	Folkestone Beds	3.8 - 5.1	1	1
Dorset: Plateau Heath, S.Haven Pan., Studland.	Bagshot Beds.	3.8 - 4.5	3	6
Godlingston Heath, Nr. Studland.	Bagshot Beds.	4.1 - 5.0	2.	4.
Kent: Hothfield Heath, Nr. Ashford,	Folkestone Beds	3.7 - 4.2	2	4
Norfolk: Kelling Heath, Nr. Weybourne.		3.7 - 4.5	2	4
S.Wales: Rhossilly Down, Gower, Glamorgan	Old Red Sandstone	3.9 - 4.5	3	5
N.Wales: Carnedd Dafydd, Ogwen Bank.	Cambrian.	3.8 - 4.2	4	8
Newborough Warren, Anglesey.	Pre-Cambrian.	5.5 - 8.0	2	4

In the following pages an account is given of the ecology, topography and geology of the areas investigated; the profile structures and characteristics are described, and tables of the species distributions are presented.

The data for the Burnt Hill area are displayed in Section B., as this area was chosen for a detailed investigation by the soil plate and a variety of other methods. The results obtained from this area (by the soil plate method) are, however, included in the general survey following the specific accounts of the various heathlands.

Staple Hill, Chobham Common, Surrey.

Geological formation: Barton Beds of the Bagshot Series. The general vegetation of this heathland area is dominated by *Molinia coerulea*, with *Ulex minor* locally abundant, whilst on the hill top (240 ft. above s.l.) *Calluna* is dominant - *Erica Cinerea* grows sparsely within this Callunetum; *Molinia* is completely excluded from this central area. *Cladonia* spp. are present but mostly the soil surface between *Calluna* plants is bare. *Calluna* in this area is dense and bush-like in habit, growing to a height of 20-28 in.

All five profiles were prepared in a small area (approximately 20 yd. square) in the centre of this Callunetum. The five profiles were sampled over a period of 18 months.

Immersion tubes were also employed for the isolation of fungi from this soil. The results of these experiments are displayed in a later section.

Description of Profiles, 1 - 5.

Horizon	Depth (in.)					pH.	Description.
	1	2	3	4	5		
A1	0-4	0-2	0-3	0-3	0-3	3.8-4	Brown-black, humus-rich sand
A2	4-9	2-13	3-12	3-12	3-12	4-4.5	Purple-brown, loose sand.
A2/c	9-16	13-19	12-14	12-18	12-16	4.5-4.8	Transition zone
C.	16-	19-	14-	18-	16-	4.8-5.2	Mottled yellow-orange and green clayey sand.

No illuviated horizons are present in area. The green colouration of the clayey-sand subsoil is due to the presence of glauconite.

Roots were abundant in A1 horizons, imparting to this surface sand a peaty, fibrous consistency. In the leached horizon roots were of frequent occurrence but were only occasionally encountered in the clayey subsoil: here, however, localised dense development of fine hair roots was infrequently observed.

Pebbles were uncommon and were never encountered below 12 in. depth.

Distribution of Species in Profile 1 (Staple Hill.)

Horizon:	A1	A2	A2/C	C	C
Sampling depth (in.)	1-4	5.6.9	12.15	18.21	24-30
Absidia orchidis	. d	. .	. .	. .	. .
Mortierella isabellina	. d	. .	. .	. .	. .
M. parvispora	a .	. .	. .	. .	. .
M. zychae	a d	. .	. .	. .	. .
Mucor ramanniunus	. d	a d	. .	. .	. .
Zygorrhynchus vuilleminii	a d	a .	a .	. .	. .
Acrostalagmus albus	a d	. .	. .	. .	. .
Beauveria bassiana	a .	a d	. .	. .	a .
? Haplaria sp (F20)	a .	. .	. .	. .	. .
Paecilomyces sp (E1)	. .	. d	. .	. .	. .
Penicillium adametzi	a d	. .	. .	. .	. .
P. fellutanum	a .	. .	. .	. .	. .
P. namyslowskii	a d	a .	. .	. .	. .
P. nigricans	a d	. .	. .	. .	. .
P. piceum	. .	. .	a .	. .	. .
P. spinulosum	. d	. .	. .	. .	. .
Sporotrichum sp (F16)	. .	. .	. .	a .	. d
Trichoderma viride	a d	a d	. .	. .	. .
? Zygodesmus sp. (F6)	a .	. .	. .	. .	. .
Sterile mycelia:- HS 3	. .	. .	. .	a .	. .
DS 1	. .	. .	. d	. .	. .
DS 3	. .	. .	. .	. .	a .
DS 4	. .	. .	. .	. .	a .
DS 6	. .	a .	. .	. .	. .
24	16	7	3	2	4

Distribution of Species in Profile 2. (Staple Hill)

Horizon:	A1	A2	A2	A2/C	C	C
Sampling depth (in.)	1.2	3-6	9.12	15.18	21.24	27.30
Mortierella parvispora	a	.	.	.	.	.
Mucor ramanniunus	a	a	.	a	a	.
Zygorrhynchus vuilleminii	.	.	.	a	.	.
Pseudoeurotium zonatum	.	.	.	a	.	.
Alternaria sp. (D20)	a	.	.	.	.	.
Beauveria bassiana	a	a	.	.	.	a
? Dematium sp. (D21)	.	.	a	.	.	.
? Monosporium sp. (F37)	.	.	.	a	.	.
Penicillium adametzi	a	a	.	.	.	.
P. cyclopium	a	.	.	.	.	.
P. piceum	.	.	a	.	.	.
P. restrictum series (E28)	.	a	.	.	.	.
P. roqueforti ? (E27)	a	.	.	.	.	.
P. spinulosum	a	a	.	.	.	.
Sporotrichum sp. (F16)	.	.	a	a	a	a
Stemphylium macrosporoideum	.	.	a	.	.	.
Stysanus medius	a	.	.	.	.	.
Trichoderma viride	a	a	a	a	.	.
Sterile mycelia: DS4	.	.	.	.	a	.
DS9	.	.	.	.	a	.
20	10	6	5	6	4	2

Distribution of Species in Profile 3. (Staple Hill)

Horizon.	A1	A2	A2	C	C	C
Sampling depth (in.)	1-3	4-6	9.12	15.18	21.24	27.30
Mucor ramanniunus	a f	a f	a f	. .	. .	. .
Beauveria bassiana	a f	. f	a .	. .	. .	. .
Cephalosporium acremonium.	. .	. .	. .	. .	a .	. .
Cladosporium herbarum	a .	. .	. .	. .	. .	. .
Penicillium adametzi	. f	. f	. .	. .	. .	. .
P. fellutanum	a f	. .	. .	. .	. .	. .
P. namyslowskii	a f	a .	. .	. .	. .	. .
P. notatum ?	. .	. .	a .	. .	. .	. .
P. spinulosum	a f	a .	. .	. .	. .	. .
Trichoderma viride	a f	a .	. .	. .	. .	. .
Sterile mycelia: DS.11	. f	. f	a f	. f	. .	. .
DS.13	a .	. .	. .	. .	. .	. .
DS.14	. .	. .	. .	. f	. .	. .
DS.18	. .	. .	. .	a .	. .	. .
DS.19	. .	. .	. .	. f	. .	a .
DS.21	. .	. .	. f	. .	. .	. .
DS.43	. .	. .	. .	a .	. .	. .
DS.44	. .	. .	. .	. .	. .	f .
HS.2	. f	. .	. .	. .	. .	. .
19	11	7	5	5	1	2

Distribution of Species in Profile 4. (Staple Hill)

Horizon.	A1	A2	A2	A2/C	C	C
Sampling depth (in.)	1-2	4-6	9.12	15.18	21.24	27.30
Mortierella isabellina	a g	a .	. .	. .	. .	. .
M. parvispora	a .	a .	. .	. .	. .	. .
Mucor ramanniunus	a g	a g	a g	. .	. .	. .
Zygorrhynchus vuilleminii	. g	. .	. .	. .	. .	. .
Coniochaeta sp. (C13)	a .	. .	. .	. .	. .	. .
Gelasinospora retispora	. g	. .	. .	. .	. .	. .
Beauveria bassiana	a .	. .	. .	. .	. .	. .
Cladosporium herbarum	a .	. g	a g	. .	. .	. .
? Monosporium sp. (F37)	. .	a g	a g	. .	. .	. .
(Oosporeae) (F48)	. .	. .	a .	. .	. .	. .
Penicillium brevi-compactum	a .	. .	a .	. .	. .	. .
P. namyslowskii	a g	. .	. .	. .	. .	. .
P. nigricans	. g	. .	. .	. .	. .	. .
P. spinulosum	a g	. g	. .	. .	. .	. .
Trichoderma viride	a g	a g	. .	. .	. .	. .
Sterile mycelia: DS.16	a .	. .	. .	. .	. .	. .
DS.17	. .	. g	. .	. .	. .	. .
DS.19	. .	. .	. .	. .	. g	. g
DS.22	. .	. .	a .	. .	. .	. .
DS.23	. .	a .	. .	. .	. .	. .
DS.24	. .	. .	a .	. .	. .	. .
DS.25	. .	. .	. .	. .	. .	a .
DS.26	. .	. .	. .	. .	. g	. g
DS.70	. .	. .	. .	. .	a .	. .
24	14	9	7	0	3	3

Distribution of Species in Profile 5. (Staple Hill.)

Horizon.	A1	A2	A2	A2/C	C	C
Sampling depth (in.)	1-3	4.5	6.9	12.15	18.21	24.27
Mortierella isabellina	a	a	.	.	.	.
M. marburgensis	a	.	.	.	.	.
Mucor ramanniunus	a	a	a	.	.	.
Zygorrhynchus vuilleminii	.	a	.	.	.	.
Gelasinospora retispora	a	.	.	.	.	.
Beauveria bassiana	a	a	a	.	.	.
Penicillium decumbens	a	.	.	.	.	.
P. spinulosum	a	.	.	.	.	.
Penicillium sp. (E67)	a	.	.	.	.	.
Trichobotrys sp. (D27)	.	.	a	.	.	a
Trichoderma viride	a	.	.	.	.	.
Sterile mycelium, C21	a	.	.	.	.	.
12	10	4	3	0	0	1

Notes: Trichoderma viride and Mucor ramannianus occurred on nearly all plates from the sampling depths within the horizons indicated.

Chobham Crossroads, Chobham Common, Surrey.

Geological formation: Barton Beds of the Bagshot Series.

Height above sea level: 220 ft.

The vegetation of this area of Chobham Common is dominated by Molinia with scattered Ulex bushes, Calluna being locally dominant. The largest area of Callunetum (about 50 yd. square) was sampled.

A shallow depression (extending from Rushy Pond on the Long Arm) transects this area of heath and terminates on the northern edge of the Callunetum which slopes gently upwards. The depression is swampy and species of Juncus, Eriophorum, Drosera and Sphagnum flourish.

The Callunetum community includes sparse Erica tetralix and Molinia, no lichens were observed and the ground between Calluna plants is bare.

Two profiles were exposed in this area, profile I was from the higher and dry ground whilst profile II was situated nearer the edge of the Callunetum on low ground which was very wet: at the time of sampling the water-table level was 19 in. beneath the soil surface at profile II. The uppermost horizon of profile II, represented by a layer of black peaty humus differed from the uppermost horizon of profile I, which was a humus-rich sandy layer. The absence of a leached horizon (as in profile II) is recorded by Tansley (1949) as being a not infrequent phenomenon.

Description of Profiles.

Profile I	Horizon.	Depth (in.)	pH.	Description
	A.1	0 - 3	3.8	Black, brownish-black, humus-rich sand.
	A.2	3 - 10	3.8-3.9	Light brown, fine sand.
	B.1	10 - 13	4.0	Brown-black, firmly compacted, humus pan
	B.2	13 - 19	4.5	Reddish-brown gritty compact sand.
	B2/C	19 - 27	4.5	Transitional zone.
	C	27 -(36)	4.5-4.8	Mottled orange and green clayey sand.
Profile II.	(A.1)	0-5.5	3.8-3.9	Brown-black, peaty moist sand.
	B.1	5.5-9	4.0	Brown-black, firmly compacted humus pan
	B.2	9-15	4.0-4.5	Reddish-brown, gritty compact sand.
	C	15-(30)	4.5	Mottled orange and green clayey sand.

Roots were abundant in the A1 horizons and frequent in the A2 horizon. No roots were observed either within or below the humus pan.

Distribution of Species in Profile I (Chobham Crossroads)

Horizon	A1	A2	B1	B2	C	C
Sampling depth (in.)	1-3	4.5. 6.9	12	15.18	21.24	27.30
Mucor ramanniunus	a .	. b	. .	. .	. .	. .
Chaetomium murorum.	. .	. .	. .	a .	. .	. .
Beauveria bassiana	a b	a b	a b	. .	. .	. .
Cladosporium herbarum	a b	. b	. .	a b	. .	. .
Fusidium sp. (F28)	. .	. .	. .	a .	. .	. .
Penicill.aurantio-candidum	. .	..	. .	. b	. .	. .
P. fellutanum	a b	..	. .	. .	. .	. .
P. namyslowskii	a .	. .	. .	. .	. .	. .
P. roseo-purpureum	. .	. .	a .	. .	. .	. .
Pen.sp.(thomii series) (E48)	a b	..	. .	. .	. .	. .
Penicillium sp. (E62)	. b	. b	a .	. .	. .	. .
Sarcinella sp. (D39)	. b	. .	. .	. .	. .	. .
Sepedonium sp. (F32)	. .	. .	. .	a .	. .	. .
Sterile mycelia: HS.6	. .	. .	. .	. b	. .	. .
HS.7	. .	a .	. .	. .	. .	. .
HS.21	. .	. .	. .	. .	. .	a .
DS.28	. .	a .	. .	. .	. .	. .
DS.30	. .	. .	. .	. b	. .	. .
DS.33	. .	. .	a .	. .	. .	. .
DS.84	. .	a .	. .	. .	. .	. .
20.	8	7	4	7	0	1

Distribution of Species in Profile 2. (Chobham Crossroads)

Horizon	A1	A1	B1	B2	C
Sampling depth (in.)	1-3	4.5	6.9	12.15	18.21.24
Mucor ramanniunus	. .	. .	a b	. .	. .
Zygorrhynchus vuilleminii	a b	. .	. .	. .	. .
Thielavia sp. (C25)	. b	. .	. .	. .	. .
Beauveria bassiana	a b	a b	. .	. .	. .
Penicillium adametzi	a b	. .	. .	. .	. .
P. namyslowskii	. b	. .	. .	. .	. .
P. rolfsii ? (E75)	a .	. .	. .	. .	. .
P. spinulosum	. .	. .	. b	. .	. .
Pullularia pullulans	. b	. b	. .	. .	. .
Stachlydium sp. (D31a)	. b	. .	. .	. .	. b
Trichobotrys sp. (D27)	a b	a b	a b	. .	. b
Trichoderma viride	a b	a .	. .	. .	. .
Sterile mycelia: DS.38	. .	. .	. .	. .	. b
DS.67	. .	a b	. b	. .	. .
14	10	5	4	0	3

Notes: Colonies of Beauveria bassiana were extremely numerous on all soil plates prepared from the horizons where it is indicated as present. Mucor ramanniunus occurred sparsely.

Ship Hill, Chobham Common, Surrey.

Geological formation: Bagshot Beds of the Bagshot Series.
Height above sea level: 175 ft.

The area sampled is a flat plain below Ship Hill, the vegetation of which is uniform over a large area - Calluna and Erica tetralix being co-dominant; Molinia occurs in isolated tussocks and the dwarf furze (Ulex minor) is of scattered yet even distribution. Between ericaceous shrubs the ground is bare except for a sparse development of Cladonia spp.

The area is damp, as indicated by the prevalence of E. tetralix, receiving the drainage water from the surrounding high ground. A series of ditches, however, apparently provides efficient drainage and prevents the development of bog conditions.

Only one profile was exposed and sampled in this area: immersion tubes were also employed and the results of this latter method are displayed in a later section.

Description of Profile.

Horizon.	Depth (in.)	pH.	Description.
A.1	0-1.5	3.6	Brown-black humus-rich sand
A.2	1.5-8	3.8-4.0	Yellow-brown, fine, soft sand
B.1	8-11	4.0-4.5	Chocolate brown very firmly compacted humus pan.
B.2	11-14	4.5	Reddish-brown, gritty sand
B2/C	14-24	4.5-4.8	Gradual transition to -
C	24-(33)	4.8	yellow-orange clayey sand.

Roots were extremely abundant in the surface horizon, where the mass of fine fibrous roots imparting a peaty consistency. Roots were observed frequently in the leached horizon but were apparently absent below this level.

Distribution of Species in Profile I (Ship Hill)

Horizon	A1	A2	A2	B1	B2	C	C
Sampling depth (in.)	1	2-4	5.6	9	12	15.18	21.24
Mucor ramannianus	. . . . .	.c.	...	...	...	...	...
Aspergillus ruber ? (C17)	...	..g	...	...	...	...	...
Beauveria bassiana	ac.	acg	.cg	...	...	...	..g
Cephalosporium sp (F.31)	.c.	...	...	...	...	...	...
Cladosporium herbarum	a..	.cg	a..	...	...	...	...
Penicillium adametzi	...	ac.	...	...	...	...	...
P. fellutanum	acg	a.g	...	...	...	...	...
P. namyslowskii	acg	a.g	..g	...	...	...	...
P. spinulosum	..g	...	...	...	...	...	...
P. thomii	..g	...	...	...	...	...	...
Pullularia pullulans	.c.	...	...	...	...	...	...
Trichobotrys sp.(D27)	...	.c.	.cg	...	...	...	...
Trichoderma viride	a.g	.c.	...	...	...	...	...
Torula sp. (D.19)	...	...	...	...	...	...	..g
(Demetiaceae)(D29)	...	a..	...	...	...	...	...
(")(D35)	...	...	...	...	.c.	...	...
Sterile mycelia: HS.6	...	...	...	...	...	.c.	.c.
DS.31	...	...	...	...	...	...	.c.
DS.33	...	...	.cg	...	...	...	a.g
DS.34	...	a.g	a.g	...	...	...	...
DS.47	...	a..	...	...	...	...	...
DS.68	...	...	...	.c.	...	...	...
DS.4	...	...	..g	...	...	...	...
23	9	11	8	1	1	1	5

Puttenham Common, Near Puttenham, Surrey.

Geological formation: Sandy Beds of the Folkestone Beds (Lower Greensand)

Height above sea level: 150 ft.

Puttenham Common is approximately 2 square miles in area. The vegetation is varied within the limits of South-eastern English heaths and commons. Small copses of Silver Birch and stands of Scots Pine are widely distributed. Molinia provides the dominant ground flora in the damper regions; bracken is completely dominant in certain areas and appears to be spreading vigorously. The 'spine' of the common is provided by a range of high ground running roughly parallel with the Hog's Back. This range is dominated by ericaceous shrubs: Calluna and Erica cinerea being co-dominant. No pure Calluna stands could be found. Calluna and Erica occur in small stunted tussocks, with few projecting shoots, of the type described by Farrow (1916) and indicating intensive rabbit grazing. Rabbit droppings were abundant. Two profiles were prepared at well separated locations on the hill range and the samplings of each were separated in time by a period of one year. Both profiles were exposed under Calluna-Erica growth of the type described. Cladonia spp. provided a sparse surface flora; however much of the soil between the ericaceous tussocks was barren, except where small yet dense stands of Polytrichum juniperinum were developed.

Description of Profiles.

	Depth (in.)	pH.	Description.
Profile I	0 - 6	3.9-4.0	Medium brown, humus-rich sand
	6 -(36)	4.0-5.0	Light brown sand, becoming progressively lighter in colour with increase in depth.

Profile II Identical with Profile I

Roots were abundant in the topmost six inches, giving a peaty consistency to the horizon. Below 6 in. roots occurred frequently but decreased in frequency with increase of depth until at 30 inches no roots were observed,

Distribution of Species in Profile I (Puttenham Common)

Horizon:	A1	A2	A2	A2	A2
Sampling depth (in.)	1-6	9.12	15.18	21.24	27.30
Absidia orchidis	a	.	.	.	.
A. spinosa	a	.	.	.	.
Mortierella marburgensis	a	.	.	.	.
Mucor ramannianus	a	a	a	a	a
Zygorrhynchus vuilleminii	a	a	.	.	.
Thielavia sp. (C19)	a	.	.	.	.
Beauveria bassiana.	a	.	.	a	a
Cephalosporium curtipes ? (F29)	a	.	.	.	.
Dicoccum asperum	a	.	.	.	.
Gliomastix convoluta v.felina	a	.	a	a	.
Penicillium adametzi	a	a	.	.	.
P. brevi-compactum	a	a	.	.	.
P. cyclopium	a	.	.	.	.
P. nigricans	a	a	a	a	.
P. spinulosum	a	.	.	.	.
Penicillium sp. (E62)	a	.	.	.	.
Trichoderma viride	a	a	a	.	.
17	17	6	4	4	2

Notes: Colonies of Mucor ramannianus were numerous on all plates from 15 to 30 ins., and were present on all soil plates.

Zygorrhynchus and T. viride occurred on every plate prepared from the horizons where they are recorded as present.

Distribution of Species in Profile 2. (Puttenham Common)

Horizon	A1	A2	A2	A2	A2	A2
Sampling depth (in.)	1-6	9.12	15.18	21.24	27.30	33.36
Absidia orchidis	a c	. .	. .	. .	. .	. .
Mortierella marburgensis	. c	. .	. .	. .	. .	. .
M. (stylosporic sp.) (A41)	a .	. .	. .	. .	a .	. .
Mucor hiemalis	a .	. .	. .	. .	. .	. .
M. ramannianus	a c	a c	a c	a c	a c	a c
Zygorrhynchus vuilleminii	a c	. .	. .	. .	. .	. .
Beauveria bassiana	. c	. .	. .	. .	. .	a .
? Monosporium sp. (F37)	. .	a .	. .	a .	. .	. .
Penicillium adametzi	. c	. .	. .	. .	. .	. .
P. cyclopium	a .	. .	. .	. .	. .	. .
P. nigricans	a c	a c	. .	. .	. .	. .
P. phoeniceum	. c	. .	. .	. .	. .	. .
P. spinulosum	a c	. .	a .	. .	. .	. .
P. thomii	. .	. .	a .	. .	. .	. .
Penicillium sp. (E62)	a c	. .	. .	. .	. .	. .
Penicillium sp. (E80)	. c	. .	. .	. .	. .	. .
Pullularia pullulans	. c	. .	. .	. .	. .	. .
Trichoderma viride	a c	. .	. .	. .	. .	. .
Sterile mycelium, C.21	. c	. .	. .	. .	. .	. .
Sterile mycelium, DS.	. .	. .	. .	. .	. .	a c
20	17	3	3	2	2	3

Notes: M. ramannianus occurred on all soil plates prepared, and Pen. nigricans occurred on all plates to 9 in.

Blackheath, Near Albury, Surrey.

Geological formation. Sandy Beds of the Folkestone Beds, Lower Greensand.

Height above sea level: 350 ft.

The greater part of this heath vegetation is dominated by Calluna; small exposed areas of the soil surface are colonised by Cladonia spp. and mosses, of which Dicranum scaparum is dominant. Ulex europaeus is locally dominant and small stands of Betula alba also occur.

In the area sampled Calluna was dominant, Cladonia and Dicranum occurred on the exposed soil surfaces between Calluna patches.

Only one profile was exposed and sampled.

Description of Profile.

Horizon.	Depth (in).	pH.	Description.
A.1	0-1.5	3.8	Dark-brown, humus-rich sand
A.2	1.5-22	4.0-4.2	Grey-brown sand becoming lighter in colour with increase in depth.
B.1	22-25	4.0	Brown-black, firmly compacted, yet friable humus pan.
B.2	25-31	4.1-4.5	Reddish-brown, gritty sand.
B2/C	31-36	4.8-5.0	Transition zone to -
C	36-(46)	5.0-5.1	Orange-broad coarse sand.

Distribution of Species in Profile I (Blackheath).

Horizon	A1	A2	A2	A2	B1	B2	C
Sampling depth (in.)	1	2-6	9.12	15-21	24	27-36	39
Mort. marburgensis	.	a	.	.	.	.	.
Mucor hiemalis	a	.	.	.	.	.	.
M. ramannianus	.	a	a	a	a	.	.
Chaetomium bostrychodes	.	a	.	.	.	.	.
Dicoccum asperum	.	a	.	.	.	.	.
Penicillium adametzi	.	a	.	.	.	.	.
P. fellutanum	.	a	.	.	.	.	.
P. namyslowskii	a	.	.	.	.	.	.
P. spinulosum	a	a	.	.	.	.	.
P. terlikowskii	.	.	a	.	.	.	.
Trichoderma viride	a	a	.	.	.	.	.
(Moniliaceae) (F.26)	.	a	.	.	.	.	.
Sterile mycelium, DS	.	.	.	a	.	.	.
13	4	9	2	2	1	0	0

Notes: M. ramannianus occurred on every soil plate from 5 to 24 in., and was, with few exceptions, the only fungus occurring on these plates.

Hothfield Heath, Nr. Ashford, Kent.

Geological formation: Sandy Beds of Folkestone Beds, Lower Greensand.

Height above sea level: 50 ft.

The vegetation of this area consists mainly of grasses; Agrostis tenuis and Festuca ovina being co-dominant. In certain regions the grasses are overgrown and suppressed by bracken. Stands of pine and solitary oaks are of scattered distribution.

Traversing this area is a shallow depression, the trough of which is swampy and here Juncus sp., Polytrichum commune and Leucobryum glaucum predominate. The vegetation on the gently sloped banks of this depression shows marked zonation: at the higher level the grasses give way to a narrow belt of silver birch and common gorse, and the lower levels are dominated by a band of Molinia, Dactylis glomerata and gorse (which on both banks of the depression provides a boundary zone of the central swampy Juncetum.) Between the two distinct belts on the sloping banks occurs a narrow strip, 10-15 yds. wide, of Callunetum. This Callunetum is pure. The Calluna, in habit, is dense and hummocked, growing to a height of 6 in., and appears to be fairly heavily grazed: between hummocks the black, barren soil surface is exposed.

Two profiles were prepared within the Callunetum on one of the banks of this depression, and separated by a distance of 30 yds.

Description of Profiles.

	Horizon	Depth (in.)	pH.	Description.
Profile I	A.1	0-2.5	3.7	Brown-black, humus-rich sand.
	A1/A2	2.5-6	3.7-3.8	Transitional zone to -
	A2	6-14	3.8-4.0	Light grey-brown soft sand.
	B	14-22	4.0-4.1	Brown-black firmly compact pan.
	B/C	22-24	4.1-4.2	Transitional zone to -
	C	24-(33)	4.2	Orange brown clayey-sand.
Profile II	A.1	0-3.5	-	Brown-black humus rich sand.
	A.2	3.5-10	-	Almost white, fine sand.
	B.	10-13	-	Brown-black firmly compacted pan.
	B/C	13-14	-	Transitional to -
	C	14-(30)	-	Yellow-orange clayey-sand.

Roots showed a similar distribution to that in other podsol profiles: abundant in A1 horizon, frequent in the A.2 and very sparse below this level. No roots were observed below the 24 inch level.

Distribution of Species in Profile I. (Hothfield Heath)

Horizon:	A1	A1/A2	A2	B	C
Sampling depth (in.)	1.2	3-6	9.12	15-21	24.27
Absidia orchidis	. c	. .	. .	. .	. .
Mortierella parvispora (A40)	a c	. .	. .	. .	. .
Mucor ramannianus.	a c	a c	. .	. .	. .
Zygorrhynchus vuilleminii	. c	. .	. .	. .	. .
Beauveria bassiana	a c	a c	. .	. .	. .
Cladosporium herbarum	. .	a .	. .	. .	. .
Penicillium namyslowskii	a c	a c	. .	. .	. .
P. restrictum	. .	. .	a .	. .	. .
P. spinulosum	a c	a c	. .	. .	. .
Penicillium sp. (E67)	a c	. .	. .	. .	. .
Sarcinella sp. (D39)	. .	. c	. .	. .	. .
Scopulariopsis sp. (F35)	a .	. .	. .	. .	. .
Trichobotrys sp. (D27)	. .	a c	. .	. .	. .
Trichoderma viride	a c	. .	. .	. .	. .
(Demetiaceae) (D37)	. .	. .	. c	. .	. .
(Oosporeae) (F41)	. .	. .	. c	. .	. .
Sterile mycelia: HS.3	. .	. c	. .	. .	. .
DS.40	. .	. .	. .	a c	. .
18	10	8	3	1	0

Notes: Mucor ramannianus occurred on all plates from 1 to 6 in. and B. bassiana on all plates from 2 to 6 in.

Distribution of Species in Profile 2. (Hothfield Heath.)

Horizon.	A1	A2	A2	B	C	C
Sampling depth (in.)	1-3	4-6	9	12	15.18	21-27
Absidia orchidis	b c	. .	. .	. .	. .	. .
Mortierella parvispora (A40)	b c	. .	. .	. .	. .	. .
Mucor hiemalis	b .	. .	. .	. .	. .	. .
M. ramannianus	b c	b c	b c	. .	b c	. .
Zygorrhynchus vuilleminii	b .	. .	. .	. .	. .	. .
Beauveria bassiana	. c	b c	. .	. .	. .	. .
Cladosporium herbarum	. c	. .	. .	. .	. .	. .
Penicillium adametzi	b c	. .	. .	. .	. .	. .
P. namyslowskii	b c	. .	. .	. .	. .	. .
P. spinulosum	b c	. .	. .	. .	. .	. .
Penicillium sp. (E67)	b .	. .	. .	. .	. .	. .
Penicillium sp. (E74)	b c	. .	. .	. .	. .	. .
Trichobotrys sp.(D27)	. .	b c	. .	. .	. .	. .
Trichoderma viride	b c	. .	. .	. .	. .	. .
14	13	3	1	0	1	0

Notes: Mucor ramannianus and Beauveria bassiana occurred on all soil plates from the horizons indicated.

Plateau Heath, South Haven Peninsula, Nr. Studland.

Geological formation: Bagshot Beds of the Bagshot Series.
Height above sea level - less than 50 ft.

The physiography of the S. Haven Peninsula has been described by Diver (1933) and an account of the ecology of this area has been given by Good (1935). Diver states: "It may reasonably be assumed that heath vegetation has existed.... through an indefinite period; but all that can be said definitely is that such a distribution was present in 1849"

Profiles I and II were prepared from soils dominated by Calluna, growing in vigorous bushy habit to a height of 24 ins.; Erica cinerea and Ulex minor are sparse associates, and Cladonia spp. provide a sparse surface flora.

The vegetation of the area from which profile III was sampled has a damper facies; Calluna and Erica tetralix are co-dominant, and in small depressions in this area Juncus spp. and Sphagnum occur. Leucobryum glaucum is also present together with Cladonia spp.

Profiles I and II were prepared close together and were about half a mile distant from Profile III.

Description of Profiles.

	Horizon	Depth (in.)	pH	Description.
Profile I.	A1	1-3.5	3.8	Dark brown peaty sand
	A1/A2	3.5-6.0	4.1	Grey sand.
	A2	6.0-19.0	4.4	Loose white sand, flint-like stones, up to 1 ft. in length, frequent.
	B	19.0-22.0	3.9	Dark brown firmly compacted layer.
	C	22.0-(42.0)	4.5	Yellow-orange clayey-sand, stones frequent
Profile II.	Similar to Profile I except for the following slight differences in horizon depths:-			
	A1	0-4.5		
	A1/A2	4.5-9.0		
	A2	9.0-19.5		
	B	19.5-22.0		
Profile III.	A1	1.0-6.0	3.9	Brownish-black, sticky, peaty sand.
	A2	6.0-9.0	4.2	White sand.
	B	9.0-14.0	4.3	Reddish-brown, sticky, zone deposition.
	B/C	14.0-15.0	4.4	Transition zone.
	C	15.0-(30.0)	4.5	Greyish-white clayey sand, with yellow-orange mottling.

Distribution of roots in all three profiles was similar: they occurred in great abundance in the A1 horizons, giving this layer a fibrous texture, and were frequent in the A2 horizons below which roots were rare.

Distribution of Species in Profile I. (Plateau Heath.)

Horizon.	A1	A1/A2	A2	A2	B	C
Sampling depth (in.)	1-3	4-6	9.12	15.18	21	24.30
Absidia orchidis	. .	. b	. .	. .	. .	. .
A. spinosa	a .	. .	. .	. .	. .	. .
Mortierella humilis ? (A37)	. .	. b	. .	. .	. .	. .
M. isabellina	a b	. .	. .	a .	. .	. .
M. parvispora	. .	. b	. .	. b	. .	. .
M. parvispora ? (A40)	. .	. .	. .	. b	. .	. .
M. (stylosporic sp.) (A43)	. b	. b	. .	. .	. .	. .
Mucor ramannianus	a b	a b	a b	a b	. b	. .
Gelasinospora retispora.	. .	a .	. .	. .	. .	. .
Beauveria bassiana	. b	a b	a b	a b	. .	. .
Penicillium adametzi	a .	. b	. .	. .	. .	. .
P. namyslowskii	. .	. .	a .	. .	. .	. .
P. nigricans	a b	a b	a b	. .	. .	. .
P. spinulosum	a .	a .	. .	. .	. .	. .
P. thomii	. b	. .	. .	. .	. .	. .
P. (thomii series) (E48)	a b	. .	. .	. .	. .	. .
Penicillium sp. (E62)	. .	a .	a b	. .	. .	. .
Penicillium sp. (E74)	. .	a b	. b	. .	. .	. .
Scopulariopsis sp. (F35)	. .	. b	. b	. .	. .	. .
Trichobotrys sp. (D27)	. .	. .	a b	a b	. .	. .
Trichoderma viride	a b	a .	a .	. b	. .	. .
Sterile mycelia: DS.56	. .	. b	. .	. .	. .	. .
DS.57	. .	. .	. .	. .	a .	. .
DS.58	. .	. .	. .	. .	. .	. b
24	11	15	9	7	2	1

Distribution of Species in Profile 2. (Plateau Heath.)

Horizon.	A1	A1/A2	A2	A2	B	C
Sampling depth (in.)	1-4	5.6	9.12	15.18	21	24-30
Absidia orchidis	a b	. .	. .	. .	. .	. .
A. spinosa	a .	. .	. .	. .	. .	. .
Mortierella isabellina	a .	a .	a .	a .	. .	. .
M. marburgensis	a b	. .	. .	. .	. .	. .
M. parvispora ? (A40)	. b	. .	. .	. .	. .	. .
Mucor ramannianus	a b	a b	a b	a b	. .	. .
Acrostalagmus albus	. .	. .	. b	. .	. .	. .
Beauveria bassiana	a b	. b	a b	a b	. .	. .
Cladosprium herbarum	. .	. .	. .	. b	. .	. .
Haplographium chlorocephalum	. .	. .	. .	. .	. .	a .
Penicillium adametzi	. b	. b	. .	a .	. .	. .
P. brevi-compactum	. b	. .	. .	. .	. .	. .
P. citreo-viride	. b	. .	. .	. .	. .	. .
P. namyslowskii	a b	. .	. .	. .	. .	. .
P. nigricans	a b	. .	. .	. .	. .	. .
P. thomii	a b	a b	. .	. .	. .	. .
Penicillium sp. (E62)	a b	a b	. .	. .	. .	. .
Penicillium sp. (E74)	. .	a .	. .	a .	. .	. .
Pestalotia sp. (F30)	a .	. .	. .	. .	. .	. .
Stachlydium sp. (D31)	a b	. .	. .	. .	. .	. .
Trichobotrys sp. (D27)	. .	. b	a b	a .	. .	. .
Trichoderma viride	a b	a b	a b	. b	. .	. .
Sterile mycelium: DS.53	. .	. .	. .	. .	a .	. .
23	17	9	6	8	1	1

Distribution of Species in Profile 3. (Plateau Heath.)

Horizon	A1	A1	A2	B	C	C
Sampling depth (in.)	1-3	4-6	8	9.12	15.18	21.24
Absidia orchidis	a .	a b	. .	. .	. .	. .
Mortierella stylosporic sp. (A43)	a b	. .	. .	. .	. .	. .
Botrytis cinerea	. b	. .	. .	. .	. .	. .
Cladosporium herbarum	. .	a .	. .	. .	. .	. .
Penicillium adametzi	a b	a b	. .	. .	. .	. .
P. frequentans series (E79)	a b	. .	. .	. .	. .	. .
P. namyslowskii	. b	. .	. .	. .	. .	. .
P. spinulosum	a .	. b	. .	. .	. .	. .
Scopulariopsis sp. (F57)	. .	. b	. b	a b	. .	. .
Trichybotrys sp. (D27)	. .	a b	a b	a b	. .	. .
Trichoderma viride	a b	a .	. .	. b	. .	. .
Sterile mycelia: HS.19	. .	. b	. .	. .	. .	. .
DS.53	. .	. .	. .	. .	a .	. .
DS.54	. .	. b	. .	. .	. .	. .
DS.55	. .	. b	. .	. .	. .	. .
DS.58	. .	. .	a b	. .	. .	. .
16	8	10	3	3	1	0

Notes: Profiles 1 and 2; nearly all 21-30 in. soil plates remained sterile. In both profiles Mucor ramannianus colonies were very numerous on all soil plates from 2 to 18 in.

Profile 3; M. ramannianus absent (N.B)

Godlingston Heath, Nr. Studland, Dorset.

Geological formation: Bagshot Beds of the Bagshot Series.
Height above sea level: Profile I. 100.ft. Profile II. 25 ft.
The vegetation of this heath is essentially similar to that of Plateau Heath. (See Good, 1935)

The vegetation of the area from which profile I was sampled is dominated by Calluna growing in a thick turf-like habit to a height of about 6 in. Ulex minor is of scattered distribution. Very sparse tufts of Molinia and Festuca ovina occur within this Callunetum and Cladonia spp. are present.

Profile II was sampled from the soil of a sheltered valley running steeply down to the Studland-Swanage road. Calluna is completely dominant growing in dense tall bushy habit to a height of 12-15 in. The soil surface between Calluna bushes is lightly colonised by Cladonia spp. but for the most part remains bare.

Description of Profiles.

	Horizon	Depth (in.)	pH	Description.
Profile I.	A1	1-3	4.2	Brown-black, humus-rich sand.
	A1/A2	3-9	4.4	Medium-brown, soft sand. Pebbles abundant.
	A2	9-20	4.6	Light grey, almost white sand. Pebbles occasional.
	A2/B	20-24.5	4.8	Sand becoming progressively darker in colour.
	B	24.5-29	4.8	Brown-black, firmly concreted humus pan.
	C	29-(36)	5.0	Bluish-white sandy-clay with orange red mottling

Roots were extremely abundant in the A1 horizon imparting a fibrous nature to the surface soil. In the leached horizon roots were frequent and were observed to be present beneath the pan.

Profile II.	A1	1-3	4.1	Brown-black humus-rich sand.
	(A2)	3-(39)	4.2	Medium brown soft sand
		30	4.6	gradually becoming lighter in colour. Yellow-white or almost white when dry.

No pebbles were observed throughout this profile. Roots were extremely abundant in the surface peaty layer, below this to a depth of 12 in. they were frequent and then became progressively less frequent with increase in depth. For convenience of presentation and comparison of results the plain sandy subsoil of profile II is regarded as analogous to the leached horizon of the podsols.

Distribution of Species in Profile I. (Godlingston Heath)

Horizon.	A1	A1/A2	A2	A2/B1	B1	C
Sampling depth (in.)	1-3	4-9	12-18	21-24	25-27	30
Mortierella isabellina	a .	. .	. .	. .	. .	. .
M. marburgensis	a b	. .	. .	. .	. .	. .
Mucor ramannianus	. .	a .	a b	a b	. b	. .
Coniochaeta discospora	a .	. .	. .	. .	. .	. .
Beauveria bassiana	a b	a b	a b	a .	. .	. .
Cladosporium herbarum	. .	a .	. .	. .	. .	. .
Penicillium adametzi	a b	a .	. .	. .	. .	. .
P. namyslowskii	a b	. b	. .	. .	. .	. .
P. nigricans	a b	a b	. .	. .	. .	. .
P. spinulosum	a b	a .	. .	. .	. .	. .
Penicillium sp. (E62)	a .	. b	. .	. .	. .	. .
Trichobotrys sp.(D27)	. b	. .	. b	. b	. .	. .
Trichoderma viride	a b	. .	. .	. .	. .	. .
Sterile mycelium: DS.73	. .	. .	. .	. .	a b	a b
HS.22	. .	. .	. b	. b	. .	. .
15	11	8	4	4	2	1

Notes: Mucor ramannianus was not of frequent occurrence on soil plates. Beauveria bassiana colonies were extremely numerous on all soil plates from 2 to 18 in.

Distribution of Species in Profile 2. (Godlingston Heath).

Horizon	A1	A2	A2	A2	A2	A2
Sampling depth (in.)	1-3	4-6	9.12	15.18	21.24	27.30
Mortierella isabellina	a b	. .	. .	. .	. .	. .
Mucor ramannianus	a b	a b	a b	a b	. b	. .
Coniochaeta discospora	a b	. .	. .	. .	. .	. .
Gelasinospora retispora	a .	. .	. .	. .	. .	. .
Beauveria bassiana	. .	. .	. b	. b	. .	. .
Penicillium adametzi	a b	. b	. .	. .	. .	. .
P. namyslowskii	a b	a .	. .	. .	. .	. .
P. nigricans	. b	. .	. .	. .	. .	. .
P. spinulosum	a b	. b	. .	. .	. .	. .
P. thomii series (E48)	. b	. .	. .	. .	. .	. .
Penicillium sp. (E62)	a b	. .	. .	. .	. .	. .
Penicillium sp. (E74)	a .	. .	. .	. .	. .	. .
Stachlydium sp. (D31a)	. b	. .	. .	. .	. .	. .
Trichobotrys sp. (D27)	a .	. .	. .	. .	. .	. .
Trichoderma viride	a b	. .	. .	. .	. .	. .
Sterile mycelium, DS.72	a .	. .	. .	. .	. .	. .
16	15	4	2	2	1	0

Notes: Colonies of Mucor ramannianus were extremely numerous (15-30) on all soil plates from 3 to 15 in.

Kelling Heath, Nr. Weybourne, Norfolk.

Geological formation: Glacial gravels + sands (Pleistocene).

Height above sea level: 200 ft.

Kelling Heath is a treeless windswept plateau, about 1 mile from the coast, and 2 sq. miles in area.

Over wide areas of the heath Calluna and Erica cinerea are co-dominant, and Cladonia spp. provide a sparse surface flora. Of sparse occurrence within this Calluna-Erica association are: Epilobium angustifolium, Rumex acetosella, Teucrium scorodonia and Ulex spp. Bracken is locally dominant.

Two profiles were prepared beneath patches of pure Calluna.

Description of Profiles.

	Horizon	Depth (in.)	pH	Description.
Profile I	A1	0-1.5	3.7	Dark brown humus-rich sand.
	A1/A2	1.5-5	3.9	Transitional zone.
	A2	5-10.5	4.0	Grey loose sand.
	B	10.5-12	3.9	Dark brown compact humus pan.
	B/C	12-18	4.3	Yellowish-brown transitional zone.
	C	18-(30)	4.5	Yellow-brown, coarse sand and gravel.
Profile II	Identical with profile I.			

Roots were abundant in the A1 horizon, and were of frequent occurrence to a depth of 18 in.

Stones were frequent to 18 in., below which level they were abundant.

Distribution of Species in Profile I. (Kelling Heath.)

Horizon	A1	A1/A2	A2	B	C	C
Sampling depth (in.)	1	2-5	6.9	11	12.15	18.21
Absidia orchidis	a b	. .	. .	. .	. .	. .
Mortierella isabellina	. b	. b	. .	. .	. .	a .
Mucor ramannianus	a b	a b	a b	a b	. .	. .
Beauveria bassiana	. .	. b	. .	. .	. .	. .
Cephalosporium sp. (F31)	a .	. .	a .	. .	. .	. .
Cladosporium herbarum	. .	. .	a .	. .	. .	. .
? Monosporium sp. (F37)	a .	a b	a b	. b	a b	a .
Penicillium adametzi	. b	. .	. .	. .	. .	. .
P. nigricans	a b	a b	. .	. .	. .	a .
P. spinulosum	a .	. .	. .	. .	. .	. .
Penicillium sp. (E62)	a b	. .	. .	. .	. .	a .
Trichobotrys sp. (D27)	. .	a b	. .	. .	. .	a .
Trichoderma viride	a b	a b	a .	a .	a b	. .
? Zygodesmus sp. (F6)	a .	. .	. .	. .	. .	. .
Sterile mycelia: DS.60	. .	. .	. .	. .	a b	a b
DS.63	. .	. .	. b	a b	a .	. .
DS.64	. .	. .	. .	a b	a b	. .
DS.85	. .	. .	. .	. .	a .	a b
18	11	7	6	5	6	7

Notes: Pen. nigricans was present on all plates 1-5 in. Colonies of ? Monosporium sp. (F37) were very numerous. The soil plates from beneath the humus pan were dominated by an assortment of small sterile colonies. (DS. forms)

Distribution of Species in Profile 2. (Kelling Heath.)

Horizon:	A1	A1/A2	A2	B	C	C
Sampling depth (in.)	1	2-5	6.9	11	12.15	18.21
Mortierella isabellina	a b	a .	. .	. .	. .	. .
Mucor ramannianus	a b	a .	a b	. .	a .	. .
Beauveria bassiana	. .	. b	. .	. .	. .	. .
? Chaetopsis sp. (D34)	. .	. .	. .	. .	a b	. .
Cladosporium herbarum	. .	. b	. .	. b	. .	. .
? Monosporium sp. (F37)	. .	. b	a b	a b	. b	. .
Penicillium adametzi	. .	a b	. .	. .	. .	. .
P. namyslowskii	. b	. .	. .	. .	. .	. .
P. nigricans	a b	a b	. .	. .	. .	. .
Penicillium sp. (E62)	a b	. .	. .	. .	. .	. .
Pullularia pullulans	. .	. b	. .	. .	. .	. .
Trichobotrys sp. (D27)	. .	a .	. .	. .	. .	. .
Trichoderma viride	a b	a b	a .	a b	. .	. .
Sterile mycelia: DS.60	. .	. .	. .	. .	a b	. .
DS.61	. .	. .	. .	. .	a b	. .
DS.62	. .	a .	. .	. .	. .	. .
DS.63	. .	a b	. b	. .	a .	. b
DS.64	. .	a b	. .	. .	. .	. .
DS.65	. .	a .	a .	. .	. .	. .
DS.85	. .	a b	. .	. .	a b	. .
20	6	15	5	3	7	1

Rhossilly Downs, Gower Peninsula, Glamorganshire.

Geological formation: Old Red Sandstone.

Height above sea level: 633 ft. (The Beacon)

The Rhossilly Downs are a range of hills at the westernmost tip of the Gower Peninsula, subjected to heavy rainfall and high winds; They rise steeply to their maximum height within half a mile of the sea-shore.

The vegetation is dominated by fescue; bracken occurs on the lower slopes, and gorse to within 50 ft. of the summit. Calluna is present only on the summit and near-summit areas, where it is co-dominant with Festuca ovina. The Calluna habit is a short dense turf, 6-8 in. tall, through which the fescue grows. Small patches of Polytrichum juniperinum are of frequent occurrence. The whole area is grazed by sheep and rabbits.

Rocky outcrops, often quite large, are abundant on these higher slopes and the preparation of profiles was difficult; in many places it was impossible to dig below 4 in. where the soil formed merely a thin covering to the underlying rock. Three profiles were exposed, however, the soil from these representing a filling between large rock fragments.

Description of Profiles.

	Depth (in.	pH	Description.
Profile I.	0-0.5		Calluna and fescue litter
	0.5-9	3.9	Brownish-black, very soft, moist clayey loam, between rock fragments.
	9-14	4.2	Yellow-brown, gritty layer.
	14-24	4.5	Reddish-brown, clayey loam.
	24-		Rock.

The surface raw humus was not sampled.

Roots were profusely developed in the upper 9 in. and were of frequent occurrence to the lowest depth at which samples could be collected.

Profile II. Prepared close to profile I and was identical with it. Samples were collected to 21 in.

Profile III.	0-6	3.8	Brownish-black moist clayey loam, between rock fragments.
	6-14	4.0	Gradual transition to -
	14-19	4.5	dark reddish-brown soil.
	19-		Rock.

Distribution of roots in profiles II and III was similar to that described for profile I

For purposes of comparison the clayey-loam soils of this area have been analogised to the A1 horizon of the podsols.

Distribution of Species in Profile I. (Rhossilly Down.)

Sampling depth (in.)	1-3	4-9	12	15.18	21.24
Absidia spinosa	a	a	.	.	.
Mortierella humilis ? (A37)	a	.	.	.	.
M. parvispora	a	a	a	a	.
Zygorrhynchus vuilleminii	a	a	.	.	.
Pseudoeurotium zonatum	.	.	.	.	a
Coniothyrium sp. (C23)	.	.	a	.	.
Penicillium adametzi	a	a	a	.	.
P. raistrickii series (E72)	a	a	.	.	.
P. spinulosum	.	.	a	.	.
P. thomii	.	a	.	.	.
Penicillium sp. (E69)	a	a	.	a	.
Penicillium sp. (E80)	.	a	.	.	a
Scopulariopsis sp. (F.35)	a	a	.	a	.
Trichoderma viride	a	a	.	.	.
14	9	10	4	3	2

Notes: Abs. spinosa, Trichoderma viride and Zyg. vuilleminii occurred on all plates from 1 to 9 in.

Mucor ramannianus was not isolated.

Distribution of Species in Profile 2. (Rhossilly Down.)

Sampling depth (in.)		1-3	4-9	10-12	15.18	21
Absidia orchidis		a c	. c	. .	. .	. .
Abs. spinosa		a .	a c	a .	. .	. .
Mortierella humilis ?	(A37)	. c	. .	. .	. .	. .
M. parvispora		. c	. .	a .	a c	. .
Mucor hiemalis		. .	a c	a .	. c	. .
Zygorrhynchus vuilleminii		a c	a c	a .	a c	. .
Gelasinospora cerealis		a c	. .	. .	. .	. .
Acrostalagmus cinnabarinus ?	(F39)	. .	. c	. .	. .	. .
(Oosporeae)	(F40)	. .	. .	. .	a .	. c
(")	(F42)	. .	. .	. .	a .	. .
Penicillium adametzi		a c	. c	a .	. .	a .
P. cyclopium		. .	. c	. .	. .	. .
P. nigricans		. .	a .	a c	. .	. .
P. melinii ?	(E70)	. .	. .	. c	. .	. .
P. raistrickii series	(E72)	. .	a .	. .	. .	. .
P. spinulosum		a .	a .	a .	. .	. .
P. thomii		. .	a c	a c	. .	. .
P. thomii series	(E48)	. .	a .	. .	. .	. .
Penicillium sp.	(E62)	a c	a .	. .	. .	. .
Penicillium sp.	(E67)	. c	. .	. .	. .	. .
Penicillium sp.	(E69)	a .	. c	. c	. .	. .
Penicillium sp.	(E80)	. .	a c	a c	a c	. .
Trichoderma viride		a c	a c	a c	. c	. .
Sterile mycelia:	C.21	. .	a c	. .	. .	. .
	HS.6	. .	. .	. .	. c	. .
	DS.83	. .	. .	. .	a .	. .
26		12	17	12	9	2

Distribution of Species in Profile 3. (Rhossilly Down.)

Sampling depth (in.)	1-3	4-6	9.12	15.18
Absidia orchidis	a .	. .	. .	. .
Abs. spinosa	. c	a c	. c	. .
Mortierella humilis ? (A37)	a c	. .	. .	. .
M. parvispora	. c	a c	. c	a c
Mucor hiemalis	a .	. .	. .	. .
M. ramannianus	. .	. c	. .	. .
Zygorrhynchus vuilleminii	a c	a c	a c	a c
Gelasinospora cerealis	a c	. .	. .	. .
Penicillium adametzi	. .	. c	. .	. c
P. nigricans	a c	. c	. .	. .
P. raistrickii series (E72)	a .	. .	a .	a c
P. restrictum	. .	. .	. .	a .
P. spinulosum	a c	. .	a .	. .
P. thomii	. .	a c	a .	. .
Penicillium sp. (E62)	a c	. .	a .	. .
Penicillium sp. (E69)	a c	a .	. c	. .
Penicillium sp. (E80)	. c	. .	. .	. .
Pullularia pullulans	. .	. c	. .	. .
Scopulariopsis sp. (F35)	. .	a c	. c	a c
Trichoderma viride	a c	a c	a c	a c
20	14	11	10	7

Notes: Most soil plates were dominated by Absidia spp., Trichoderma and Zygorrhynchus. In this series of experiments only one colony of Mucor ramannianus was observed.

Ogwen Bank, Carnedd Dafydd, Nr. Bethesda, N. Wales.

Geological formation: Sandstone, slates and conglomerates of Cambrian age.

Height above sea level: 700 - 1200 ft.

The vegetation and ecological features of this area have been described by Campbell (1949), who says of the Carneddan: ".... mostly grassland dominated by Nardus stricta and changing on the drier, less-peaty N.E. slopes to fescue .... at lower elevations Calluna tends to form the principal cover". The Calluneta sampled would, according to the classification given by Stapledon (1936) fall into the category of Heather Moor or Heather Fell.

The two profiles, I and II, on the Ogwen Bank, were prepared from soils dominated by Calluna and Festuca ovina, where the Calluna is confined to dense patches within the grass vegetation. The ling seldom attains a height greater than 2 in., which is probably due largely to grazing. Vaccinium myrtillus occurs within these Calluna patches.

Profiles III and IV were prepared on the higher and more exposed slopes above Tyny-Maes at the N.W, foot of Carnedd Dafydd. Nardus stricta is dominant: Calluna locally dominant and appears to be confined largely to shallow soil pockets on the abundant rocky outcrops. Vaccinium myrtillus and a flora of various mosses (Leucobryum glaucum, Dicranum scaparum, Hypnum schreberi, and Rhacomitrium lanuginosum) were associated with the Calluna.

Description of Soil Profiles.

	Depth	pH	Description.
Profile I	0-4	3.8	Rich brown peaty loam
	4-		Rock
Profile II	0-3	3.9	Rich brown peaty loam.
	3-4	4.2	Grey clay-like material; superficial weathered layer of the underlying rock.
	4-		Rock.
Profile III	0-5	3.8	Rich reddish-brown peaty loam.
	5-		Rock
Profile IV	1-4	3.8	Rich reddish-brown peaty loam.
	4-		Rock.

In all four areas sampled the surface was covered by a deposit of raw humus, which in the two samples from Tyny-Maes was about 2 in. thick and composed mainly of moss material. This layer was not sampled. The whole area is grazed by sheep and rabbits.

Roots were abundant throughout all soils sampled.

(For purposes of comparison of profile distributions the soils of this locality are considered analogous to the A1 horizon of the podsols.)

Distribution of Species in Profile 1. (Ogwen Bank)

Sampling depth (in.)	1	2	3	4
Absidia orchidis	a .	. .	. .	. .
Abs. spinosa	a c	a c	a c	a c
Mortierella humilis ? (A37)	. c	. .	. .	. .
M. hygrophila	. .	. .	. .	a .
M. parvispora ? (A40)	. .	. c	. .	. c
Mort.(stylosporic sp.) (A43)	a c	. c	a .	a c
Mucor jansseni	. .	a .	. .	a .
Cephalosporium humicola	. .	. .	a .	. .
Cephalosporium sp. (F31)	. .	. .	. c	. .
Penicillium adametzi	a c	a c	a c	a .
P. melinii ? (E70)	a .	a .	a .	a .
P. nigricans	a c	a c	a c	a c
P. raistrickii series (E72)	. .	. .	a .	a .
P. thomii	. .	a .	. c	. .
Trichoderma viride	a c	a c	a c	. c
15	8	9	10	10

Notes: Abs. spinosa, P. adametzi, P. nigricans, and Trichoderma occurred on nearly every soil plate prepared.

Distribution of Species in Profile 2. (Ogwen Bank).

Sampling depth (in).	1	2	3	4
Absidia spinosa	a .	. c	a .	. .
Mortierella (stylosporic sp.) (A43)	. .	. .	. .	a .
Penicillium adametzi	a c	a c	a c	a c
P. melinii ? (E70)	a .	a .	. .	. .
P. nigricans	. .	a c	a c	. .
Penicillium sp. (E67)	. .	. c	. .	. .
Trichoderma viride	a .	a c	a c	a c
Sterile mycelium, DS.50	. .	. .	. .	a .
8	4	6	4	4

Distribution of Species in Profile 3. (Ogwen Bank)

Sampling depth (in.)	1	2	3	4	5
Absidia spinosa	. .	a b	. b	. .	. .
Mortierella minutissima	. .	. b	. .	. .	a .
Mort. (stylosporic sp.) (A.43)	a b	. .	. .	. .	. .
Mucor jansseni	. .	. .	. .	. .	a .
Pseudoeurotium zonatum.	. .	. .	. .	a b	a .
Beauveria bassiana ? (F52)	. .	. .	. .	. b	. .
Cephalosporium humicola	a b	. .	. b	a b	a b
Penicillium adametzi	. b	a b	a b	. .	a b
P. lividum	a .	. .	. .	. .	. .
P. spinulosum	a b	. .	. .	. .	. b
Pullularia pullulans	. b	. .	. .	. .	. .
Trichobotrys sp. (D27)	. .	. .	. .	. b	. .
Trichoderma viride	a b	a b	a .	a .	. b
Sterile mycelium, C21	. .	a b	. .	. .	. .
14	7	5	4	5	7

Distribution of Species in Profile 4. (Ogwen Bank)

Sampling depth (in.)	1	2	3	4
Absidia orchidis	. c	. .	. .	. .
Abs. spinosa	a c	a c	a c	. c
Mortierella humilis ? (A37)	. c	. .	. .	. .
Mort. (stylosporic sp.) (A43)	a .	. .	. c	. .
Penicillium adametzi	a c	a c	. .	a c
P. melinii ? (E70)	. .	a .	. .	. c
P. nigricans	a c	a c	a c	a c
P. raistrickii series (E72)	. .	a .	. .	. .
P. spinulosum	. .	. .	. c	. c
P. thomii	. .	. .	. .	a .
Trichoderma viride	a c	a c	a c	a c
11	7	6	5	7

Newborough Warren, Anglesey.

Geological formation: Blown sands overlying strata of Pre-Cambrian origin.

Height above sea level: Very little.

Newborough Warren is a region of sandy wastes and dunes, of approximately 8 sq. miles in area, on the southernmost corner of the island of Anglesey.

Calluna does not occur in pure stands, but only within quite complex plant associations; it is limited to the areas behind the grey fixed dunes.

Profile I was prepared in an area intermediate between slack and fixed dune (Calluna in Salicetum). The species present are listed below in order of percentage frequency: *

100	Salix repens	70	Agrostis stolonifera
	Potentilla erecta		Equisetum varigatum
	Prunella vulgaris		Hydrocotyle vulgaris
90	Calluna vulgaris		Agrostis tenuis
	Carex flacca		Carex caryophila
	C. pulicaris		Juncus articulatus
	Lotus corniculatus	60	Leontodon hirsutus
	Poa pratensis	Bryophytes:	
	Festuca rubia		Campylium stellatum 80
80	Triolium repens		Fissidens adiantorides 60

(Only those spp. with frequency greater than 50% are listed)

Calluna vulgaris often occurs in larger patches (of several sq. yd.) where it is locally sub-dominant (Calluna-Salicetum) or even dominant. (Fixed Callunetum with Salix.)

The whole of this Salix - Calluna - Carex association is closely grazed and plants seldom attain more than one or two inches in height.

At the time of sampling (12th May '53) the water table was at 22 in. depth.

The profile (I) was prepared beneath Callunetum in which Salix was sub-dominant

The area in which profile II was prepared is somewhat higher than the former and nearer to the grey fixed dunes. The spp. present are listed below in order of percentage frequency. *

100	Salix repens		Bryophytes:
85	Calluna vulgaris	100.	Rhytiadelphus triquetrum
80	Carex arenaria	95	Hylocomium splendens.
65	Poa pratensis		
60	Potentilla erecta		

Calluna is locally dominant where it occurs in large hummocks, within which a deep surface flora of mosses (Hylocomium) develops and gives rise to a layer of raw humus, 1 - 2 in. deep, about the aerial shoots of the Calluna plants. This area is not completely submerged during winter months, but the water is not far removed from the surface.
At the time of sampling the water table was 24 in. below the surface.

The profile II, prepared in this area was immediately beneath a large Calluna hummock, which itself was pure; but it was noted that the extensive root system of Salix ramified through the soil of this small area.

Descripton of Profiles.

	Depth.	pH	Description.
Profile I.	0-2	5.5	Brownish-black peaty sand.
	2-3.5	5.9	Transition zone.
	3.5-(24)	6.0-8.0	Brownish-yellow sand flecked with orange-red.
Profile II.	0-4	6.5	Dark brown peaty sand.
	4-5.5	7.0	Transition zone.
	5.5-(22)	7.0-7.5	Brownish yellow sand.

Roots were abundant in surface layers, but were very infrequently encountered below 9-12 in.

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* Species lists and frequency data kindly provided by D. Ranwell Esq. B.Sc.

Distribution of Species in Profile 1. (Newborough Warren)

Sampling depth (in.)	1.2	3	4-6	9.12	15.18
Mortierella humilis ? (A37)	a b	a .	a .	. .	. .
M. minutissima	a b	a b	. b	. .	. .
Mucor ramannianus	. .	. .	a .	. .	. .
Thielaria sp. (C26)	a b	. .	. .	. .	. .
Beauveria bassiana	. b	. .	. .	. .	. .
Cephalosporium acremonium	a b	a .	a .	a b	. .
Cephalosporium sp. (F31)	a b	. .	. .	. .	. .
Cladosporium herbarum	. b	. .	. .	. .	. .
Gliomastix convoluta v. felina	. b	. .	. .	. .	. .
Penicillium melinii ? (E70)	. b	. .	. .	. .	. .
Penicillium sp. (E62)	a b	. .	. .	. .	. .
Penicillium sp. (E67)	. .	a b	a b	a b	. .
Penicillium sp. (E69)	a .	. .	a b	. .	. .
Pestalotia sp. (F43)	a .	. .	. .	. .	. .
Pullularia pullulans	. b	. .	. .	. .	. .
Trichoderma viride	a b	a b	a b	. .	. .
16.	14	5	7	2	0

Notes: The numbers of colonies of Penicillium spp. on soil plates increased with depth to 9 in. This is the reverse of the normal distribution of Penicillia in soil profiles.

Only one colony of M. ramannianus was observed.

Distribution of Species in Profile 2. (Newborough Warren)

Sampling depth (in.)	1-4	5	6	9.12	15.18
Mortierella humilis ? (A37)	a b	a b	. .	. .	. .
M. minutissima	a b	a b	a .	a .	. .
Mucor ramannianus	a b	a .	. .	. .	. .
Sporormia intermedia	. b	. .	. .	. .	. .
Beauveria bassiana	a b	a b	a b	. b	. .
Cephalosporium acremonium	. b	a b	a b	. b	. .
Cladosporium herbarum	a .	. .	. .	. .	. .
Gliomastix convoluta v. felina	a b	. .	. .	. .	. .
Penicillium cyclopium series (E42)	. b	. .	. .	. b	. .
P. lividum	a .	. .	. .	. .	. .
P. phoeniceum	a .	. .	. .	. .	. .
P. spinulosum	a .	. .	. .	. .	. .
P. thomii	a b	. .	. .	. .	. .
Penicillium sp. (E67)	a b	a b	a b	. b	. .
Sarcinella heterospora	a .	. b	. .	. .	. .
Trichoderma viride	a b	. .	. .	. .	. .
16	16	7	4	5	0

Notes: Colonies of both Mortierella spp. were numerous on soil plates from 1 to 5 in.: colonies of M. ramannianus were few at these levels. Penicillia, particularly E67, showed similar anomalous distribution to that in Profile I.

4. CORRELATION AND DISCUSSION OF RESULTS.

(a) Groups and Species of fungi isolated.

The species isolated are listed below. Following each specific name are two numbers; the first is a constancy figure representing the number of heathland soils from which the fungus has been isolated (maximum 13), the second figure is the frequency of occurrence in 39 profiles, and is expressed as a percentage.

The majority of sterile mycelia are divided into two groups: one in which the mycelium is dark-coloured (DS) and the other light coloured, or hyaline, (HS)

Where there is doubt as to identity the collection number is given.

PHYCOMYCETES.

Absidia orchidis (Vuill.) Hagem. 8:56

A. spinosa Lendner. 4:26

Haplosporangium decipiens Thax. 2:10

Mucor hiemalis Wehm. 4:13

M. jensseni Lendner 1:5

M. ramannianus Moller 12:82

Mortierella hygrophila Linn. 1:2

M. isabellina Ond. 4:41

M. marburgensis Linn. 6:20

M. minutissima van Tieg. 2:8

M. parvispora Linn. 4:26)
M. parvispora ? (A40) 4:15) 6:33

M. zychae Linn. 1:2

PHYCOMYCETES.

M. humilis ?Linn.(A37) 4:20

M. (stylosporic) sp.(A41) 1:2

M. (stylosporic) sp.(A43) 2:15

Piptocephalis cylindrospora ? Bain. (A23) 1:5

Zygorrhynchus vuilleminii Namysl. 5:31

ASCOMYCETES.

Aspergillus ruber series (C17) 1:2

Chaetomium bostrychodes Zopf. 1:2

C. murorum Cain 1:5

Coniochaeta discospora (Auwers) Cain 1:5

Coniochaeta sp. (C13) 1:2

Gelasinospora cerealis Dowd. 2:10

Gelasinospora retispora Cain 3:10

Pseudoeurotium zonatum v. Beyma 3:8

Sporormia intermedia Auwers. 1:5

Thielavia terricola (G. & A.) Emmons 1:5

Thielavia sp. (C19) 1:2

Thielavia sp. (C25) 2:5

Thielavia sp. (C26) 1:2

FUNGI IMPERFECTI.

Sphaeropsidales.

Coniothyrium sp. (C23) 2:10

Pyrenochaeta sp. (C24) 1:2

Phoma sp. (C2) 1:2

Phoma sp, (C22) 1:2

FUNGI IMPERFECTI.

Moniliales.

Acrostalagmus albus Preuss 2:5

A. cinnabarinus ? Cda. (F39) 1:2

Alternaria sp, (D20) 1:2

Beauveria bassiana (Bals.) Vuill. 10:56

Cephalosporium acremonium Cda. 2:8

C. curtipes? Sacc. (F29) 1:2

C. humicola Oud. 1:5

Cephalosporium sp. (F31) 4:10

? Chaetopsis sp. (D34) 2:10

Cladosporium herbarum Link ex Fries. 9:56

? Dematium sp, (D21) 2:5

? Dematium sp. (D32) 1:2

Dicoccum asperum Cda 2:5

Fusidium sp. (F28) 1:2

Gliomastix convoluta (Harz) v felina Mason 2:8

? Haplaria sp, (F20) 2:5

Haplographium chlorocephalum (Fres) Gr. 1:2

? Monosporium sp, (F37) 4:36

Oosporeae: F41 1:2

F42 1:2

F48 1:2

Oedocephalum sp. (F34) 1:2

Paecilomyces sp. (E1) 1:2

FUNGI IMPERFECTI.

Moniliales.

Penicillium adametzi Zal.	12:87
P. aurantio-candidum Dierckx.	1:2
P. brevi-compactum Dierckx.	4:13
P. cyclopium Westling	4:18
P. cyclopium series (E42)	1:2
P. citreo-viride Biourge	2:8
P. decumbens Thom	1:2
P. fellutanum Biourge	5:18
P. frequentans series (E79)	1:2
P. lividum Westling	2:5
P. melinii ? Thom (E70)	4:15
P. namyslowskii Zal.	9:64
P. nigricans Bainier	8:41
P. notatum ? Westling (E34)	1:2
P. phoeniceum v. Beyma	2:5
P. piceum Raper & Fennel	1:5
P. roqueforti series (E27)	1:2
P. restrictum Gil. & Abb.	3:10
P. restrictum series (E28)	1:2
P. raistrickii series (E72)	2:13
P. rolfsii ? (E75)	2:5
P. rosco-purpureum Dierckx.	1:2
P. spinulosum Thom.	13:82
P. terlikowskii Zal.	1:2
P. thomii Maire	8:31

FUNGI IMPERFECTI.

Moniliales.

P. thomii series (E48) 5:13

Penicillium sp. (E62) 8:41

P. " (E67) 6:23

P. " (E69) 2:10

P. " (E74) 3:13

P. " (E80) 2.10

Pestalotia sp. (F30) 1:2

Pestalotia sp. (F43) 1:2

Pullularia pullulans (de Bary) Berk. 8:38

Sarcinella heterospora Sacc. 1:2

Sarcinella sp. (D39) 3:10

Scopulariopsis sp. (F35) 4:15

Scopulariopsis sp. (F57) 1:2

Sporotrichum sp. (F16) 1:5

Stachlydium sp. (D31) 3:15

Stachlydium sp. (D31a) 3:8

Stemphylium macrosporoideum (Berk & Broome) Sacc. 1:2

Stysanus medius Sacc. 1:2

Torula sp. (D19) 1:2

Trichobotrys sp. (D27) 9:59

Trichoderma viride Pers ex Fr. 13:97

? Zygodesmus sp. (F6) 3:8

Unidentified Species:-

(D29) 1:2; (D35) 1:2; (D37) 1:2; (D38) 1:2;

(D44) 1:2; (D45) 1:2; (F26) 1:2; (F40) 1:2.

STERILE MYCELIA.

Scerotial form C18 1:5

Chlamydosporic form C21. 5:26

Dark coloured sterile mycelia: DS. 1-85 (with few omissions)

Total number isolates: 64

Light coloured, or hyaline sterile mycelia: HS 2 - 22 (with omissions)

Total number isolates: 9

Phycomycetes, although common in these soils, were represented only by Zygomycetes species. The absence of Oomycetes does not appear to be due to any shortcoming of the soil plate method since the presence of these fungi could not be demonstrated by any method employed in this investigation: Both the soil plate and the immersion tube method have been shown to isolate readily oomycetous fungi, but neither method isolated them from Calluna-heathland soils. Special baiting methods were also unsuccessful. One of the commonest of all fungi to be isolated, Mucor ramannianus, belongs in this group (Zygomycetes) but other species of Mucor were not of common occurrence on soil plates. Species of Mortierella were common, and often troublesome owing to their vigorous habit of growth. Two species of Absidia and Zygorrhynchus vuilleminii, were of frequent occurrence: both species of Absidia, although heterothallic were observed to produce zygospores on soil plates. One parasitic species, Piptocephalis cylindrospora, was encountered twice from separate profiles of Ship Hill soil: in both instances the fungus was parasitic on species of Penicillium, in culture it was shown to parasitise Mucor ramannianus.

Thirteen species of Ascomycetes were isolated: none was of constant occurrence, although two species of Gelasinospora occurred in five of the heathland soils and results indicate that they were locally common. Thielavia was the commonest genus encountered in this group.

The great majority of species isolated were Fungi Imperfecti, of which the Penicillia were the most numerous. Of some interest was the common occurrence of Beauveria bassiana, present in ten of the thirteen soils, and in over half of the profiles sampled: members of this genus are parasitic on insects, and the distribution of this species may well be associated with that of certain members of the soil microfauna, (see Section B). Trichoderma viride occurred in all soils sampled, and Trichobotrys (species indeterminate) D27 and Pullularia pullulans were of common occurrence. Somewhat over half of the species of this group were each isolated from one area only and most of these from only one profile; a few, although by no means constant, were locally of common occurrence.

The largest group of isolates were the sterile mycelia. Of the 'DS' and 'HS' isolates none showed a constancy greater than two, often, however, forms were present in all profiles sampled from any one heathland area: thus DS85 was present in both profiles from Kelling Heath and in all ten profiles from Burnt Hill, Chobham Common. All sterile mycelia occurring on soil plates were isolated and cultured on several media: only two of them finally sporulated. Most of these isolates produced dark-coloured, small and very slowly growing colonies. Their growth, occurrence and distribution in the soil is discussed below, and in a later section.

(b) Distribution of Fungi in Thirteen Heathland Soils.

Species of fungi showing the highest constancy (i.e. to constancy of 3, representing occurrence in over 20% of the heaths examined), are listed in Table 1. The selection of constancy 3 as the lower limit is arbitrary.

Table 1. Constancy of Species in Thirteen Heathland Soils.

Constancy 13.
- Trichoderma viride
- Penicillium spinulosum

Constancy 12.
- Mucor ramannianus
- Penicillium adametzi

Constancy 10.
- Beauveria bassiana

Constancy 9.
- Cladosporium herbarum
- Penicillium namyslowskii.
- Trichobotrys sp. (D27)

Constancy 8.
- Absidia orchidis
- Penicillium nigricans
- P. thomii
- Penicillium sp. (E62)
- Pullularia pullulans

Constancy 6.
- Mortierella marburgensis
- Penicillium sp. (E67)

Constancy 5.
- Mortierella isabellina
- Penicillium fellutanum
- P. thomii series (E48)
- Zygorrhynchus vuilleminii
- Sterile mycelium, C21.

Constancy 4.
- Absidia spinosa.
- Cephalosporium sp. (F31)
- Mortierella parvispora.
- M. parvispora ? (A40)
- M. humilis ? (A37)
- Mucor hiemalis
- Penicillium brevi-compactum.
- P. cyclopium.
- P. melinii ? (E70)
- Scopulariopsis sp. (F35)
- ? Monosporium sp. (F37)

Constancy 3.
- Gelasinospora retispora
- Penicillium restrictum
- Penicillium sp. (E74)
- Pseudoeurotium zonatum
- Stachlydium sp. (D31)
- Stachlydium sp. (D31a)
- Sarcinella sp. (D39)
- ? Zygodesmus sp. (F6)

Of the species included in this list Cladosporium herbarum and Penicillium spinulosum were common laboratory contaminants; their occurrence and distribution, however, were so constant that they are included in the results.

It is interesting to compare these results with those obtained by Jeffries et al (1953) in an investigation of Calluna-heathland soils by a dilution plate method, for their list of species includes over 50% of the (determinate) constant species recorded here. Also 30% of Warcup's (1951) list of species from acid grassland heath are here recorded as common in Calluna-heathland.soils.

Whilst the constants give some idea of the quantitative composition of the fungal flora of these soils, the experimental data (pages36-90) show that the quantitative composition, at both group and species level, is variable: the relative (percentage) compositions of the fungal floras in 16 areas of the 13 heaths are recorded in Table 2. The dominance of Hyphomycete species, and in particular the Penicillia, in all soils is clearly demonstrated, while the number of Ascomycetes is constantly very small and in two heaths none was recorded. In contrast to the constantly high and low numbers of species recorded respectively in these two latter groups, the Zygomycetes and sterile mycelia show pronounced variation: A greater number of forms of sterile mycelia than any other group were isolated from Staple Hill soil; while in some heaths they approach (in number of forms)

Table 2. Percentage Composition of the Fungus Floras in Sixteen Areas of the Thirteen Heathland Soils.

Heathland Area	Habitat Type *	Phycomycetes	Penicillia	Other Moniliales	Ascomycetes	Sterile mycelia
Blackheath	A	21.4	42.8	21.4	7.1	7.1
Godlingston H. I	"	20.0	33.3	26.6	6.6	13.3
Burnt Hill	"	11.1	23.8	31.7	4.8	28.5
Staple Hill	"	11.7	22.6	25.8	4.7	35.2
Chobham X-roads I	B	5.0	30.0	25.0	5.0	35.0
Kelling Heath	"	12.0	20.0	40.0	-	28.0
Plateau H. I & II	"	25.0	31.2	28.1	3.1	12.5
Hothfield Heath	C	23.8	28.6	38.0	-	9.5
Chobham X-roads II	"	14.3	28.6	35.7	7.1	14.3
Plateau Heath III	"	12.5	25.0	31.2	-	31.2
Ship Hill	"	4.3	21.7	39.0	4.3	30.4
Godlingston H. II	D	12.5	43.8	25.0	12.5	6.2
Puttenham C.	"	29.1	37.5	25.0	4.2	4.2
Newborough Warren	—	13.6	40.8	36.4	9.1	-
Carnedd Dafydd	E	33.3	33.3	25.0	4.2	4.2
Rhossilly Down	"	23.3	43.3	20.1	6.7	6.7

* Habitat type: see Table 3 and in text.

the dominance of the Moniliales, in others they are few or completely absent. A similar, variable distribution is shown in the numbers of Zygomycete species recorded, and it is perhaps not surprising that while the relative percentages of Moniliales and Ascomycete species remain more or less constant, the Zygomycetes and sterile mycelia vary in relation to one another. But it appears that the factor determining the relative abundance of these two groups is not one of chance; the ratio, Zygomycete: sterile mycelia, can be correlated with the effects of the particular soil characteristics of each area (and with the method of isolation employed) on the numbers of sterile mycelial forms isolated. As this relationship is concerned with the vertical distribution of sterile mycelia rather than distribution in area it is discussed in the next subsection.

The relationships existing between the occurrence of individual species of fungi and the 13 heathland areas when these areas were studied as separate communities - or when grouped on the basis of their geological derivations, or again on the basis of their higher plant vegetations - were not more readily described than in terms of their grouped habitat factors. For whilst no relationship was discernible between the occurrence of species of fungi and the geological formations from which the various heathland soils were derived, the type of vegetation is itself related to soil characteristics. For this reason the major ecological factors of each heath (and in some cases the areas about certain

profiles within a heath) have been collected and summarised in Table 3.

Table 3. Summary of Habitat Characteristics.

Heathland-Area.	Soil Type			Callunetum			Moisture Relations				Habitat-type	No. of Profiles.
	Podsol	Sand	Loam	Pure	+ Erica	Mixed	Dry	Damp	Water table	High Rainfall		
Blackheath	+			+			+					
Burnt Hill	+			+			+				A	17
Godlingston, I	+			+			+					
Staple Hill	+			+			+					
Chobham Xroads, I	+				+		+					
Kelling Heath	+				+		+				B	5
Plateau H. I & II	+				+		+					
Hothfield Heath	+			+				+				
Chobham Xroads,II	+				+			+	+		C	5
Plateau H, III	+				+			+				
Ship Hill	+				+			+				
Godlingston H, II		+		+			+				D	3
Puttenham Common		+			+		+					
Newborough		+				+		+	+			
Carnedd Dafydd			+			+				+	E	7
Rhossilly			+			+				+		

The 16 areas which have been sampled are readily grouped into 5 habitat types. The heaths of England fall into the first four of these habitat types (A, B, C and D) of which three (A, B and C) are podsols. The Calluneta of South and North Wales, essentially similar, are included in the fifth habitat type (E). The Callunetum from Newborough Warren is retained as a separate type.

On this basis of study the species distribution can be related, in many cases, to the grouped habitat features. The distribution of the most constant species in these 5 habitat types is recorded in Table 4., and some indication of the frequency of occurrence of species is given, (calculated from the number of profiles, in each habitat, from which a species was isolated).

Seven species of fungi occurred in all habitat types: Absidia orchidis, Trichoderma viride, Penicillium adametzi, P. spinulosum, Pullularia pullulans, Trichobotrys sp. (D27). and Mucor ramannianus. Of these species the first-named five were of more or less equal frequency in all habitat types, but the last-named two species were not: Trichobotrys sp. was rarely found in D & E soils; Mucor ramannianus was absent from E soils, with the exception of one colony observed in the profile experiments from Rhossilly soils, and, although marked as 'abundant' in C soils, was absent from one area (Plateau Heath, III) and of very sparse occurrence in another, (Chobham Crossroads, II). Of abundant occurrence in A,B & D, all dry and well drained soils, (M. ramannianus often

Table 4. Distribution of Most Constant Species in Five Habitat Types.

(+++ = abundant: ++ = frequent: + = occasional.
N.W. = Newborough Warren. p = present).

Habitat Type	A	B	C	D	E	N.W
Trichoderma viride	+++	+++	+++	+++	+++	p
Pen. spinulosum	+++	+	+++	+++	++	p
Trichobotrys sp. (D27)	+++	+++	+++	+	+	.
Pen. adametzi	+++	+	++	++	+++	.
Absidia orchidis	++	++	++	++	++	.
Pullularia pullulans	++	+	+	+	+	p
Zygorrhynchus vuilleminii	+	.	++	++	++	.
Pen. restrictum	+	.	+	+	+	.
Pen. nigricans	+	+	.	+++	++	.
Penicillium sp. (E62)	+	+++	.	++	+	p
Scopulariopsis sp. (F35)	+	+	+	.	+	.
Pen. thomii	+	+	+	.	++	p
Mucor ramannianus	+++	+++	+++	+++	(+)	p
Beauveria bassiana	++	+++	+++	+++	.	p
Pen. brevi-compactum	+	+	+	+	.	.
Pen. namyslowskii	+++	+++	+++	+	.	.
Penicillium sp. (E74)	+	+	+	+	.	.
Mortierella parvispora ? (A40)	.	+	++	.	+	.
Cephalosporium sp. (F31)	.	+	+	.	+	p
Absidia spinosa	.	+	.	+	+++	.
Mucor hiemalis	.	.	+	+	+	.
Sterile mycelium, C.21	++	.	.	+	+	.
Pen. cyclopium	+	.	.	++	+	.
Stachlydium sp. (D31(a))	+	.	+	+	.	.
Penicillium sp. (E67)	+	.	+	.	+	p
Mortierella parvispora	+	+	.	.	++	.
Stachlydium sp. (D31)	+	+	.	.	+	.
Pen. thomii series (E48)	+	+	.	.	+	.
Gelasinospora retispora	+	+	.	+	.	.
Mortierella isabellina	++	+++	.	+	.	.
? Monosporium sp. (F37)	++	+	.	+	.	.
Sarcinella sp. (D39)	+	+	+	.	.	.
Pen. fellutanum	+	+	+	.	.	.
Mortierella humilis ? (A37)	.	+	.	.	++	p
Pseudoeurotium zonatum	+	.	.	.	+	.
Penicillium sp. (E70)	+	.	.	.	++	p
Mortierella marburgensis	+	.	.	++	.	.
? Zygodesmus sp. (F6)	+	+	.	.	.	.

accounted for 20-30 colonies on many of the plates from these soils), this abundance (as measured by numbers of colonies on plates) decreased markedly with increase in the soil moisture content and the fungus was invariably absent from soils which were constantly wet and inadequately drained. It had been observed during the sampling of many heathland habitats that where Erica tetralix, an indicator of damper soil conditions, entered into the Calluna community, Mucor ramannianus showed a decline in abundance.

Both Beauveria bassiana and Trichobotrys sp. (D27) show a similar distribution to that of M. ramannianus, abundant in all habitats except E. The moisture content of the soil, however, appeared not to affect these two species as it did M. ramannianus; indeed Beauveria bassiana was often more abundant (in numbers of colonies) on soil plates prepared from damper habitats, thus presenting an inverse relationship with M. ramannianus in regard to soil moisture conditions. Neither Beauveria nor Trichobotrys were commonly isolated from the surface peaty soil levels, but both were frequently isolated from leached horizons, where, together with M. ramannianus they were often the only species present. This vertical distribution may represent a true habitat relationship or a failing of the soil plate method: all three are slow growing species and would be suppressed on plates by the more vigorous fungi abundant in the surface soils. Nevertheless it remains that these species could be isolated from the leached sandy

horizons which are lacking in the E type soils. The distribution of ? Monosporium sp. (F37), present only in the podsolic soil groups A, B and D, and restricted to the subsurface leached sands, could be accounted for in a similar manner.

Absidia spinosa, unlike Absidia orchidis, was of rare occurrence in all soils except E, where it was abundant.

Other Phycomycetes did not show such constancy in distribution: Mucor jensseni was isolated only from Carnedd soils (E) but did not appear to be numerous there; Mucor hiemalis was isolated rarely from three soil groups; Zygorrhynchus vuilleminii although locally common (e.g. at Staple Hill, Puttenham and Rhossilly) showed no distribution that could be correlated with the more obvious and known habitat features; Mortierella species were also of irregular distribution throughout the habitat types with the exception of Mortierella isabellina, which was restricted to the dry soils and in particular the podsol groups A and B, where it occurred frequently in the surface horizons.

The distribution of Penicillium species mostly showed little relation to the five habitat types: some, particularly P. brevi-compactum and P. namyslowskii, were commonly isolated from Southern English heaths and were absent from the Welsh Calluneta, whilst others: P. lividum, P.raistrickii series (E72) and Penicillium sp. (E69) were isolated from Carnedd, Rhossilly and Newborough soils only. Penicillium sp. (E67) was of sporadic occurrence in the English heaths and was of

frequent occurrence at Newborough. Penicillium sp. (E80) was very common in Rhossilly soils whilst it was only isolated from one other heath, (Puttenham). P. nigricans was of widespread occurrence and was locally abundantly isolated from Carnedd, Puttenham and Kelling Heath soils.

Species of Moniliales, other than the Penicillia and those species already mentioned, were of irregular occurrence. Many of the species were isolated from one profile only, and even then were seldom numerous, often being represented on the soil plates by one or two colonies. It is a remarkable fact that almost without exception the once-recorded demetiaceous species were isolated from the subsurface horizons of A, B & C type soils - sparsely colonised soils which, when plated, permit their contained fungi to develop on the agar medium free from competition.

Competition on soil plates appeared to be a factor of the utmost importance in determining the results obtained by the plating method, and this factor is probably largely responsible for the apparent distribution of many slowly growing species. This point is well demonstrated by the occurrence of the sterile mycelial forms.

Table 5 records the average number of sterile mycelial forms isolated from each profile of the five habitat types.

Table 5. Occurrence of Sterile Mycelia in Five Soil Habitats.

A	B	C	D	E
3.3	4.8	3.0	0.4	0

These forms are shown to be confined to the podsolic soil groups A, B and C where they occur mostly beneath the illuviated horizons, their distribution is discussed in more detail in the next subsection. Of this group of fungi only one was at all constant in occurrence - the sterile mycelium, C.21. This "species" differs markedly from all other sterile mycelia isolated in being a very rapidly growing form, perhaps the most rapid of all species that were isolated. Its mycelium was at all times submerged in the medium and its characteristic broad septate hyphae with abundant production of terminal chlamydospores was easily recognised. This fungus was of widespread occurrence and was isolated from Puttenham Common, Chobham Common (Staple Hill and Burnt Hill), Rhossilly and Carnedd.

Some special comment is necessary on the differences in the fungal flora of Welsh Calluneta and the English heaths. The soils and the higher plant vegetation of Carnedd and Rhossilly differ markedly from those of the English heaths. These habitats cannot be termed heaths, but rather are they heather moors. The peat is not formed from ling and the climatic conditions are such that Calluna retains a precarious dominance in an association of many species. It is then perhaps not surprising that some of the species of fungi characteristic of the English heathland soils are absent, whilst other species are of greatly increased frequency of occurrence; of these latter species Absidia spinosa, Absidia orchidis, Zygorrhynchus vuilleminii,

Trichoderma viride, Penicillium nigricans, P. adametzi, Mortierella parvispora and Mortierella humilis (A37) are outstanding. The similarity of the general composition of the fungal floras of these two areas, separated by a distance greater than a hundred miles, demonstrates well the relationship existing between the soil microflora and the habitat type.

That the species and composition of the fungal flora of the Newborough Warren Calluneta closely resemble those of English heaths (rather than those of Carnedd), further demonstrates this relationship, for differences in the habitats are not great. The soils of Newborough are essentially similar to those of type D and to the leached horizons of the podsol groups (A, B and C): leached, base deficient fine sands, of acid reaction. Although the subsoils are subjected to periodic saturation by base-rich water, the water table does not rise to the upper, leached, acid layers, which remain base deficient. (D.S. Ranwell 1952, unpublished data). Both profiles were prepared in areas in which Calluna was dominant although the general vegetation of the area was mixed and contained a variety of species.

(d) Vertical Distribution of Fungi.

For purposes of comparison of the vertical distribution of fungi in the varied types of soil profile which have been examined, the terminology applied to podsols has been somewhat freely adapted; the humus-rich, surface layers in all soils are termed 'A1' horizons and the leached sands, poor in humus,

whether from podsols or soils of type D (see previous subsection) are referred to as 'A2' horizons. The designations B, B1, B2 and C are used in their correct sense.

In all profiles examined the number of species decreased with increase of depth (see experimental data on pages 36-90), and showed a close relationship with the distribution of plant roots. Even within the upper A1 horizon a decrease in number of species with increase in depth was noted, although the method of presentation of the experimental data does not always show this relationship clearly. (But see Section B).

The vertical distribution of the most constantly occurring species is recorded in Table 6, where occurrence of species is represented as a percentage of their presence in the total number of the particular horizons examined.

The four species which occurred in all types of soil horizon fall naturally into two groups: Trichoderma viride, representing the first group, was present in every A1 horizon examined, with only one exception, and was decreasingly abundant in horizons of increasing depth being recorded from only three of the twenty-eight C horizons examined; as was mentioned above, Mucor remannianus, Trichobotrys sp. (D27) and ? Monosporium sp. (F37), representing the second group, were all most abundant in the A2 horizon. The distribution in area of these three species was probably a reflection of their preference for the A2 horizons, or the facility with which they were isolated from these horizons. A notable feature of the vertical distribution of these four species is the almost

Table 6. Occurrence of Constant Species in Soil Horizons.

Horizon Type	A1	A2	B1	B	B2	C
No. of horizons examined	40	32	14	8	14	28
Trichoderma viride	97.5	62.3	14.3	37.5	7.1	10.7
Mucor ramannianus	62.5	96.7	57.0	87.5	21.4	17.8
Trichobotrys sp. (D27)	42.4	53.0	42.8	12.5	7.1	14.3
? Monosporium sp.(F37)	12.5	42.7	21.4	25.0	7.1	10.7
Penicillium spinulosum	77.5	25.0	7.1	.	.	.
P. namyslowskii	60.0	34.2	7.1	.	.	.
Beauveria bassiana	47.4	46.8	7.1	.	.	3.5
Penicillium sp. (E62)	35.0	12.5	7.1	.	.	3.5
Pen. nigricans	40.0	9.4	.	.	.	3.5
Mortierella isabellina	37.5	22.0	.	.	.	7.0
Mort. parvispora	25.0	12.5	.	.	.	3.5
Pen. adametzi.	75.0	37.0	.	.	.	.
Absidia orchidis	47.5	3.1	.	.	.	.
Pullularia pullulans	35.0	9.4	.	.	.	.
Zygorrhynchus vuilleminii	25.0	12.5	.	.	.	.
Pen. thomii	22.5	3.1	.	.	.	.
Sterile mycelium, C.21	20.0	15.6	.	.	.	.
Mort. humilis (A37)	20.0	6.2	.	.	.	.
Scopulariopsis sp.(F35)	12.5	6.2	.	.	.	.
Mort. marburgensis	12.5	3.1	.	.	.	.
Mort. parvispora ? (A40)	10.0	6.2	.	.	.	.
Absidia spinosa	25.0	.	.	.	.	.
Mucor hiemalis	12.5	.	.	.	.	.

perfect constancy with which lowest numbers of isolates were recorded in the (B2) iron pan, the very firmly concreted, gritty, almost humus-free horizon of mature podsols.

Burges (1950) suggested that the vertical distribution of certain species in sandy horizons may be correlated with the downward movement of their spores due to percolation of water. He has, however, been unable to demonstrate this effect through the illuviated horizons of podsols (unpublished preliminary observation) owing to their firmly compact mechanical nature. And it is possibly due to the 'filtering action' of these pans on spore suspensions in the soil, or to the lateral drainage occurring over the upper surface of these horizons, that the majority of species were not isolated either from the pans, or beneath them, whilst occurring commonly in the A1 and with decreased frequency in the A2 horizons.

Beauveria bassiana and the sterile mycelium C21 were almost as frequent in A2 as in surface horizons. All other common species, except those already mentioned, were most abundant in the surface, peaty, humus-rich soils. The anomalous occurrence of four species in the C horizon is perhaps relatable to root distribution (see Section B).

Only two common species were isolated from the A1 horizon alone, Absidia spinosa and Mucor hiemalis. That A. spinosa occurs in all depths in several profiles from Carnedd and Rhossilly, where the soil is classed as an 'A1' type, tended to indicate that this fungus was not restricted in

distribution by strongly aerobic tendencies.

Ascomycetes, as a group, were fairly strictly confined to the A1 horizons: of 26 recorded isolates, 21 were from this surface horizon and 4 from the A2 horizon.

The distribution of the dominant groups of fungi are recorded in Table 7. In this analysis the average number of species occurring in the various horizons has been calculated from the experimental data, and from these numbers the percentage frequency of occurrence in the various horizons has been derived. All groups, with exception of the sterile mycelia, were most frequently isolated from the A1 horizons; species of Mortierella and Penicillium being strictly confined to these horizons,

Table 7. Distribution of some Groups of Fungi in the Soil Horizons.

Horizon	A1	A2	B1	B2	B	C
Mortierella spp.	83	14	0	0	0	3
Penicillium spp.	77	15.5	3	2	2.5	0
Moniliales (other than Penicillium)	47	28	8	4	9	4
Sterile mycelia	11	9	19.5	13.5	16	30

This table demonstrates the inverse relationship in distribution existing between the sterile mycelial forms and the remaining three groups. The percentage composition of the dominant groups of fungi in 16 areas of 13 heaths was

analysed and recorded in Table 2, and some note was made of the great variation in proportions represented by the sterile mycelia. These data are again presented in Table 8, where the sterile mycelia of 16 heaths are recorded in order of decreasing abundance, and it is shown that they are more or less wholly confined to the podsolic soil types.

Thus the distribution in area of the sterile mycelial forms was closely related to their distribution in depth, i.e. their preference for the illuviated horizons and in particular the parent clayey sands. An alternative reason for the distribution of sterile mycelia may well be simply that they could not compete, on plates prepared from surface soils, with the sporing forms - which, almost without exception, were more vigorous in growth. But whether or not these forms were of more frequent occurrence in the lower than in the upper horizons the fact remains that they were almost the sole inhabitants of the lower soil layers of podsols.

Table 8. Sterile Mycelial Componant (expressed as %age) of the Fungus Flora of 16 Heathland Areas.

Heathland Area	%age abundance	Profile Type
Staple Hill	35.2	Podsol
Chobham X-roads I	35.0	"
Plateau H. III	31.2	"
Ship Hill	30.4	"
Burnt Hill	28.5	"
Kelling H	28.0	"
Chobham X-roads II	14.3	"
Godlingston H. I	13.3	"
Plateau H. I & II	12.5	"
Hothfield H.	9.5	"
Blackheath	7.1	"
Rhossilly	6.7	Non-podsol
Godlingston H. II	6.2	"
Carnedd	4.2	"
Puttenham C.	4.2	"
Newborough	0	"

Bisby, James & Timonin (1935) found anaerobic and low temperature fungi to be more common in the B & C soil horizons and suggested that fungi differing physiologically and even morphologically may develop during a long sojourn deep in the soil. These investigators wrote: "we were at first inclined

to think that fungi from lower horizons more frequently failed to produce spores than those from upper horizons, but such is not really the case, although spore production may be delayed".

It appears to be possible, from the present investigation, that these mycelial forms, which are nearly all dark-coloured and very slow growing, represent a miscellaneous group of fungi physiologically adapted to the conditions of subsoil life, and which may have lost the ability to produce spores. Whilst it was demonstrated in observation employing the Rossi-Cholodney slide technique (discussed in a later section), that perhaps these results were strongly biased by the method of isolation, a more detailed investigation of these subsoil inhabitants, however, might well prove rewarding.

The results recorded in Tables 6 and 7, suggest that the illuviated horizons were limiting the distribution of spore-producing fungi, if only by providing a check to the downward movement of their spores. For this reason the number of sporing species of fungi occurring at similar soil depths in non-podsolic soils and in podsolised soils beneath the pans, were collected, averaged, and compared. The results are recorded in Table 9.

Table 9. Effect of the Illuviated Horizons on the Number of Species of Spore-Producing Fungi.

Depth in inches:	12	15	18	21	24	27	30
(a) In Non-podsols	3.3	2.0	3.0	2.3	1.3	1.0	1.3
(b) In podsols beneath the pans	2.0	0.5	1.5	0.5	0.5	0	0.5

Although a reduction in numbers of species occurred in all soils beneath the illuviated horizons, the two sets of averages, when analysed by Fischer's variance ratio test, do not differ significantly.

PART II

SECTION B. INVESTIGATION OF THE FUNGUS FLORA OF A LIMITED AREA OF CALLUNA-HEATHLAND SOIL BY A VARIETY OF METHODS.

CONTENTS.

Introduction.

The investigations described in the preceding section supplied evidence of both a certain constancy in the composition of the fungus flora of a number of Calluna-heathland soils, and of a differentiation in the distribution of certain species and groups of fungi. The information obtained was, however, very limited - while lists of species were recorded the state in which they were present in the soil remains unknown, as do their microhabitats.

Again, many of the results are perhaps considerably biased by the method of isolation. The majority of the species isolated had a large sporing capacity and many of the more slowly growing forms were recorded only in the absence of vigorously growing species. Thus the total information concerning distribution is partly positive, partly negative, and indeed may be only partly true.

Other investigators (Chesters 1948, Jensen 1934-5, Nicot and Chevaugeon (1949) have demonstrated the limited value of plating methods which supply only a partial picture of the soil microflora and that this, if accepted as being complete, is misleading. These conclusions were derived from the complementary and contradictory evidence provided by the use of two or more methods of isolation and examination.

From these considerations it was decided that investigations of the fungus floras of differing heaths, of necessity by the use of only one method, could only yield very

limited information concerning the soil fungus flora.

The type of investigation finally designed, and described in the following pages, involved the use of as many methods of isolation and observation as could be handled conveniently in the examination of the fungus flora of one small area of heathland soil. The soil plate was used as the standard plating method, and profiles were sampled at monthly intervals for a period of 10 months so that local variations and differences due to season might be demonstrated. To obtain information concerning the active mycelial constituent of the fungus flora, and to determine at the same time whether the active mycelial growth of the fungi was subject to seasonal variation, the immersion tube method (Chesters 1948) was employed; the slide trap method devised by C. La Touche (1948), and modified by the present writer, was used as a secondary and corroborative method of study of these active mycelia. Since the work already described had suggested that the vertical distributions of fungi and plant roots were related the fungus flora of the root surfaces was investigated for comparison with that of the soil. The examination of the fungal species present in rabbit dung, abundant on the soil surface of the area, was for the correlation and corroboration of certain results obtained early in the investigation. Finally the Rossi-Cholodney slide and other microscopical techniques were employed for the observation of the growth of fungi in the soil, and, it was hoped, also to provide a means

of assessing the relative values of the data contributed by the various isolation methods.

Subsection (i) The Habitat.

Location of Site: Burnt Hill, Chobham Common, Surrey.

A small area (740 sq.yd.) of Callunetum was selected and its boundary marked by wooden stakes. This area lies within a large expanse of heathland occurring on a gently sloping plain, facing S.S.W., the gradient of which is approximately 1 in 20. This plain is less than half a mile south of Sunningdale, bounded on one side by the Sunningdale-Chobham Road and on the other side by the Wentworth Golf Course. Although the site is designated 'Burnt Hill' it is, in fact, at the N.W. foot of this hill.

Vegetation: The sloping plain, mentioned above, covers an area of approximately 50 acres, its vegetation is dominated by Calluna vulgaris. Within this Callunetum are small stands of silver birch and Scots pine, the dwarf gorse (Ulex minor) is of sporadic occurrence over the whole area, and Molinia is locally subdominant.

In the small area selected for investigation Calluna is dominant and provides the sole higher plant vegetation with the exception of U. minor which is of sparse occurrence. The Calluna grows in a bushy habit, 12 - 18 in. in height. The soil surface between Calluna bushes is barren except for a sparse development of Cladonia spp. (See Plates 1 & 2)

Biotic Factors. Rabbits are fairly abundant over the whole plain and the limited encroachment of Molinia appears to be due to heavy rabbit grazing. Whilst no effects of grazing

Burnt Hill, Chobham Common, Surrey.

Two views of the Calluna vegetation of the area investigated.

Plate 1. Southern aspect

Plate 2 Northern aspect

are apparent in the small selected area it is traversed by rabbit runs and rabbit dung is abundant on the soil surface. The biotic factor was found to be rather more serious than had been anticipated when rabbits removed and chewed the cotton wool plugs from immersion tubes, while ants removed the agar, nested and reproduced in them.

The heather has not been fired for at least ten years.

Profile Structure: The sandy soil, derived from the Bagshot Beds, is podsolised, and a description of the various horizons is given below.

A1 Horizon: Humus-rich sand containing profuse development of fine fibrous roots which impart a fibrous consistency to this layer. The soil is black when moist, chocolate brown when dry.

A2 Horizon: Light coloured, greyish-brown sand of loose texture.

B1 Horizon: A firmly compacted dark brown, humus-rich layer, somewhat sticky when moist, friable when dry.

B2 Horizon: Yellowish or reddish-brown, very firmly concreted layer. Gritty in texture.

C Horizon: Clayey sands, sticky in texture. The colour is variable, brick-red, deep orange or yellow brown. Large pockets of light grey clay are common.

Table 10: Variation of Horizon Depths in 10 Profiles.

Profile No.	Depth in inches. A1	A2	B1	B2	Depth to which profile exposed. (in.)
1	0-3	3-15	15-20	20-28	32
2	0-4	4-15	15-20	20-28	34
3	0-4.5	4.5-14			36
4	0-3	3-14	14-17	17-22	32
5	0-3	3-12	12-15	15-17	32
6	0-2.5	2.5-16	16-22	22-26	36
7	0-2.5	2.5-16	16-20	20-27	33
8	0-3.5	3.5-19	19-22	22-26	36
9	0-3.5	3.5-14	14-19	19-22	30
10	0-3.5	3.5-15	15-22	22-25	32

The structure of profile 3 was anomalous: two humus horizons, at 14-20 in. and 23-26 in., were revealed separated by light grey clay with pockets of reddish-brown colouration; this clay was also observed beneath the lower humus pan and continued to a depth of 34 in., where the normal parent material, yellow orange clayey-sand, was encountered.

Apart from this atypical profile the structure was found to be very constant apart from slight variations in depths and thicknesses of the various horizons as recorded in Table 10. The soils of this area were extremely acid in reaction. The pH values for the various horizons are recorded in Table 11.

Table 11. pH Values for the Podsol Horizons.

Horizon	pH Range.
A1	3.6 - 3.8
A2 (upper)	3.7 - 4.0
A2 (lower)	3.8 - 4.4
B1	4.0 - 4.6
B2	4.0 - 4.7
C	4.3 - 4.9

Soil Composition: The mechanical analysis of soils from the 5 horizons are recorded in Table 12.

Table 12. Mechanical Analysis of Soil Horizons.

	A1	A2	B1	B2	C
Coarse sand	? 30.75	17.23	21.63	36.61	36.54
Fine sand	? 49.93	75.39	65.08	55.19	39.34
Silt	-	4.00	6.75	2.75	12.75
Clay	-	3.75	4.75	3.75	10.00
Loss on ignition	13.43	1.38	2.97	1.97	1.91
Hygroscopic moisture *	1.61	0.25	0.78	0.71	0.97
Total	-	102.00	101.96	100.98	101.51
Moisture holding capacity *	71.3	29.5	35.4	30.37	36.00

* Expressed as percentage of air-dry soil.

The soils from the surface horizon (A1) were not analysed completely as it was found impossible to bring about complete oxidation of the humus matter short of ignition. The percentages of fine and coarse sand were determined but these values are rather high owing to the presence of humus.

Since these soils were air dried and sieved, to remove all particles of diameter greater than 2 mm., the analysis gives no indication of the great mass of fibrous root material in the A1 horizon which, it is estimated, contributes about one quarter of its bulk.

In all profiles examined stones were of infrequent and sparse occurrence.

Distribution of Roots in Relation to the Profile Horizons.

That the form of the rooting system of a given species depends largely on the prevailing edaphic conditions is well illustrated by Calluna, whose root system is always closely related to the soil horizons.

The fairly stout rootstock of Calluna, often 1 in. in thickness, is usually buried just beneath the soil surface and lies parallel to it. From the rootstock the aerial shoots arise in profusion forming the compact base of the Calluna bush. Adventitious roots develop from the submerged parts of the aerial system.

The main and primary lateral roots, seldom greater than 3 mm. diameter, and seldom exceeding three in number from each rootstock, grow more or less vertically downwards through

the leached sand, decreasing in diameter until at the A2/B1 interface very few roots are found to exceed 0.5 mm in diameter. These roots are heavily suberised and branch sparingly in the leached horizons, the branches which are formed remain short and give rise to few hair roots. (Root hairs are absent in Calluna.)

Where these roots enter the B1 horizon profuse branching takes place giving rise to local dense masses of very fine rootlets and hair roots. It is possible to isolate these roots only by washing out the humic soil material, when a dense and intricately woven mat of repeatedly branching and very delicate roots is left: some of these roots are young and extending, and under the microscope are quite transparent, the majority, however, are yellowish-brown, the epidermis disintegrated, the endodermis and outer bast parenchyma being lightly suberised. (See Beijerinck, 1940). These roots seldom exceed 0.1 mm diameter.

Very few roots pass through the B1 horizon and enter the gritty, firmly concreted iron pan, the few that do so are little branched and seldom penetrate far. Roots have been observed in the clayey sand of the C horizon but only rarely.

Another and more important system of roots is developed in the surface, humus-rich, A1 horizon. In this zone the root development is very dense, for a great number of repeatedly branching fibrous roots are developed, largely from the rootstock, but also from the upper portions of the

main and primary lateral roots, and, adventitiously, from the bases of the aerial shoots. Most of the roots in the A1 horizon grow horizontally, more or less parallel to the soil surface, seldom entering the A2 horizon, profusely branching and finally giving rise to the delicate hair roots.

Thus the root system of Calluna in this area of soil can be divided into 3 distinct habits:

(1) The dense, horizontal system of roots, profusely branched and imparting a fibrous, consistency to the surface A1 soil horizon.

(2) The little-branched, heavily suberised primary lateral roots which grow more or less vertically downwards through the A2 horizon.

(3) The local dense ramifications of exceedingly fine hair roots in the B1 horizon.

Prevailing Temperatures.

Investigations of the fungus flora of the Burnt Hill soils were continued over a period of sixteen months, from October 1952, to January 1954. During this period no data were collected relating to the prevailing climatic conditions. The data collected at the Royal Horticultural Society Gardens at Wisley, however, have been obtained; The mean monthly temperatures, together with the number of days during each month in which ground frost was reported, are recorded in Table 13. The Wisley Gardens are less than eight miles distant from the Burnt Hill site.

Table 13. Mean Monthly Temperature and Number of Days Ground Frost in each Month: October 1953 - January 1954.

Year:	1952			1953				
Month:	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
(a) Mean temp. (°F)	48.5	39.5	36.7	37.5	39.3	42.5	47.3	55.9
(b) No. days with ground frost.	8	18	24	19	19	23	14	7

Year:	1953							1954
Month:	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.
(a)	58.6	61.0	63.1	57.3	50.6	46.9	45.2	37.1
(b)	0	0	0	0	6	7	11	23

Subsection (ii). Investigation of the fungus flora by the Soil Plate Method.

The methods of collecting soil samples, isolating micro fungi, recording species and presenting results were described in Section A.

In the soil plate experiments of this investigation four isolating media were employed:

Czapek-Dox + 0.5% yeast extract, pH adjusted to 4.5 (a)

" " " " " + Rose bengal, pH unadjusted (b)

Soil extract agar, pH 4.5 (see Section A) (c)

Czapek-Dox + 0.5% yeast ext., pH 4.5, glucose as carbon source (g)

The index letters, a, b, c and g, following each type of medium are used in the presentation of the results. A set of soil samples (from the various levels in each profile) was collected, each set was plated with three media in three separate experiments. In each of these profile experiments soil from every level was plated in triplicate.

The 10 profiles, in this investigation, were prepared and sampled at monthly intervals; the respective times at which each profile was sampled are listed below:

Profile 1,	October, 1952.	Profile 6,	March, 1953.
" 2,	November "	" 7,	April "
" 3,	December "	" 8,	May "
" 4,	January 1953.	" 9,	June "
" 5,	February "	" 10,	July "

As mentioned above, 3 sets of soil plate experiments were

prepared from each set of soil profile samples. Thus a total number of 30 experiments were carried out. The results of these 30 soil plate experiments are recorded in the following pages.

Distribution of Species in Profile 1. (Burnt Hill)

Horizon.	A1	A2	A2	B1	B2	C
Sampling depth (in.)	1-3	4-6	9.12	15.18	21-27	30
Absidia orchidis	acg	...	...	...	...	...
Haplosporangium decidiens	ac.	...	...	...	...	...
Mortierella isabellina	acg	..g	...	...	...	...
Mucor ramannianus	acg	acg	acg	acg	a..	...
Piptocephalis cylindrospora	.c.	...	...	...	...	...
Gelasinospora cerealis	..g	.c.	...	...	...	...
Thielavia terricola	.c.	...	...	...	...	...
Beauveria bassiana	acg	acg	...	...	...	...
Cladosporium herbarum	.c.	...	...	a..	...	...
Penicillium adametzi	.cg	..g	...	...	...	...
P. cyclopium	a..	...	...	...	...	...
P. fellutanum	acg	acg	...	...	...	...
P. melinii ? (E70)	..g	...	...	...	...	...
P. namyslowskii	.cg	a..	...	...	...	...
P. nigricans	a..	...	...	...	...	...
P. spinulosum	acg	...	...	...	...	...
Penicillium sp. (E62)	a.g	...	...	...	...	...
Pullularia pullulans	acg	acg	...	...	...	...
Stachlydium sp. (D31)	...	a..	.c.	...	...	...
Trichobotrys sp. (D27)	ac.	...	acg	.cg	...	...
Trichoderma viride	acg	.cg	...	...	...	...
Sterile mycelia: DS.35	...	...	.c.	.c.	...	...
DS.45	...	...	...	...	ac.	...
DS.66	...	...	...	...	a..	...
DS.85	.c.	...	..g	.c.	acg	ac.
25	21	10	5	5	4	1

Distribution of Species in Profile 2. (Burnt Hill)

Horizon:	A1	A2	A2	B1	B2	C
Sampling depth (in.)	1-3	4-9	12.15	18.21	24.27	30.33
Absidia orchidis	acg	...	...	...	...	...
Mortierella isabellina	...	...	...	...	...	..g
M. marburgensis	..g	...	...	...	...	...
M. parvispora	.c.	...	...	...	...	...
Mucor ramannianus	..g	acg	acg	acg	...	...
Piptocephalis cylindrospora	..g	...	...	...	...	...
Gelasinospora cerealis	..g	...	...	...	...	...
Cladosporium herbarum	...	..g	.c.	.c.	...	...
? Dematium sp. (D21)	...	.c.	...	...	...	...
? Haplaria sp. (F20)	...	...	...	...	...	.c.
? Monosporium sp. (F37)	...	...	acg	.c.	...	...
Oedocephalum sp. (F34)	...	...	.c.	...	...	..g
Penicillium adametzi	acg	.c.	.c.	...	...	...
P. cyclopium	...	...	...	..g	...	...
P. Fellutanum	acg	acg	...	...	...	...
P. Namyslowskii	acg	.c.	...	...	...	...
P. spinulosum	acg	a..	..c	...	...	...
Penicillium sp. (E62)	...	a..	...	...	...	...
Pullularia pullulans	..g	...	...	...	...	...
Scopulariopsis sp. (F35)	..g	acg	...	...	...	...
Stachlydium sp. (D31)	...	.cg	...	...	...	...
Trichobotrys sp.(D37)	a.g	acg	a.g	..g	...	...
Trichoderma viride	acg	..g	...	...	..g	...
(Demetiaceae) (D44)	...	...	...	...	.c.	...
Sterile mycelia: DS.36	...	...	a..	...	...	...
DS.85	...	a..	...	...	...	acg
(Chlamydospore form) C.21	.cg	a.g	...	...	...	...
(Sclerotial form) C.18	a..	...	...	...	...	...
28	16	14	8	5	2	4

Distribution of Species in Profile 3. (Burnt Hill.)

Horizon	A1	A2	A2	B1	B2?	B1	B2/C
Sampling depth (in.)	1-4	5.6	9.12	15.18	21	24	27-33
Absidia orchidis	acg	...	...	...	...	...	...
Mucor ramannianus.	acg	acg	acg	acg	acg	acg	.cg
Coniothyrium sp.(C23)	ac.	...	...	...	...	...	...
? Monosporium sp. (F37)	...	...	ac.	acg	...	...	...
Pen. namyslowskii	acg	...	...	...	...	...	...
P. rolfii ? (E75)	ac.	...	...	...	...	...	...
P. spinulosum	acg	...	...	...	...	...	...
Pullularia pullulans.	ac.	...	...	...	...	...	...
Pyrenochaeta sp.(C24)	...	.c.	...	...	...	...	...
Sarcinella sp.(D39)	...	...	...	...	...	...	.c.
Trichobotrys sp. (D27)	acg	acg	acg	a..	..g	.c.	acg
Trichoderma viride	acg	.c.	...	.c.	...	...	...
Sterile mycelia: DS.48	...	...	...	.cg	...	...	...
DS.85	...	...	a.g	...	.cg	a.g	acg
(Chlamydo.form) C21	acg	...	...	...	...	...	...
15	10	4	4	5	3	3	4

Distribution of Species in Profile 4. (Burnt Hill.)

Horizon:	A1	A2	A2	B1	B2	C
Sampling depth (in.)	1-3	4-6	9.12	15	18.21	24-30
Absidia orchidis	acg	...	...	...	...	...
Mortierella isabellina	acg	...	...	...	...	...
Mucor ramannianus	acg	acg	acg	acg	...	...
Thielavia sp. (C25)	.c.	...	...	...	...	...
Cladosporium herbarum	.c.	a..	...	...	...	...
? Monosporium sp.(F37)	...	acg	acg	..g	.c.	...
Penicillium adametzi	acg	...	...	...	...	...
P. brevi-compactum	ac.	...	...	...	...	...
P. cyclopium	.cg	...	...	...	...	...
P. namyslowskii	a.g	...	...	...	...	...
P. restrictum	.c.	...	...	...	...	...
P. spinulosum	acg	...	...	...	...	...
Penicillium sp. (E62)	a..	...	...	...	...	...
Penicillium sp. (E67)	.c.	...	...	...	...	...
Pullularia pullulans	a.g	...	...	...	...	...
Stachlydium sp. (D31)	...	..g	...	...	...	...
Trichobotrys sp.(D27)	acg	acg	acg	...	...	...
Trichoderma viride	acg	...	...	...	...	...
Sterile mycelia: DS.46	ac.	...	...	...	...	...
DS.69	...	...	...	acg	...	...
DS.85	acg	...	a..	...	...	a..
21	18	5	4	3	1	1

Distribution of Species in Profile 5. (Burnt Hill).

Horizon:	A1	A2	A2	B1	B2	C	C
Sampling depth (in)	1-3	4-6	9.12	13	15	18.21	24.27
Absidia orchidis	..g	...	...	...	...	...	...
Mort. isabellina	acg	...	acg	...	...	...	...
M. parvispora	acg	...	...	...	...	...	...
Mucor ramannianus	...	acg	acg	acg	acg	.cg	...
Cladosporium herbarum	.cg	...	...	...	...	...	...
? Chaetopsis sp.(D34)	...	.c.	...	.c.	...	...	...
? Monosporium sp.(F37)	...	...	ac.	...	...	...	...
Penicillium adametzi	acg	...	...	...	...	...	...
P. citreo-viride	ac.	...	...	...	...	...	...
P. cyclopium	a..	...	...	...	...	...	...
P. namyslowskii	acg	acg	acg	ac.	...	...	...
P. spinulosum	a..	a..	a.g	...	...	...	...
Penicillium sp.(E67)	.c.	...	...	...	...	...	...
? Phoma sp. (C22)	a..	...	...	...	...	...	...
Pullularia pullulans	.c.	...	...	...	...	...	...
Stachlydium sp.(D31a)	...	...	acg	...	...	...	...
Trichobotrys sp.(D27)	acg	acg	acg	.c.	...	...	...
Trichoderma viride	acg	...	acg	acg	...	...	.c.
(Demetiaceae) (D45)	.c.	...	...	...	...	...	...
Sterile mycelia: DS37	ac.	...	...	...	...	...	...
DS39	...	...	...	...	...	.c.	...
DS.85	..g	a..	...	...	...	ac.	acg
(Chlamydo form) C21	...	a.g	...	...	...	...	...
23	17	7	8	5	1	3	2

Distribution of Species in Profile 6. (Burnt Hill.)

Horizon:	A1	A2	A2	B1	B2	C
Sampling depth (in.)	1.2	3-6	9-15	18.21	24	27-33
Mucor ramannianus	ab.	abc	abc	abc	...	...
Thielavia terricola	..c	...	...	...	...	...
Coniothyrium sp. (C23)	...	...	a..	...	...	...
? Monosporium sp.(F37)	...	...	abc	...	...	...
Penicillium adametzi	.bc	...	...	...	...	...
P. brevi-compactum	...	..c	...	...	...	...
P. namyslowskii	ab.	a..	...	...	...	...
P. spinulosum	abc	...	...	...	...	...
P. thomii	a.c	...	...	...	...	...
Penicillium sp. (E62)	a.c	.b.	...	...	...	...
Pullularia pullulans	..c	...	..c	...	...	...
Trichobotrys sp. (D27)	...	abc	.bc	...	...	...
Trichoderma viride	abc	ab.	a..	...	...	...
Sterile mycelia: DS.19	...	...	...	...	...	..c
DS.31	...	...	...	...	...	.bc
DS.45	...	...	...	...	...	abc
DS.85	...	...	...	a..	...	...
(Chlamydo. form) C.21	...	...	abc	...	...	...
18	9	6	7	2	0	3

Distribution of Species in Profile 7. (Burnt Hill.)

Horizon:	A1	A2	A2	B1	B2	C
Sampling depth (in.)	1.2	3-6	9-15	18	21.24	27.30
Mortierella isabellina	a..	...	...	...	...	...
M. parvispora ? (A40)	...	a..	...	...	...	...
Mucor ramannianus	abc	abc	abc	abc	..c	..c
Beauveria bassiana	.bc	...	...	...	...	...
Cladosporium herbarum	...	.b.	...	...	...	...
? Monosporium sp. (F37)	...	abc	abc	...	...	...
Penicillium adametzi	abc	.bc	...	...	...	...
P. citreo-viride	..c	...	...	...	...	...
P. namyslowskii	a..	...	...	...	...	...
P. restrictum	...	..c	...	...	...	...
P. spinulosum	abc	...	...	...	...	...
Scopulariopsis sp.(F35)	ab.	...	...	...	...	...
Trichobotrys sp. (D27)	...	abc	...	...	...	...
Trichoderma viride	abc	ab.	abc	...	...	..c
Sterile mycelia: DS.49	...	.bc	...	...	...	...
DS.85	...	...	...	...	...	ab.
(Chlamydo.form) C.21	ab.	a.c	...	...	...	...
17	10	10	3	1	1	3

Distribution of Species in Profile 8. (Burnt Hill.)

Horizon:	A1	A2	A2	A2	B1	B2	C
Sampling depth (in.)	1-3	4-6	9.12	15.18	21	24	27.30
Absidia orchidis	a..	...	...	...	...	...	...
Haplosporangium decipiens	a..	...	...	...	...	...	...
Mort. isabellina	a..	...	...	...	...	...	...
Mucor ramannianus	abc	abc	abc	abc	...	...	...
? Chaetopsis sp.(D34)	...	...	...	...	...	...	.b.
Cladosporium herbarum	...	..c	...	...	...	...	...
? Monosporium sp.(F37)	a..	...	a.c	...	...	...	...
Penicillium adametzi	abc	..c	...	...	...	...	...
P. namyslowskii	abc	.b.	...	...	...	...	...
Trichobotrys sp.(D27)	.bc	abc	abc	.bc	...	...	...
Trichoderma viride	abc	...	...	...	...	...	...
Sterile mycelia: DS.42	...	...	...	.b.	a.c	a.c	...
DS.45	...	...	...	...	...	...	..c
DS.85	...	...	...	...	...	..c	..c
14.	9	5	3	3	1	2	3

Distribution of Species in Profile 9. (Burnt Hill.)

Horizon:	A1	A2	A2	B1	B2	C
Sampling depth (in.)	1-3	4-6	9.12	15.18	21	24.27
Absidia orchidis	.bc	...	...	...	...	...
Haplosporangium decipiens	a.c	...	...	...	...	...
Mortierella isabellina	abc	abc	...	...	...	...
M. parvispora	abc	.bc	...	...	...	a..
Mucor ramannianus	.bc	abc	abc	abc	...	...
? Chaetopsis sp. (D34)	...	.bc	...	...	...	...
Cladosporium herbarum	..c	...	...	...	...	...
? Monosporium sp.(F37)	...	a.c	abc	...	...	...
Penicillium adametzi	abc	abc	a..	...	...	...
P. namyslowskii	ab.	ab.	...	...	...	...
P. spinulosum	ab.	...	...	...	...	...
P. thomii series (E48)	...	.b.	...	...	...	...
Pullularia pullulans	abc	abc	a..	...	...	...
Sarcinella sp. (D39)	a..	...	...	...	...	...
Sepedonium sp. (F32)	a.c	...	...	...	...	...
Stachlydium sp.(D31)	.bc	...	...	...	...	...
Trichobotrys sp. (D27)	.b.	ab.	abc	...	...	...
Trichoderma viride	abc	...	a..	...	...	...
(Demetiaceae) (D38)	...	...	...	..c	...	...
Sterile mycelia: DS.51	...	...	...	a..	...	...
DS.52	...	...	...	..c	...	...
DS.85	...	...	...	ab.	...	...
(Chlamydosp. form) C.21	ab.	...	ab.	...	...	...
22	15	10	7	5	0	1

Distribution of Species in Profile 10. (Burnt Hill.)

Horizon:	A1	A2	A2	B1	B2	C
Sampling depth (in.)	1-3	4-6	9.12	15.18	21.24	27
Mortierella marburgensis	a.c	...	...	...	...	...
Mucor ramannianus	abc	abc	abc	abc	...	...
Thielavia terricola	..c	...	...	...	...	...
Cladosporium herbarum	.bc	a..	.b.	...	...	...
? Monosporium sp. (F37)	...	.bc	..c	..c	...	...
Penicillium adametzi	abc	...	...	...	...	...
P. namyslowskii	abc	...	...	...	...	...
P. spinulosum	abc	...	...	...	...	...
Pullularia pullulans	.bc	..c	.b.	...	...	...
Trichobotrys sp. (D27)	.b.	.b.	.bc	a..	...	...
Trichoderma viride	abc	...	...	...	...	...
? Zygodesmus sp. (F6)	a..	...	...	...	...	...
Sterile mycelia: HS.20	...	...	...	...	...	.b.
DS.59	...	...	...	a..	...	...
DS.85	...	...	a..	..c	...	...
(Sclerotial form) C.18	.b.	...	...	...	...	...
16.	12	5	6	5	0	1

Discussion and Correlation of Results.

1. The Groups and Species of Fungi Isolated.

The species of fungi isolated are listed below. Each specific name is followed by two numbers (underlined): the first number is a constancy * figure representing the number of profiles in which the species was present (maximum number 10), and the second is the percentage frequency of occurrence in 30 experiments.

The sterile mycelial forms are not listed in full; all but four forms were present in only one profile.

The collection number is given in brackets, following the name of those species whose identity is in doubt.

PHYCOMYCETES.

Absidia orchidis (Vuill.) Hagem.	7:53
Haplosporangium decipiens Thaxter	3:13
Mortierella isabellina Oud.	7:50
M. parvispora Linn.	3:23
M. parvispora ? (A40)	1:3
M.marburgensis Linn.	2:10
Mucor ramannianus Möll	10:100
Piptocephalis cylindrospora Bainier	2:7

ASCOMYCETES.

Gelasinospora cerealis Dowding	2:10
Thielavia terricola (G & A) Emmons	2:7
Thielavia sp. (C25)	1:3

* N.B. The term 'constancy' is here used to represent occurrence in 10 profiles; in Section A it was used to represent occurrence in 13 heathland area.

FUNGI IMPERFECTI.

Sphaeropsidales.

Coniothyrium sp. (C23)	3:13
Phoma sp. (C22)	1:3
Pyrenochaeta (C24)	1:3

Moniliales.

Beauveria bassiana (Bals.) Vuill.	2:17
? Chaetopsis sp. (D34)	3:10
Cladosporium herbarum Link ex Fr.	8:46
Dematium sp. (D21)	1:3
Dematium sp. (D32)	1:3
? Haplaria sp. (F20)	1:3
? Monosporium sp. (F37)	9:77
Oedocephalum sp. (F34)	1:7
Penicillium adametzi Zal.	9:83
P. brevi-compactum Dierckx.	2:10
P. citreo-viride. Biourge	2:10
P. cyclopium Westling.	3:17
P. fellutanum Biourge	2:20
P. melinii ? (E70)	1:3
P. namyslowskii Zal.	10:87
P. nigricans. Bainier	2:7
P. restrictum Gil. & Abb.	2:7
P. rolfsii ? (E75)	1:7
P. spinulosum Thom.	9:73
P. thomii. Maire	1:7

FUNGI IMPERFECTI.

Moniliales.

P. thomii series (E48)	1:3
Penicillium sp. (E62)	4:27
Penicillium sp. (E67)	2:7
Pullularia pullulans (de Bary) Berk.	8:50
Sarcinella sp. (D39)	2:7
Scopulariopsis sp. (F35)	2:17
Stachlydium sp. (D31)	4:23
Stachlydium sp. (D31a)	1:10
Trichobotrys sp. (D27)	10:100
Trichoderma viride Pers ex Fries.	10:98
? Zygodesmus sp. (F6)	1:3

Unidentified species:-

(D38) 1:3; (D44) 1:3; (D45) 1:3

STERILE MYCELIA.

Sclerotial form, C.18 2:7

Chlamydospore producing form, C21. 6:53

Dark-coloured sterile mycelia; DS.45 3:17

DS.85 10:80

Sixteen other sterile mycelial forms (DS & HS) were isolated, each from only one profile.

The Phycomycetes, represented only by Zygomycete species, were few in number, although three species of this group were among the commonest fungi to be isolated: Mucor ramannianus, Absidia orchidis and Mortierella isabellina. The occurrence of Piptocephalis cylindrospora has been noted previously (p. 97).

Only three species of Ascomycetes were isolated and of these none was common.

Species of the Fungi Imperfecti were the commonest of all isolates. Forty-two species were isolated, of which twenty-two were Penicillia. Only about one quarter of these species were of constant occurrence.

Twenty forms of sterile mycelia were isolated of which only four were isolated from more than one profile.

Basidiomycetes and Oomycetes were not represented in these isolations.

Of the fifty-five sporing species isolated, twenty-five were isolated from the soils of at least three other heaths.

The distribution of numbers of species of varying constancy of occurrence is recorded in Table 14, where it is shown that the species fall naturally into two groups - those of high and of low constancy. Very few species occurred in the middle ranges of constancy (4 - 7) and 17% of total isolates were of high constancy.

Table 14. Distribution of Species of Varying Constancy of Occurrence.

Constancy	No.of St.mycelial forms.	No.of sporing species	Total No.
1	16	25	41
2	1	12	13
3	1	5	6
4	0	2	2
5	0	0	0
6	1	0	1
7	0	2	2
8	0	2	2
9	0	3	3
10	1	4	5
Totals.	20	55	75

The sterile mycelial forms were very largely of constancy one, although reference to the experimental data shows that these forms were often present in two or three of the experiments from any one profile.

The species which occurred in three or more of the ten profiles are listed below (Table 15), together with three species of constancy two which are of some interest in relation to the investigations described below.

Table 15. List of Species in Order of Constancy of Occurrence

Constancy 10.

Mucor ramannianus
Penicillium namyslowskii
Sterile mycelium, DS.85
Trichobotrys sp. (D27)
Trichoderma viride

Constancy 9.

? Monosporium sp. (F37)
Penicillium adametzi
P. spinulosum

Constancy 8.

Cladosporium herbarum
Pullularia pullulans

Constancy 7.

Absidia orchidis
Mortierella isabellina

Constancy 6.

Sterile mycelium C21.

Constancy 4.

Penicillium sp. (E62)
Stachlydium sp. (D31)

Constancy 3.

? Chaetopsis sp. (D34)
Coniothyrium sp. (D23)
Haplosporangium decipiens.
Mortierella parvispora
Penicillium cyclopium
Sterile mycelium, DS.45

Constancy 2.

Beauveria bassiana
Gelasinospora cerealis
Mortierella marburgensis, etc.

Of the twenty-four species listed, eighteen were recorded in at least three other heathland soils, and the remaining six in one other heathland soil. Thus not one of these common species was restricted to Burnt Hill.

No relationship between the occurrence of species and the

season could be determined. This absence of evidence of seasonal variation is probably related to the method which is not suitable for its exhibition and which enables no distinction to be made between the phases of fungal activity and the inactivity in the soil.

That no indication is given of the activity of fungi isolated detracts from the value of this method as a means of ecological study. For the enumeration of a 'soil fungal flora' which may be considered part of the plant community, it is essential to distinguish between the active and inactive components of the fungus flora. While the higher plant community may be defined as a collection (including one or many species) of plants growing together and having a certain unity (Tansley 1949), such a definition is not easily applicable to the fungus flora. The plant ecologist acknowledges only those plants which are growing, whereas the fungal ecologist is presented with a mass of species which could grow if certain nutrient, climatic and soil conditions were to be fulfilled. The 'certain unity' which Tansley describes in its simplest form as being a 'common habitat' (the soil), to a large extent determines those species which shall grow and flourish: it is one of the fundamental problems of fungal ecology to determine which actually provides the common habitat of the species isolated - the soil itself or the agar medium and method. Once a soil sample is collected and plated with a nutrient agar medium,

that medium becomes the common habitat and determines which of the species, present in the soil sample, shall flourish on the plate. Perhaps many of the species which flourish would normally have remained dormant and eventually died in the soil without ever having been actively concerned in the soil fungal community. There is, theoretically, no limit to the number of fungal species which can occur in any soil type (see Brierley, 1927), but there is a limit to the number which can exist in the form of actively growing mycelium: this limit being determined by the nutrient material which is continuously being added to the soil, by the ability of the fungi to tolerate the conditions of the macrohabitat, and also by their ability to compete for the microhabitat. Apart from those species of fungi which become active only under certain well defined soil conditions (the zymogenous component of the fungus flora), there exist species which are more or less constantly active (the autochthonous flora) - indigenous species which characterise the soil and (conversely) whose presence may be considered a reflection of the macrohabitat with its contained standard and constant set of microhabitats. It is the purpose of fungal ecological studies of macrohabitats to determine this autochthonous component of the fungus flora and to relate it to the macrohabitat.

Several earlier workers have attempted to delimit the constant component of the fungus flora by accepting only those species of fungi which were isolated more than a certain number of times, this figure being an arbitrary one. Thus, Bisby, James and Timonin (1933) decided that ..... "if a

fungus is present to the extent of 1% or more of the total number isolated it is safe generally to conclude that it is an active soil fungus" Whether indeed it is safe to reach such a conclusion is questionable, especially so when the method employed (the dilution plate) selectively favours the isolation of species with a high sporing capacity. The soil plate method was designed by Warcup to reduce this selective effect, but even so it is apparent from the lists of species that a large proportion of them are species of Moniliales, and especially of Penicillia - which are among the most vigorous spore-producing species, and form a large proportion of the total number of species isolated in this study (i.e. more than 40%). Most of these Penicillium species are represented by more than 1% of the total number of isolates. The use of the ecological concept of constancy probably provides a sounder basis for such a deliniation of species than does the percentage of total isolates, since it does not take into account the very localised abundance likely to be found with species of high sporing capacity. Thus if those fungi of constancy three or more are arbitrarily chosen as being representative of part of the (autochthonous) soil fungus flora of the Burnt Hill site, the percentage of Penicillium species is reduced to twenty-four.

While there remains no definite proof that even these very constant species are active members of the fungus community, evidence in favour of this conclusion can be

derived from an analysis of the components of the fungus floras of the various horizons. Disregarding the fresh habitats provided on the disintegration of plant roots and animals within the soil, all newly colonisable habitats are introduced at the surface layers; one would expect the deeper soil horizons to provide more stable nutritional and environmental conditions and, therefore, a more stable and less variable fungus flora. Newman and Norman (1943), in their study of the activity of subsurface populations, have demonstrated that such a relatively more stable community does exist. The percentage components of high and low constancy (sporing) species in three soil levels are recorded in Table 16, where it is shown that the constant species provided a larger proportion of the fungus flora in the deeper soil levels.

Table 16. Distribution of Species of Varying Constancy in three Soil levels.

Constancy.	A1.	A2.	B1/B2/C.
1 - 5	69.2	57.7	50.0
6 - 10	30.8	42.3	50.0

This evidence tends to support the view that these constantly occurring species can be related to the macro - rather than the micro-habitat, and to the autochthonous rather than the zymogenous flora.

3. Vertical Distribution of Fungi.

The distribution of the most constantly occurring species in the various soil horizons is recorded in Table 17. Many of the features of this distribution were discussed in Section A.

Mucor ramannianus, Trichobotrys sp. (D27) and ? Monosporium sp. (F37) were all most commonly isolated from the A2 horizon, and on all soil plates prepared from the sands of this horizon these three species were invariably present. Mucor ramannianus dominated the fungi occurring on these plates and often as many as thirty colonies of this fungus were counted on many of them. No species, other than M. ramannianus, was of frequent occurrence in the illuviated horizons - indeed very few species were isolated from them.

The majority of species occurred most commonly in the A1 horizon and were isolated less frequently with increase of depth. It is interesting to note that the distribution of the 'A1/A2 fungi' in depth was not strictly related to their gloiosporic or xerosporic habits, (cf. Burges 1950). Absidia orchidis, a very common fungus in this area of soil, was entirely confined to the A1 horizon.

The vertical distribution of the sterile mycelial forms was discussed in the preceding section; the apparent general confinement of these forms to the lower soil horizons was well illustrated in the Burnt Hill soils. Table 18 records the distribution of sterile mycelial forms, in three soil levels, as a percentage of the total number of species isolated from these levels.

Table 17: Occurrence of Constant Species in Profile Horizons.

(Expressed as %age occurrence in total number of horizons examined).

	A1	A2 (upper)	A2 (lower)	B1	B2	C
Trichoderma viride	100	27	24	11	4	7
Mucor ramannianus	76	100	100	89	21	10
Sterile mycelium, DS.85	17	7	15	19	15	47
? Monosporium sp. (F37)	3	33	67	11	4	.
Trichobotrys sp. (D27)	57	80	79	18	.	.
Pen. namyslowskii.	80	30	6	7	.	.
Pen. adametzi	83	23	3	.	.	.
Pen. spinulosum	80	7	9	.	.	.
Pullularia pullulans	47	23	9	.	.	.
Sterile mycelium, C21	30	20	15	.	.	.
Mort. isabellina	23	13	9	.	.	3
Coniothyrium sp. (C23)	7	.	3	.	.	.
Stachlydium sp. (D31)	7	4	3	.	.	.
Mort. parvispora	23	7	.	.	.	3
Beauveria bassiana.	17	10	.	.	.	.
Penicillium sp. (E62)	17	7	.	.	.	.
Scopulariopsis sp. (F35)	10	10	.	.	.	.
Gelasinospora cerealis	7	3	.	.	.	.
Absidia orchidis	50	.	.	.	.	.
Haplosporangium decipiens	17	.	.	.	.	.
Mort. marburgensis	10	.	.	.	.	.
Thielavia terricola	10	.	.	.	.	.
Sterile mycelium, C18	7	.	.	.	.	.
? Chaetopsis sp. (D34)	.	7	.	4	.	4
Sterile mycelium, DS 45	.	.	.	.	7	13

Table 18. Distribution of Sterile Mycelial Forms.

Horizon:	A1.	A2.	B1/B2/C.
Percentage:	7.1	16.1	50.0

Two rapidly spreading sterile mycelia, C18 and C21, were of most commonly isolated form in the A1 horizon; their growth habit, however, is markedly different from that of the 'DS' and 'HS' forms.

In the edited experimental data (p.135-44) the fungi isolated from the A1 horizon were recorded without reference to their actual depth in inches. Since the vertical distribution of species within this horizon agreed closely with their tendency to occur in the A2 horizon, the original experimental data have been collected and are recorded in Table 19. The first six species in this list were isolated only from the A1 horizon, and the table shows that all of these species were wholly or mostly confined to the uppermost inch of this horizon. Trichoderma viride, although recorded from all soil horizons, was most commonly isolated from the A1 and occurred constantly only in the uppermost inch of this horizon. Penicillium species also tended to occur most frequently in the uppermost two inches. Both Mucor ramannianus and Trichobotrys sp. (D27), of very frequent occurrence in the A2 horizon, were almost absent from the surface inch (in fact these two species were vert rarely recorded on soil plates together with Trichoderma viride.) The three sterile mycelia, DS.37, DS.46 and DS.85, were all isolated from the lowermost

Table 19. Distribution of Species in the A1 Horizon.

(Expressed as percentage of the maximum possible number of records)

Depth in inches.	1	2	3
Mortierella marburgensis	10	.	.
Gelasinospora cerealis	7	.	.
Sterile mycelium, C.18	7	.	.
Absidia orchidis	50	7	.
Haplosporangium decipiens	13	10	.
Thielavia spp.	10	7	.
Scopulariopsis sp. (F35)	7	3	.
Trichoderma viride	100	57	27
Pen. namyslowskii	73	50	30
Pen. spinulosum	63	37	20
Pullularia pullulans	30	20	7
Mortierella parvispora	17	10	10
Pen. adametzi	60	67	40
Mortierella isabellina	27	47	20
Sterile mycelium, C21	10	17	10
Penicillium sp. (E62)	7	10	3
Mucor rmannianus	7	60	70
Beauveria bassiana	.	7	10
Trichobotrys sp. (D27)	.	20	43
Sterile mycelium, DS.37	.	.	7
" DS.46	.	.	7
" DS.85	.	.	17

level of the A1 horizon.

Thus the distribution of species in the various soil horizons was reflected by their occurrence within these horizons. The rapidly growing species were for the most part confined to the A1 horizon, within which they were shown to be more or less confined to the uppermost inch. These distributions appear to be related closely to the growth rates of the species and hence also to the likelihood of their isolation by the soil plate method. While the distribution patterns for rapidly growing species. (e.g. Trichoderma, Mortierella spp. C.18 and C21, Gelasinospora sp. and Absidia sp.) are acceptable - those of the slowly growing species, in particular Mucor ramannianus, Trichobotrys sp. and Beauveria bassiana, are probably greatly modified by the method of isolation. The method permits the isolation of slowly growing species only in the absence of more rapidly growing ones, and all results need to be critically examined with this factor in mind.

Subsection (iii). Investigation of the Fungus Flora by the Immersion Tube Method.

The immersion tube method was devised by Chesters (1940) as a means of isolating fungi from soil in situ, under the actual conditions of soil moisture and temperature. All other methods previously described had involved the removal of soil samples to the laboratory before they were plated, either directly - as in Waksman's and Warcup's method, or indirectly - as in the dilution plate method.

Chesters considers that the immersion tube easily isolates active spreading mycelium, or active localised mycelium, which happens to come into contact with the capillary inlets of the immersion tube. He considers that a control of the isolated fungi may be affected at the point of penetration.

Phycomycetes, including species of Pythium, Mortierella, Mucor and Zygorrhynchus, and certain species of Fungi Imperfecti were commonly isolated by this method; Penicillia were rarely isolated (Chesters,1948). Chesters found the fungi isolated to be dependent upon the soil type and the isolating medium.

Apart from these two reports published by Chesters (1940 and 1948) only one other paper has been presented concerning investigations involving the use of this method - by Nicot and Chevaugeon (1949). These workers criticise the existing plating methods of isolation, and emphasise the importance of distinguishing between the fungi occurring in the soil as active mycelia and those occurring as inactive

spores or resting stages; they agree with Chesters' conclusion that only those fungi existing in the soil as active mycelia can be isolated by the immersion tube method.

In this present investigation the immersion tube method was employed in a similar manner to that described by Chesters (1948), but with one difference: the core of agar (removed from the medium within the immersion tube) was simply cut into four pieces and placed in one petri-dish. To reduce the number of plates required for each immersion tube from four to one was a necessary economy; possibly certain fungi which had penetrated the agar medium were suppressed, in some cases, by crowding on the plates, but it is doubted that the general results were affected by this procedure.

The agar media employed in routine isolations were:

Soil extract agar. (As described in Section A)
Czapek-Dox + yeast ext., pH 4.5 (" " ")
Corn-meal agar, pH 5.0 (Difco brand)
Plain (water) agar (3%)

1. Investigation of the Fungi Occurring in the Surface Soils.

Thirty-six immersion tubes were buried in the surface soils of the Burnt Hill site every month from October 1952, to January 1954, with the exception of the following months; November 1952, July and December 1953.

Fungi were not isolated from all of the tubes immersed. It was found that the temperature greatly affected the necessary period of incubation in the soil - during winter months often twenty-one days or more were required before the

tubes were infected. Single immersion tubes were removed after varying periods of time and examined for the presence of mycelia; only when mycelium was observed in these tubes were the remaining tubes collected and often several remained uninfected. A few of the tubes were spoiled by certain biotic agencies (rabbits and ants) and some were crushed in the soil during periods of very low temperature. In all, 386 immersion tubes were successfully employed in this investigation.

The four media were used in a more or less equal number of immersion tubes during every month. This number was not quite constant, however, as usually some of the tubes were spoiled during the sterilisation processes. It was always necessary to prepare more immersion tubes than were required. Whilst the results were rewarding, the method, for routine isolation work, was found to be laborious and wasteful of materials.

When immersed the tubes were arranged in three or four groups (or 'nests'), well separated in distribution over the site. Each nest consisted of an equal proportion of tubes containing the four media. The arrangement of tubes in distributed nests was to facilitate determination of the distribution of fungi - to determine whether fungi were only locally active within the area, or were generally active over the whole area.

The species of fungi which were isolated in this

investigation are listed below. Following each specific name is a figure representing the number of times the fungus was isolated, the maximum number possible for each species being 386.

PHYCOMYCETES.

Absidia orchidis (Vuill.) Hagem.	3
Haplosporangium decipiens Thaxter	6
Mortierella bainieri Const.	6
M. hygrophila Linneman	1
M. marburgensis Linneman	36
M. parvispora Linneman	4
M. parvispora ? (A40)	9
M. pulchella Linneman	3
M. zychae Linneman	98
Mucor hiemalis (+-) Wehmer	34
M. hiemalis group (A20)	1
M. mucedo ? (A39)	1
M. piriformis Fischer	9
M. saturninus Hagem	6

ASCOMYCETES.

Gelasinospora cerealis Dowding	10
G. retispora Cain.	9
Sordaria destruens (Shear) Hawker.	2
S. humana (Fuckel) Winter	3

FUNGI IMPERFECTI.

Botrytis cinerea Pers.	30
Dicoccum asperum Cda.	1
Epicoccum sp. (D35)	17
Phoma sp. (C22)	14
Trichderma viride Pers ex Fr.	222
Zygodesmus sp. (F6)	1
(Moniliales) (F26)	1

STERILE MYCELIA.

Sclerotium-producing form, C18.	94
Chlamydospore-producing form, C21.	27
Basidiomycete mycelium, C34.	2

The Phycomycetes, represented by four genera: Mortierella, Mucor, Haplosporangium and Absidia, provided a greater number of species than any other group of fungi. Mortierella marburgensis, M. zychae and Mucor hiemalis were of common occurrence. No Oomycete species were isolated, although corn-meal agar was employed for this purpose (see Chesters, 1948)

Species of the Ascomycetes were not abundant, although two species of Gelasinospora were isolated on several occasions.

Only seven species of Fungi Imperfecti were isolated of which four species were of common occurrence: Trichoderma viride, Phoma sp. (C22), Epicoccum sp. (D35) and Botrytis cinerea, the first-named of these fungi accounted for more

than one third of the total number of isolations. No Penicillia were isolated in this investigation.

Three sterile mycelial forms were isolated; two were of common occurrence, the other, a Basidiomycete mycelium (C34), was isolated only twice.

(a) Effect of Agar Medium on the Species of fungi Isolated.

The numbers of times each species occurred in the four isolating media were collected and expressed as a percentage of the total number of isolations possible. The important features of these records are presented in Table 20. The distributions of these percentage isolation figures were analysed by the Chi-squared test, and the probabilities of the chance occurrence of these distributions are recorded in the fifth column of Table 20. Several of these distributions show a probability of less than 5% and it must be concluded that the media employed influenced the species of fungi isolated. (see Chesters, 1948)

— See over

Table 20: Occurrence of Fungi in Four Isolating Media.

(Expressed as %age of total number of isolations possible)

Agar medium:	Plain	Soil Ext.	Czapek	Corn-meal	Probability
Mortierella spp. (Total)	53.2	28.1	38.7	32.0	5 - 2% *
Mort.marburgensis	8.4	3.7	16.8	8.6	5 - 2% *
Mort. zychae	38.5	18.3	13.5	21	1 - 0.1% *
Mucor spp. (Total)	3.5	14.7	30.1	11.0	$<$ 0.1% *
M. hiemalis.	1.4	9.7	20.5	8.6	$<$0.1% *
Ascomycete spp. (Total)	3.5	8.5	7.2	7.4	$>$5%
Sterile mycelium, C.18	26	25.5	15.7	28.5	$>$5%
Fungi Imperfecti (Total)	68.1	80.5	82.9	72.8	$>$5%
Trichoderma viride.	59	57.3	54.0	56.8	$>$5%

Plain agar exerted a selective effect on the isolation of Mortierella species when they were considered as a group although one species, Mortierella marburgensis, was favourably isolated by Czapek-Dox agar. The selective isolation of M. zychae (the commonest species of this genus to be isolated) by plain agar was great.

Mucor species also were significantly affected by the isolating medium: a higher number of isolations of Mucor spp. (and Mucor hiemalis) was recorded from Czapek-Dox agar than from the three remaining media together.

From the data accumulated no other groups of fungi, or commonly occurring species, were significantly affected by the isolating medium.

(b). Distribution of Active Species in Area.

The burial of the immersion tubes of each experiment in groups, or nests, was mentioned above, and from this treatment it has been possible to derive information concerning the distribution of these active fungi within the Burnt Hill area.

The number of isolates of each species of fungus from each nest in thirteen experiments were tabulated, and, although it is not possible to present all of these results, those from six experiments are recorded in Table 21. These results, including only those fungi which were of common occurrence in the experiments, demonstrate well the general distribution patterns.

Only very rarely were species restricted to any one nest of immersion tubes (i.e. to any one very small area within the site); even species which were isolated only two or three times in an experiment were almost invariably isolated in two or three well separated nests. Exceptions to this distribution are illustrated in Table 21: by Mucor saturninus and Mucor hiemalis in Experiment 7 and Phoma sp. (C22) in Experiment 4. But it also shows that Mucor hiemalis, whilst isolated only three or four times in Experiments 4 and 6 respectively, was more or less evenly distributed between the nests in these experiments.

9.

Table 21: Distribution of Species in Immersion Tube 'Nests'

Experiment No. :—	4				5				6			7			8				9			
Nest:—	A	B	C	D	A	B	C	D	A	B	C	A	B	C	A	B	C	D	A	B	C	D
No. of immersion tubes:—	9	10	10	4	8	6	6	1	10	10	13	12	10	10	9	5	10	10	10	10	9	5
Mort.marburgensis	2	4	3	3	1	3	2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
M. parvispora ? (A40)	.	.	.	.	2	1	0	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
M. pulchella	0	1	1	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
M. zychae	5	5	4	2	2	2	1	0	3	6	4	5	4	3	0	2	1	0	1	2	1	0
Mucor hiemalis	1	1	1	0	.	.	.	.	2	1	1	0	2	0	.	.	.	.	.	.	.	.
M. saturninus	.	.	.	.	.	.	.	.	.	.	.	5	0	1	.	.	.	.	.	.	.	.
Absidia orchidis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	0	0	1
Gelasinospora cerealis	.	.	.	.	.	.	.	.	1	1	0	.	.	.	.	.	.	.	.	.	.	.
G. retispora	0	1	2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Phoma sp. (C22)	0	2	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Sterile mycelium, C18	4	1	0	0	2	5	3	1	6	2	4	3	4	1	1	2	1	1	1	0	1	1
" " C21	.	.	.	.	4	0	2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Thus the evidence indicates that the species of fungi isolated were generally present, in an active state, over the whole area and did not occur in small regions or 'flushes'.

c. Seasonal Distribution of Species.

The complete analysis of the results obtained over a period of sixteen months is recorded in Table 22, where the isolation figures are given as percentages of the total number of isolations recorded in each month. Some of these data are re-presented, in graphical form, in Fig. 1.

The most prominent feature of these results is the very marked seasonal variation in the occurrence of Trichoderma viride; these results are highly significant either when analysed alone by the Chi-squared test, or when analysed on the basis of the occurrence of Trichoderma relative to that of all other species. Trichoderma viride was virtually absent from isolations during the winter months (October 1952 to March 1953 and January 1954), when low temperature prevailed (see Table 13.), and was abundantly represented in isolations made during the warmer summer months. (Compare Figs. 1 & 2.)

In contrast to Trichoderma, species of Mortierella and Mucor were of greatest occurrence during the winter months. That the seasonal variation of species of the latter two genera is a real one is, however, questionable: the isolation of these two groups of fungi may well be related to the activity of Trichoderma, which, on plates, rapidly suppressed the growth of these and other species, and would probably

Fig.2. Prevailing Temperatures, October 1952-January 1954.

(Data from Table 13.)

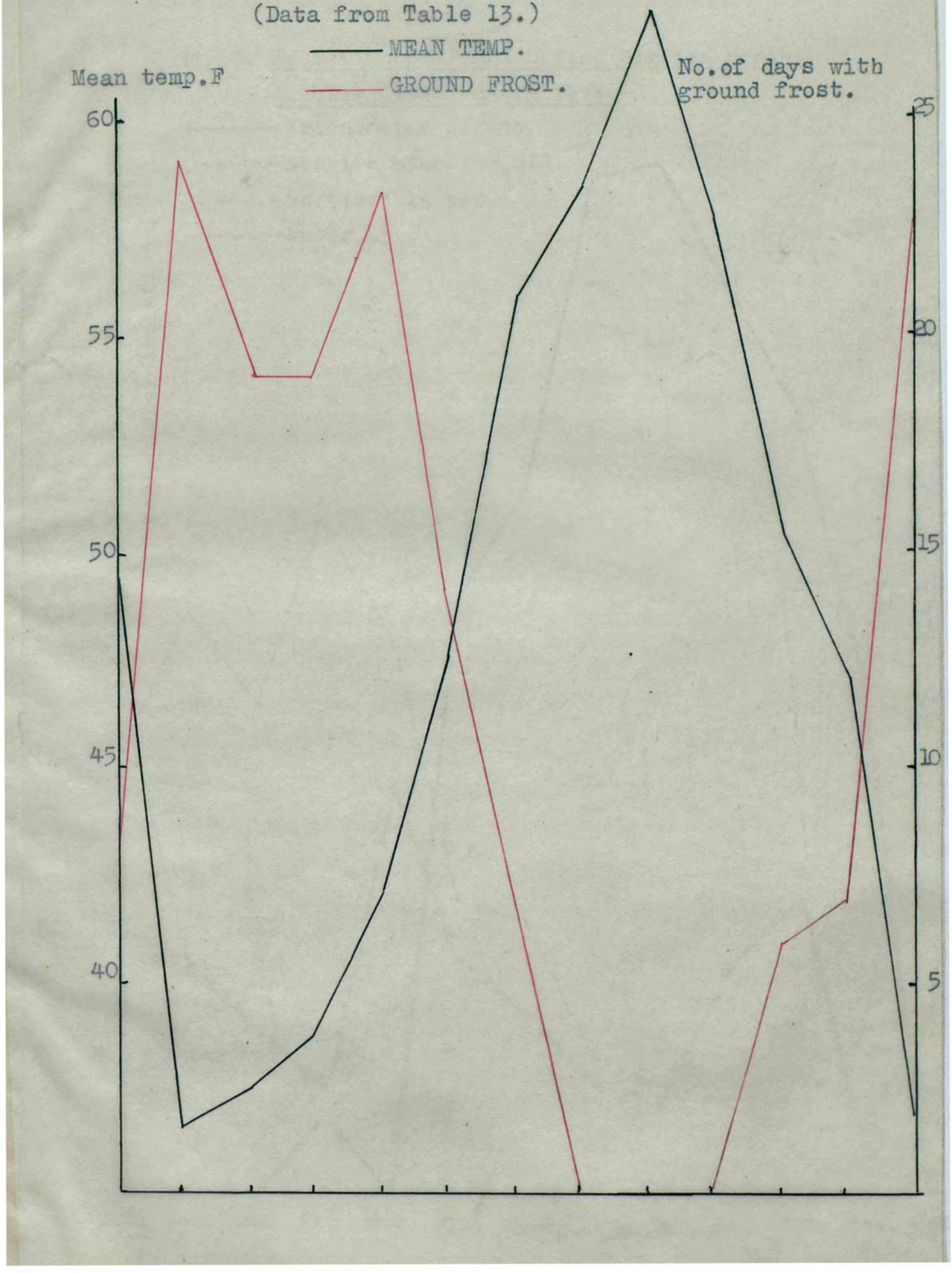
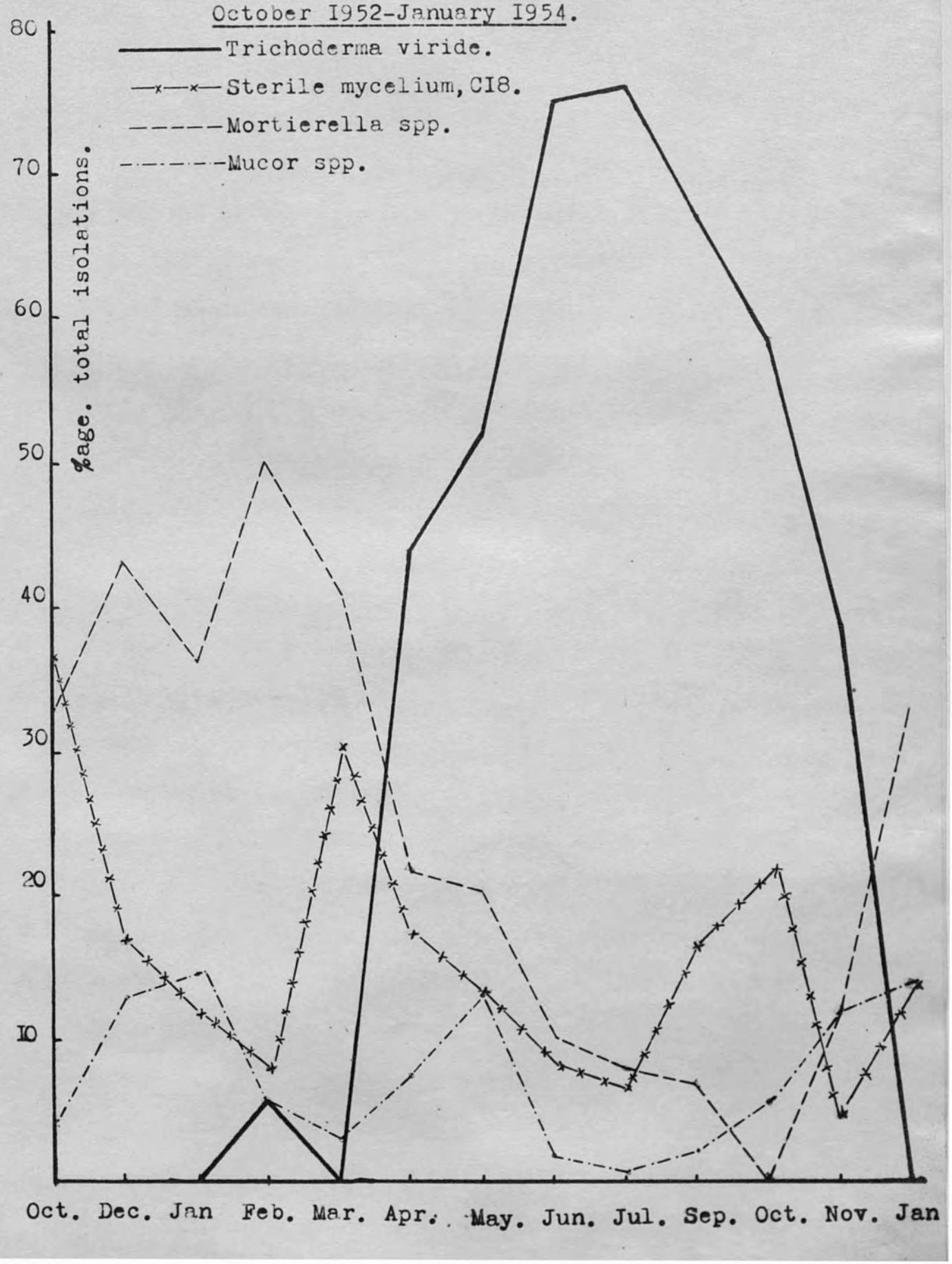


Fig. I. Isolation of Fungi During Sixteen Months, October 1952-January 1954.



behave similarly at the points of entry, or within, the immersion tube.

Nicot and Chevaugeon (1949) commenting upon the suppression of the other species by Trichoderma, concluded that the vigorous growth of this fungus, together with its inhobitory properties, was responsible for the isolation of this species alone. (Trichoderma accounted for 85% of their total isolations by the immersion tube method).

It is of interest to note here that Chesters (1948) isolated Trichoderma by the immersion tube method very infrequently, although it was constantly isolated by plating methods. Chesters considered that its failure to appear in immersion tube isolations was due to one or more of the following factors: (a) that its spread in the soil was restricted to its immediate medium, (b) the competition of faster growing species in the capillaries of the immersion tube, and (c) that vigorous sporulation in the soil causes early cessation of vegetative growth,

Nicot and Chevaugeon when considering their own and Chesters' results decided that the limitation of Trichoderma to its immediate medium (organic debris and root material), was the reason for its absence in Chesters' investigations, and for its abundance in their own. For the French investigators were studying the fungi in a soil so rich in organic material that they considered it inevitable that this material would occur in close juxtaposition to the capillary inlets when the immersion tube was buried, and thus permit the abundant

Table 22: Isolation of Species during Sixteen Months.

(Figures for each species given as percentage of total number of isolations recorded in each month)

List of Species	Oct. 1952	Dec. 1952	Jan. 1953	Feb. "	Mar. "	Apr. "	May "	June "	Aug. "	Sept. "	Oct. "	Nov. "	Jan. 1954	Total
Absidia orchidis	-	-	-	-	-	1.5	-	-	4.5	-	-	-	-	6
Haplosporangium decipiens	-	-	-	1.5	-	1.5	-	-	-	-	-	6	-	9
Mortierella bainieri	-	1.5	2.5	-	-	-	-	-	-	-	-	-	11	15
M. hygrophila	-	-	2.5	-	-	-	-	-	-	-	-	-	-	2.5
M. marburgensis	21	17	2.5	19	16.5	1.5	-	-	-	-	-	-	-	77.5
M. parvispora	-	1.5	-	3	-	1.5	-	-	-	-	-	-	-	6
M. parvispora ? (A40)	-	-	2.5	-	11	-	-	2	-	-	-	-	8.5	24
M. pulchella	-	-	2.5	3	-	-	-	-	-	-	-	-	-	5.5
M. zychae	12.5	23	24	25	14	19	20	8.5	8.5	7	-	12	14	187
Mucor hiemalis	-	8	7	4.5	3	6	3	2	-	2.5	5.5	9	14	64.5
M. hiemalis group(A20)	4	-	-	-	-	-	-	-	-	-	-	-	-	4
M. mucedo	-	-	2.5	-	-	-	-	-	-	-	-	-	-	2.5
M. piriformis	-	5	5	1.5	-	1.5	-	-	-	-	-	3	-	16
M. saturninus	-	-	-	-	-	-	10	-	-	-	-	-	-	10
Gelasinospora cerealis	12.5	1.5	-	1.5	-	3	2	2	2	-	-	-	-	24.5
G. retispora	-	-	2.5	4.5	-	-	-	2	-	-	4	3	-	16
Sordaria destruens	-	-	-	-	-	-	-	-	-	-	4	-	-	4
S. humana	-	-	-	-	-	-	-	-	-	4.5	-	1.5	-	6
Botrytis cinerea	-	2	2.5	12	3	1.5	-	-	-	2.5	4	12	13.5	53
Dicoccum asperum	4	-	-	-	-	-	-	-	-	-	4	-	-	8
Epicoccum sp. (D35)	-	3	5	4.5	3	1.5	-	-	2	-	-	6	2.5	27.5
(Moniliaceae) (F26)	-	1.5	-	-	-	-	-	-	-	-	-	-	-	1.5
Phoma sp. (C.22)	-	6	2.5	3	3	-	-	-	-	-	-	-	16.5	31
Trichoderma viride	4	-	-	6	-	44	52	75	76	67	58	39	-	421
? Zygodesmus sp.(F6)	-	1.5	-	-	-	-	-	-	-	-	-	-	-	1.5
Sterile mycelia:	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sclerotial form, C.18	37.5	17	12	8	30	17.5	13.5	8.5	6.5	16.5	21	4.5	14	206
Chlamydosporic form, C21	4	9	24	1.5	16.5	-	-	-	-	-	-	3	2.5	60.5
Basidiomycete, C34	-	1.5	-	-	-	-	-	-	-	-	-	-	2.5	4
Total.	99.5	99	99.5	99	100	100	100	100	99.5	100	100	99	99	-

isolation of Trichoderma.

Unfortunately, in neither of these investigations is the period in which the work was done stated; the contrasting results which were obtained could, perhaps, in the light of this present investigation, be due to a marked seasonal variation in the growth of Trichoderma viride. Also it should be remarked that the surface soils of the Burnt Hill site are extremely rich in organic debris and living roots, yet the isolation of Trichoderma was not constant.

Of the three factors which may limit the growth, but not necessarily the isolation, of Trichoderma, the first appears to be the most acceptable; it is shown in a later subsection that Trichoderma was present on the surfaces of roots occurring in soils from which it was rarely isolated. That competition of other species restricts the entry of Trichoderma into immersion tubes seems unlikely, when its complete domination of these species is demonstrated by plating methods. Vigorous sporulation may well cause early cessation of vegetative growth, but no signs of such sporulation were observed in the plating methods already described; Trichoderma, in the soil plate studies, was shown to be largely confined to the uppermost inch of soil, and if vigorous sporulation had occurred it seems improbable that such a large mass of gloiospores would remain confined in this surface layer (Burges, 1950).

The present investigation indicates that Trichoderma

grows vigorously in the surface soils, especially in association with organic debris and roots, and whilst so growing suppresses the isolation (by such methods as have been employed) of other species of fungi; that it is inactivated by low temperature (and in particular appears to be sensitive to ground frost - see Table 13 and Fig. 2.) and remains dormant and inactive during colder periods.

No species of fungi were isolated more or less constantly throughout the sixteen months of this investigation, except the sclerotium-forming sterile mycelium, C.18. This fungus alone appeared to be unaffected, in isolation, by the vigorous growth of Trichoderma viride, and it is noteworthy that it was the only species to be isolated which checked the growth of Trichoderma on agar media. The growth rates of these two fungi (C18 and Trichoderma) were more or less equal, and when their two colony margins came into contact the growth of both was arrested (see Plate 3): the colony margin of Trichoderma showed marked hyphae accumulation (A, Plate 3) which is clearly demonstrated in the photographs (where it is stained by the rose-bengal contained in the agar medium). No concentration of the hyphae of C18 was observed, but a heavy brown pigmentation of the medium was always associated with its colony margin (B, Plates 4 and 5). Usually the colonies appeared to remain static for seven days or more and then the scleroria of C18 were observed to develop within the Trichoderma colony (see Plate 5). Often Trichoderma

Plate 3.

The upper half of the medium is colonised by T. viride, the lower by sterile mycelium, C18.

A: Line of dense accumulation of Trichoderma hyphae.

B: Vigorous sporulation of Trichoderma.

Plate 4.

The mycelium of T. viride encloses two colonies of C18, along the line of contact hyphal accumulation is clearly shown.

C: Brown pigmentation of the medium caused by sterile mycelium, C18.

Plate 5.

The petri-dish pictured in Plate 4 but fourteen days later. Sterile mycelium, C18 has invaded the Trichoderma colony.

C: Brown pigmentation of medium caused by C18.

sporulated vigorously along the line of contact (C, Plate 3.)

The chamydospore-producing sterile mycelium, C21, was not recorded during the period of abundant isolation of Trichoderma: the reason for this could be attributed to the difficulty of observing its sparse and widespread submerged mycelium, in the presence of Trichoderma, on plates, as well as to any seasonal variation in its occurrence or isolation.

Mucor species, which tended to be isolated with reduced frequency during the period of vigorous Trichoderma growth, were often observed, on plates, to be actively parasited by Trichoderma viride.

2. Vertical Distribution of Species.

The immersion tube, when buried in the normal vertical position, only sampled the fungi growing in the surface three inches of the soil. This present investigation was designed to determine which species of fungi were actively growing at greater depths in the soil, and whether any 'layering' of mycelial activity occurred within the surface three inches.

The investigation, which was of a very limited nature, involved the preparation of small trenches and the horizontal burial lf immersion tubes at various soil depths. The standard immersion tube was not suitable for this type of study since the capillary inlets are arranged spirally around the tube; thus some of the inlets would be facing upwards and soil particles would have tended to fall directly onto

the agar surfaces within the capillaries, also they would not permit a precise definition of the depth of isolation. For these reasons 'one-sided' immersion tubes were used, in which three capillary inlets were placed in line with the longitudinal axis of the tube; this arrangement enabled the tubes to be buried (horizontally) with all three inlets facing downwards, and with all inlets at one specific depth in the soil.

In all experiments plain agar was used as the isolating medium.

The designs of these experiments are described below:

Experiment 1. (August, 1953) A shallow trench was prepared and twelve immersion tubes were buried, horizontally, at a depth of 1 in., and ten immersion tubes at a depth of 3 in. After an incubation period in the soil of more than two weeks the 1 in.-level tubes were observed to be infected and all tubes were collected and plated. The results are recorded in Table 23.

Table 23. (For explanation see text).

	1 in.	3 in.
Trichoderma viride	10	0
Mortierella zychae	2	0

Experiment 2. (September, 1953.) Five immersion tubes were buried at each of the following depths: 5, 7, 9, 11, 13 in., in the A2 horizon: at the same time six immersion tubes were buried (vertically) in the bottom of the trench, into the B1 horizon. The results are recorded in

Table 24.

Table 24. (For explanation see text)

Horizon:	A2					B1
Depth in in.	5	7	9	11	13	-
Mucor ramannianus	5	3	5	5	5	0
Trichoderma viride	0	1	0	0	0	0
?Monosporium sp.(F37)	0	0	0	1	0	0

Experiment 3. Five immersion tubes were buried at each of the following depths: 2, 3, 4 in. (representing the A1/A2 transition zone). The results are recorded in Table 25.

Table 25. (For explanation see text).

Depth in in.	2	3	4
Mucor ramannianus	0	4	5
Penicillium namyslowskii	0	1	0
Trichoderma viride	5	1	0

Although these experiments were so limited, the results are of interest.

Experiment 1 demonstrated the difference in the fungal growth rate in the uppermost inch of soil from that of the succeeding inch: that only the uppermost series of immersion tubes was infected indicated that in normal, vertically immersed tubes, infection occurred most rapidly through the uppermost capillary inlets, and perhaps the agar medium was wholly colonised before fungi growing in lower levels could

infect the medium. Competition not only between fungi in the capillary inlets, but also between those fungi already established in the medium and those attempting to enter may be of considerable importance. The results also demonstrated that Trichoderma was only active, or was considerably more active, in the uppermost inch of soil, (cf. results from soil plate experiments)

The mycelial activity of Mucor ramannianus was demonstrated by its almost constant isolation from the A2 horizon. These results conformed closely with those obtained by the soil plate method, and also illustrated well the effect of competition of faster growing species in excluding M. ramannianus from the routine surface soil isolations. For although M. ramannianus was isolated at the 3 in. level by horizontally immersed tubes, it was never isolated by immersion tubes buried vertically in the soil surface, yet sampling to a depth of 3 in. Again, the isolation of Penicillium namyslowskii showed that it was probably due to competition that its frequent isolation from soil plates was not reflected by the surface soil immersion tube investigations.

Subsection (iv). Investigation of the Fungus Flora by a Slide-Trap Method.

A simple method of isolating fungi actively growing in the soil was described by C. La Touche (1949), in which two cavity slides were clipped together in such a manner as to form an enclosed, shallow, elliptical chamber in which agar medium was contained. These ~~chambar~~ 'slide-traps' were pushed horizontally, or vertically, into the soil, and, during a period of incubation in the soil, fungal hyphae penetrated between the slide surfaces, entered the small chamber and colonised the agar medium. The slide-traps were then removed from the soil, returned to the laboratory, opened, and the agar medium - after being cut into small pieces - was plated.

Apart from the names of a few species which he isolated by this method, La Touche gave no information concerning its efficacy as compared with other methods, except to emphasise its simplicity. He considered the method to be advantageous in that bacterial contamination was slight, and that vegetative and reproductive structures could be studied in situ: among the disadvantages of the method he included the suppression of certain species of fungi by those more rapid in growth, and the penetration of spores and bacteria when the surrounding soil was flooded.

1. Investigation of the Fungus Flora by the La Touche Slide-Trap Method.

The slide-trap method was employed in an investigation of

the fungus flora of Burnt Hill soils; throughout this investigation, during which eighty slide-traps were buried, plain water agar was used as the isolating medium.

The results obtained by this method were disappointing; many of the traps remained uninfected and, in comparison with the immersion tube method, only very few species were isolated. Of the eighty slide-traps used, sixty were immersed in the soil surface (A1 horizon), and twenty in the sandy soils of the A2 horizon. The results are recorded in Table 26.

Table 26. Fungi Isolated by the La Touche Slide-Trap Method.

	No. of Isolations.	
Horizon.	A1.	A2.
Beauveria bassiana	-	1
Cephalosporium sp. (F33)	1	-
Mucor ramannianus	-	7
Penicillium spp.	4	-
Trichoderma viride.	31	-
No. traps remaining sterile	24	12
Total	60	20

Fungal hyphae were observed to be present in the great majority of slide-traps after their removal from the soil, but mostly these hyphae had penetrated only a very short distance into the traps. La Touche, mentioned that he incubated such lifted traps in a moist chamber until mycelial growth was observed in the agar medium, and this procedure was adopted in the present investigation. Some of the hyphae

continued to grow and, eventually, colonised the agar medium: without exception - in slide traps from A1 soils - these were hyphae of Trichoderma viride. In most of the traps the penetrant hyphae grew no further. On no occasion was more than one species of fungus isolated from a slide-trap.

From the results and observations it appeared that the distance the fungal hyphae had to grow, between the glass slide surfaces, was too great for the majority of species. Because of this factor the method very strongly favoured the isolation of vigorously growing species, and the quantity of agar medium contained in the traps was so small that it enhanced further this selective effect - species which perhaps even infected the agar medium first, were later suppressed by more vigorous species, either within the trap or when the agar was plated with a nutrient medium.

The method, however, was so simple to use that modified slide traps were devised and tested. The ultimate objectives in these modifications were firstly, to reduce to a minimum the distance between the soil and the agar medium, and secondly to increase the amount and area of the agar medium within the traps. Eventually a modified slide trap was designed which proved to be an efficient and simple method for isolating actively growing mycelia; its construction and the results obtained by its use are described below.

2. A Modification of the La Touche Slide-Trap.

(i) Construction of the Slide-Trap.

The modified slide-trap (see Fig. 3) consists of a shallow

chamber and a glass microscope slide, which acts as a cover, held in position by wire paper clips fashioned for the purpose.

The shallow chamber (Fig.3,Y) was constructed from 'Perspex' (I.C.I. unplasticised polymethyl methacrylate) - attempts to use glass for this purpose were unsuccessful owing to the lack of sufficiently strong and heat resisting cements.

I.Slide-Trap prepared for immersion.

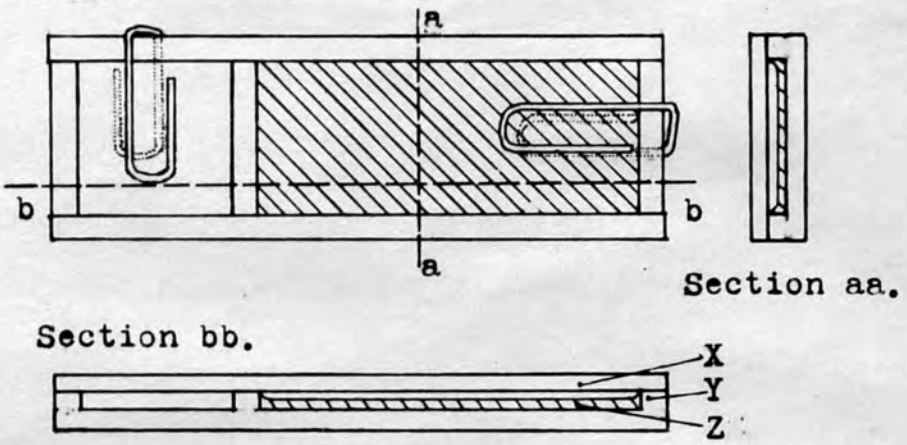


Fig.3. Modified La Touche Slide-Trap.

A 'Perspex' strip (3 in. x 1 in. x 3/32 in.) formed the base of the shallow chamber, and narrow lengths of the same material (⅛ in. x 3/32 in.) were fixed around the edges of the base to form the four walls of the shallow chamber. Chloroform was used to weld the 'Perspex' surfaces.

The shallow chamber was unequally divided internally by a cross piece; the larger part of the chamber (2 in. x ¾ in.) provided the 'active' isolating portion and contained the agar medium (Fig. 3, Z), whilst the smaller part protruded above the soil when the slide-trap was immersed.

(ii) Preparation of the Slide-Trap.

The 'Perspex' shallow chamber was stored in absolute alcohol when not in use, and was sterilised by flaming before use. Plain agar (approximately 1 cc.) was placed in the larger part of chamber by means of a sterilised pipette; the agar was added whilst hot (60-65°c) so that it spread evenly to form a thin film over the floor of the chamber. The chamber was then covered by a sterilised glass microscope slide (Fig.3, X) and, when the agar had solodified, this was fixed in position by two wire paper clips, as illustrated in Fig. 3. The prepared slide trap was finally lightly flamed and placed in a sterilised container. All manipulations were carried out under a sterile hood.

Slide traps were always prepared just before they were required for immersion: the processes of sterilisation, addition of the agar medium and sealing occupied very little time - twenty-five slide traps could be prepared, without difficulty, in thirty minutes.

(iii) Immersion.

The slide-trap was (vertically) immersed into a pre-made, small, slot-shaped hole in the soil surface to such a depth that the larger part of the shallow chamber, containing the

agar medium, was completely submerged - the small dividing cross-piece being at soil surface level. The hole was prepared by means of a stout knife, or a square-ended chisel blade of similar proportions to those of the slide trap.

Slide traps were buried in groups and each group was protected against rain by an inverted U-shaped piece of zinc sheeting.

(iv). Isolation of fungi from the slide-trap.

After the necessary period of incubation in the soil - determined by the periodic removal of sample slide-traps - the slide-traps were carefully withdrawn from the soil and returned to the laboratory. The period of incubation required for infection was two or three days less than that for immersion tubes.

It was found possible to observe penetrant fungal hyphae under a wide-field binocular microscope, to mark them, and to isolate each one, or group of hyphae, separately. This procedure, however, was rather time-taking and possible only when a small number of traps were used.

In routine isolations, the slide-traps were opened under a sterile hood and the agar medium around the edges of the chamber only was removed. Usually the agar medium around the perimeter was cut into nine small pieces (see Fig.3. II): four pieces from the upper part of the isolation chamber, which sampled the fungi in the uppermost inch of soil (Fig.3, II, 1,2,3 & 4) and five pieces from the lower part of the

of the isolation chamber, which sampled fungi in the second inch of soil (Fig.3, II; 5,6,7,8 & 9). These two groups of agar strips were plated in separate petri-dishes, thus enabling a rough distinction to be made between those fungi which penetrated the slide-traps from the upper and lower levels of soil sampled.

Czapek-Dox solution agar with 5% yeast extract, adjusted to pH4.5, was used throughout as the plating medium.

3. Species of Fungi Isolated by the Modified Slide-Trap Method.

The species of fungi which were isolated from surface soils by seventy-four modified slide-traps are listed below. The relative (percentage) frequency of isolation of each species is stated after the specific name.

PHYCOMYCETES.

Absidia orchidis (Vuill.) Hagem	0.5
Haplosporangium decipiens Thaxter	5.4
Mortierella bainieri Const.	1.5
M. hygrophila Linneman	0.5
M. isabellina Oud.	2.4
M. marburgensis Linn.	2.9
M. parvispora Linn.	7.3
Mortierella parvispora ? (A40)	0.5
M. pulchella Linn.	0.5
M. zychae Linn.	14.2
Mucor hiemalis Wehmer	0.5
M. piriformis Fischer.	0.5

FUNGI IMPERFECTI.

Sphaeropsidales.

Coniothyrium sp. (C23)	1.5
Coniothyrium sp. (C35)	0.5
Phoma sp. (C22)	8.6
Penicillium namyslowskii Zal.	6.3
Trichoderma viride Pers ex Fr.	22.0
Zygodesmus sp. (F6)	0.5

Moniliales.

Botrytis cinerea Pers.	1.5

STERILE MYCELIA.

Sclerotium-producing form, C18.	10.7
Chlamysporic mycelium, C21	1.0
Basidiomycete mycelium, C34	10.7
White mycelium, HS21	0.5

Of the twenty-three species isolated, twelve were species of Phycomycete, seven were Fungi Imperfecti and four were ~~represented in these isolations~~. sterile mycelia.

Mortierella was the genus most abundantly represented, and Mortierella zychae was the second most commonly isolated species - Trichoderma viride was the first most commonly isolated species. Only one Penicillium species, P. namyslowskii, was recorded: this species was isolated in thirteen traps of three experiments. The sterile mycelia, C18 and C21, were of frequent occurrence, and of some interest is the frequent isolation of the sterile, Basidiomycete mycelium, C34.

The total number of species isolated by seventy-four modified slide-traps shows a considerable increase over the three species isolated by sixty La Touche slide traps. And whilst never more than one species was isolated in each La Touche slide-trap, up to six species were isolated by modified slide-traps. The numbers of species isolated in seventy-four slide-traps are recorded in Table 27.

Table 27. Numbers of Species Isolated in Modified Slide Traps.

No. of species.	1	2	3	4	5	6
No. of traps.	11	23	19	15	5	1

4. Occurrence of Species in the Upper & Lower Parts of the Isolation Chamber.

The procedure employed in plating agar strips removed from slide traps was described above; the results thus obtained are recorded in Table 28.

Table 28. Numbers of Species & Groups of Fungi Recorded in the Upper & Lower Parts of the Isolation Chamber.

Depth sampled (in.)	0-1	0-2
Mortierella spp.	28	49
Mucor spp.	2	2
Sterile mycelium, C18	16	18
" " C21	0	2
" " C34	16	10
Spp. of Sphaeropsidales	14	11
Trichoderma viride	32	39
Penicillium namyslowskii	7	10
Other spp. of Fungi Imperfecti	9	11

All groups and species of fungi, with the exception of species of Mucor, Sphaeropsidales, and the sterile mycelium, C34, were isolated most frequently in the lower part of the isolation chamber. This result may be attributable to the greater perimeter of isolation in the lower portion of the traps (i.e. 3 in. as opposed to 2 in.) and consequently no significance can be attached to it. Conversely, however, greater significance can be attributed to increased isolation of the few species in the upper part of the isolating chamber, which sampled the fungal mycelia in the uppermost inch of soil. It must be concluded that the species of Sphaeropsidales, and perhaps of Mucor, were more active in the superficial soil layer, as was the sterile, Basidiomycete mycelium, C34. The latter result is in agreement with Chesters' (1949) conclusion that the Basidiomycetes are mainly active in the organic debris on and in the soil surface.

5. Comparison of the Modified Slide-Trap and Immersion Tube Methods.

A total of thirty-three species was isolated, from the A1 horizon, by the combined immersion tube and slide-trap methods. Of these, 18 species were isolated by both methods, ten species were isolated by the immersion tube method only and five species were isolated by the slide-trap method only. The complete results are recorded in Table 29.

The majority of species isolated were common to both sets of results, and the three species, Trichoderma viride, Mort. zychae and the sterile mycelium, C18, were those most

commonly isolated by both methods. Certain species were isolated more frequently by one method than the other - most striking of which is the sterile, Basidiomycete mycelium C34 - while other species were isolated by one method alone. The differences are mostly slight and may be due primarily to the inequality of the numbers of times with which each method was employed; these remain, however, indications that the method affects both the species isolated and their relative frequencies of isolation.

Five species were isolated by the slide trap method alone, and all five were comparatively slowly growing forms; two of them - Penicillium namyslowskii and Mortierella isabellina - were of relatively frequent occurrence. These results indicate that competition between rapidly and slowly growing species, as a factor controlling the fungi isolated, is reduced in the slide-trap method. This conclusion is further substantiated firstly, by the greatly increased relative number of isolations recorded for the sterile mycelium, C34, Phoma sp. (C22), Mortierella parvispora and Haplosporangium decipiens, all of which are among the most slowly growing of species isolated by both medods; and secondly, by the fact that P. namyslowskii was isolated by the immersion tube method from the lower A1 horizon (see Table 25) where the competition of rapidly growing species was considerably reduced.

Of the ten species of fungi which were isolated by the immersion tube method alone, it is remarkable that three were species of Mucor, and four were Ascomycetes. None of the ten

Table 29. Comparison of Species Isolated by the Slide-Trap & Immersion Tube Methods.

	Percentage frequency of Isolation.	
	Slide-trap method.	Immersion tube method.
Coniothyrium sp. (C35)	0.5	-
Sterile mycelium, HS21	0.5	-
Coniothyrium sp. (C23)	1.5	-
Mortierella isabellina	2.4	-
Pen. namyslowskii.	6.3	-
Trichoderma viride	22.0	34.0
Mort. zychae	14.2	15.3
Sterile mycelium, C18	10.7	14.5
" " C34	10.7	0.3
Phoma sp. (C22)	8.6	2.2
Mort, parvispora.	7.3	0.6
Haplosporangium decipiens	5.4	0.9
Mort. marburgensis	2.9	5.5
Mort. bainieri.	1.5	0.9
Botrytis cinerea	1.5	4.6
Sterile mycelium, C21	0.9	4.2
Mort. parvispora ? (A40)	0.5	1.4
Mort. pulchella	0.5	0.5
Mort. hygrophila	0.5	0.1
Mucor piriformis	0.5	1.4
Mucor hiemalis	0.5	5.2
Absidia orchidis	0.5	0.5
Zygodesmus sp. (F6)	0.5	0.1
Epicoccum sp. (D35)	-	2.6
Gelasinospora cerealis	-	1.5
G. retispora	-	1.4
Mucor saturninus	-	0.9
Sordaria humana	-	0.5
S. destriens.	-	0.4
Mucor, hiemalis group (A20)	-	0.1
Mucor mucedo ? (A39)	-	0.1
Dicoccum asperum	-	0.1
(Moniliales) (F26)	-	0.1

species, except Epicoccum, was isolated frequently. The sparsity of Mucor species in slide-trap isolations may be related to the isolating medium which was employed (plain agar): very few Mucor species were isolated by immersion tubes containing this medium (see Table 20). The absence of Ascomycete species in slide trap isolations, may also be associated with this factor, but is perhaps more likely explained by the limited number of slide traps used; Ascomycetes were not isolated frequently by any method.

Generally, the results obtained by the two methods were similar and some of the differences probably can be related to the divergence in the relative frequencies of application. Other factors which may be involved in bringing about these differences are: (i) the isolating media; (ii) the partially anaerobic conditions within the immersion tube as opposed to the aerobic conditions prevailing in the slide trap; (iii) the ability of fungi to grow between the flat 'Perspex' and glass surfaces into the slide trap chamber, and (iv) the ability of fungi to enter the immersion tube through the capillary inlets, where competition between species may be intense (Nicot & Chevaugeon 1949, Chesters 1948). In both methods the species of fungi isolated may be affected by the increased moisture content and decreased aeration in the soil, brought about by the immersion of a solid object into the soil (Chesters 1948). Examination of the fungus flora by Rossi-Cholodny slides also indicated that the smooth surfaces of the slide trap and immersion tube themselves, may provide

substrate favouring the rapid spread and subsequent isolation of certain species (see Subsection (vii)).

In simplicity of construction and use the slide-trap compares very favourably with the immersion tube.

Subsection (V). Microscopical Examination of the Soil Fungus-Flora by the Rossi-Cholodny Slide Method.

1. Method. The procedure described by Cholodny (1930) was closely followed in this investigation.

Clean microscope slides were inserted into an incision in the soil and were left buried for either seven or fourteen days. After removal from the soil the slides were dried at laboratory temperature and then fixed by gentle heating over a spirit flame. Larger soil particles were removed from the slide surface by gentle agitation and irrigation in water, and the slides were then washed in distilled water and stained, for one hour, in phenolic aniline blue (Jones and Mollison, 1948). Finally they were washed in distilled water, air-dried and mounted in lactophenol.

The soil slides discussed in this investigation were prepared during the months of September, October and November, 1953, and were examined during the following three months. A total of 96 soil slides was prepared from soils of the following depths: 0-1 in., 2-3 in., 5-6 in., and 9-10 in.

2. Results of the Microscopical Examinations.

(a) Occurrence of Hyaline mycelia. Almost without exception slide preparations from the surface soil layer were richly covered with fine, hyaline septate or aseptate mycelia, some stained and some unstained. A large proportion of this

mycelium was not sporulating, and, therefore, could not be identified, although in many instances sporulation accompanied mycelial development and identification of the mycelia to generic, and occasionally to specific, rank was possible.

Hyaline mycelia were mostly observed to grow over the slide surface and were seldom associated with humus, mineral particles or root fragments. Often continuous mycelia grew over considerable areas of the slide surface (1-2 sq.in.) and their distribution, in relation to the organic matter on the slide, indicated that they developed extensively where the slide was not in intimate contact with the soil.

The intensity with which mycelia were stained was extremely variable: even within one small 'colony' on the slide some hyphae could be observed which were completely unstained, while others were heavily stained. The variation in intensity of staining (demonstrated in Plates 6 and 7) could often be related to the protoplasmic content of the hyphae. Frequently hyphal tips were very heavily stained while behind the tip the intensity of staining gradually decreased. Rapid growth of these mycelial forms did not result in the production of a large mass of living mycelium. Where sporulation occurred on broadly spreading mycelia, the spore-bearing hyphae and small portions of the parent hyphae often were heavily stained whilst the remainder of the mycelium was mostly unstained and barely visible, (see Plate 8.)

The most abundant sporulating mycelia to be recorded on soil slide preparations were those of the genus Mortierella - prevalent among which was M. marburgensis. This species occurred on more than half of the preparations from surface soil and often was the most abundant mycelium on them - always sporing vigorously and easily identifiable. Sporangiophores of M. marburgensis from soil slide preparations are demonstrated in Plates 8 and 9, where the intensity of staining of the sporangiophores and 'foot-cells' is shown to be markedly greater than that of the parent hyphae.

Mortierella parvispora was of frequent occurrence; its mycelium was usually sparsely distributed over large areas of the slides but, unlike that of M. marburgensis, was little branched. This species produced scattered, single sporangiophores from stoloniferous hyphae. (Plate 10)

Other species of Mortierella were frequently recorded: M. humilis ? (A37) and a species which closely resembled M. zychae (Plate 11) were recorded on several preparations. Five Mortierella species could not be identified or related to any of those which had been isolated; among these was the Mortierella sp. illustrated in Plate 12, which was present on several slides always occurring in the manner demonstrated - a single sporangiophore developed from a large globose body bearing many rhizoidal appendages. Many coenocytic, non-sporulating mycelia, very similar to those of the Mortierella species were present and widespread on soil slide preparations.

The commonest sporulating hyphomycete mycelium was that of the Cephalosporium-type, always broadly spreading and branching vigorously. These mycelia of cephalosporic habit were not uniform and clearly represented a variety of species whose spores differed greatly in size and shape. The most abundant types produced spores similar to those of C. acremonium and C. humicola, but such identifications are open to considerable criticism: Kubiena (1938) observed that fungi from several genera adopt a cephalosporic habit of sporulation in the soil.

A species of the genus Fusidium occurred on one slide preparation (Plate 13), the mycelium of which - unlike that of most hyaline forms - was developed profusely between and within humus particles.

Sporulating mycelia of Penicillium spp. were recorded on four slide preparations on three of which the mycelia were colonising animal remains. In one instance the Penicillium mycelium was developed over an area of 2 sq.in. colonising scattered insect (?) remains: one such remnant is demonstrated (Plate 14). Plates 15 and 16 show the profuse development of Penicillium mycelium about an insect leg (?) A nematode worm was observed to be heavily colonised by at least two species of Penicillium (Plate 17) whose mycelium ramified throughout the animal's body. Only once was an identifiable, sporulating, Penicillium observed in association with humus matter.

Two other instances of fungi developing on soil animals were recorded: on one slide a nematode worm was colonised by a richly sporulating mycelium - almost certainly that of Beauveria bassiana (Plate 18), and another preparation showed a non-sporulating mycelium ramifying throughout the body of a soil mite.

Of the remaining species of fungi most commonly isolated from surface soils little was observed by this technique. Broad, coenocytic mycelia of the mucoraceous type were recorded very infrequently as mycelial fragments, and no sporulating mycelium of Trichoderma was recorded except for one small fragment whose identify was very doubtful. The fact that certain commonly isolated species were not observed, or rather could not be identified, does not in any way detract from the value of the results obtained by isolation methods. The greater amount of hyaline mycelium - mostly septate and indistinguishable from that of a host of hyphomycete species - was not sporulating on the slide preparations and could, therefore, not be identified.

The hyaline mycelial forms described above were all recorded on slide preparations from the surface soil (0-1 in.). Slides immersed at lower levels in the soil were markedly different in their mycelial content. The development of hyaline mycelia over slide surfaces reduced to negligible proportions below a depth of 6 in., except for the occasional occurrence of Mucor ramannianus. Even within the A1 horizon, at 3 in. depth, the majority of slides were almost barren of

hyaline mycelia and the only sporulating form to be observed was M. ramannianus. M. ramannianus was recorded on about one fifth of the slide preparations from the lowest level of soil sampled (10 in.).

Slide preparations from the soil surface levels showed an abundance of root fragments and black humus in the form of small particles or pellets (see Plate 20). In the lower soil levels such humus 'pellets' were absent but a delicate, light-brown, fur-like humic substance was present usually coating some of the fine sand grains. It was on such coated sand grains that Mucor ramannianus was most often observed, although not infrequently it grew over the slide surface. Plate 19 demonstrates the form in which this fungus was usually observed on soil slide preparations.

The reduction in the amount of mycelium below a depth of 3 in. - and in particular that of M. ramannianus - was considerably greater than was expected from the results of soil plate investigations.

(b). Occurrence of Dark-Coloured Mycelia.

Dark-coloured mycelia were very abundant on Rossi-Cholodny slide preparations and in habit and form they differed greatly from the hyaline mycelia.

Very rarely were dark-coloured mycelia observed to sporulate and only one sporulating mycelium was identified - Trichobotrys sp. (D27). This species occurred on a slide preparation from surface soil, the dark hyphae were in

intimate association with a humus particle from which one easily identifiable sporulating branch protruded.

Dark hyphae were present on slide preparations either in intimate association with humus particles, or as small, dead, mycelial fragments adhering to the slide surface. Never were dark-coloured hyphae observed to grow over the slide surface. Characteristically these mycelia were observed protruding from humus pellets (Plate 20), and, with favourable optical conditions, could be observed ramifying through the humus particles. Occasionally small, dense, 'knots' of dark coloured mycelium developed about humus particles (see Plate 21), loops of hyphae emerging from and re-entering the particles. More rarely such mycelia were recorded growing in close contact with dead root material (see Plate 22) but mostly skeletal root debris was uncolonised.

The most frequent occurrence of dark-coloured mycelia on slide preparations was in the form of small fragments, seldom exceeding 50μ in length, which were liberally distributed over the surfaces of all slide preparations. Such mycelial fragments were abundant at all soil depths examined. A typical field of view, containing many dark hyphal fragments, is illustrated in Plate 23, where some of the fragments are indicated.

The dark-coloured mycelia were all similar to one another in being thick-walled, septate and reddish or yellowish brown in colour: some were similar in appearance to a few of the

'DS' types of mycelia isolated in soil plate studies. None was stained, but all were readily observable. The majority of fragments were roughly broken and devoid of protoplasmic content (see Plate 24).

Often occurring in association with dark-coloured mycelia were small, reddish-brown, globose, sclerotial bodies (Plate 25) which were extremely abundant and were invariably observed embedded in humus matter, frequently in small clusters. As many as three hundred sclerotia were counted on one slide preparation.

(c) Occurrence of Fungal Spores.

Spores were of common occurrence on slides prepared from the surface soils but were apparently less abundant in deeper levels of the soil.

Most of the spores observed were dissimilar to those of any of the species of fungi isolated; many were of the small, globose, thin-walled type produced by a variety of both Phycomycete and Hyphymycete species. Ascospores of Gelasinospora cerealis and G. retispora, easily recognisable by their dimensions and heavily sculptured walls, were observed on a few slide preparations.

One of the commonest forms to occur was an acicular septate spore (see Plate 26, A) which was present on most of the slides prepared from surface soils, often in very great abundance. No fungus was isolated which produced spores of this type in culture. Plate 26 is an example of a type of

field of view, frequently observed on Rossi-Cholodny slides, containing a miscellany of spore types.

Apart from the small sporulating branch of Trichobotrys sp. (D27), mentioned above, the only dark-coloured spores observed were elongate with thickened walls and one or more transverse septa. Such spores, while not abundant, were present on slide preparations from all soil depths studied and were attached to a type of dark-coloured mycelium which was very common: this mycelium was regularly septate, thick-walled, yellowish-brown and very irregular in thickness. Three of these spore types and their irregular parent hyphae are demonstrated in Plates 27 and 28. Neither mycelium of this type nor fungi bearing spores of this type were isolated.

(d). Occurrence of Other Organisms.

Coccoid algae were present on most of the slide preparations from surface soils, often in large colonies (50-80 μ in diameter) which were always embedded in, or closely adhering to, humus matter. (see Plate 8).

Only three colonies of actinomycetes were recorded; all were Streptomyces (Plate 29). Streptomyces colonies were observed on areas of the slides free from organic matter. A similar distribution of actinomycete colonies on soil slides were noted by H.L. Jensen (1934), who concluded from this evidence that spore formation only occurred in soil spaces.

No special examination was made for bacteria and none was recorded. Bacteria appeared to be of very infrequent occurrence in these acid soils: they developed on soil plates very rarely, although they did occur, and were somewhat troublesome, on plates prepared for the isolation of root-surface fungi.

Soil animals and parts of animals were present on a few slides, all colonised by Penicillia except one - a mite which was invested by a non-sporing mycelium. Nematode worms were common, and mostly appeared to have been killed during preparation of the slides; two were observed to be colonised by fungal mycelia.

3. Discussion.

The most striking feature of these observations was the great difference in habit between the hyaline and dark-coloured mycelia: the former spreading rapidly and largely inhabiting spaces where the slide and soil were not in close contact, and the latter spreading little or not at all and colonising the humus matter. Chesters (1949) remarked that investigations of the space relationship of mycelium in the soil, with reference to organic debris, might lead to a modification of the concept of inhabitant and invader species to a concept of local and wide colonisers, and show that the inhabitant flora consists of many more members, which, although constant in the soil, have not been able to compete at isolation with the wide colonisers such as species of Mucor

and their allies. The results of the present microscopical investigation are in very close agreement with this observation.

The great decrease in the amount of hyaline mycelium below the surface two inches of soil does not closely agree with the results obtained by the plating method, and may indicate that many of the fungi isolated from the subsurface soils were present as spores and not as active mycelia. Even Mucor ramannianus was not observed frequently below 5 in. depth although many colonies of this species developed on plates prepared from all A2 horizon, and most B1 horizon soils.

The fact that the numbers of dark-coloured mycelial fragments did not greatly decrease with increase of depth is perhaps related to their thickened walls - which might make them considerably more resistant to decomposition than those fungi with thin-walled hyaline mycelia. Also dark-coloured fragments were always easily observable whilst hyaline mycelia, unless well stained, were not. These dark-coloured mycelial fragments might well have represented the mycelial activity which had occurred over a considerable period of time, whilst the evanescent nature of the hyaline mycelia was demonstrated even on soil slides which had been immersed for only seven days. (cf. Jones and Mollison, 1948).

Some fungi observed on soil slide preparations could readily be identified to generic, and a few to specific, rank. Species of Mortierella - prevalent among the immersion tube

and slide-trap isolates - were the commonest of these fungi, and it is possible that the introduction of a solid substrate (i.e. glass tubes and slides) into the soil may have brought about conditions stimulating the growth of these fungi. The abundance of Mort. marburgensis and M. parvispora on soil slides immersed during September - November, 1953, however, was not reflected by the immersion tube isolations, for during these months neither fungus was isolated.

Many fungal mycelia and spores observed on soil slides could not be identified and were dissimilar to those of species of fungi isolated: mostly these fungi were slow-growing, dark-coloured forms, some - Cephalosporium spp. and Mortierella spp. - were capable of rapid growth.

It appears from these microscopical examinations that the isolation methods reveal only a small part of the soil fungus flora and tend to exaggerate the relative importance of certain species and groups. The usefulness of the soil slide method is as great in modifying the results obtained by isolation methods as in substantiating them.

Plates 6-29.

MICRO-PHOTOGRAPHS FROM ROSSI-CHOLODNEY

SOIL SLIDE PREPARATIONS.

Plate 6. Profuse development of hyaline, septate mycelium over slide surface. Note the variation in intensity of staining, many of the hyphae are unstained and barely visible. (X 160)

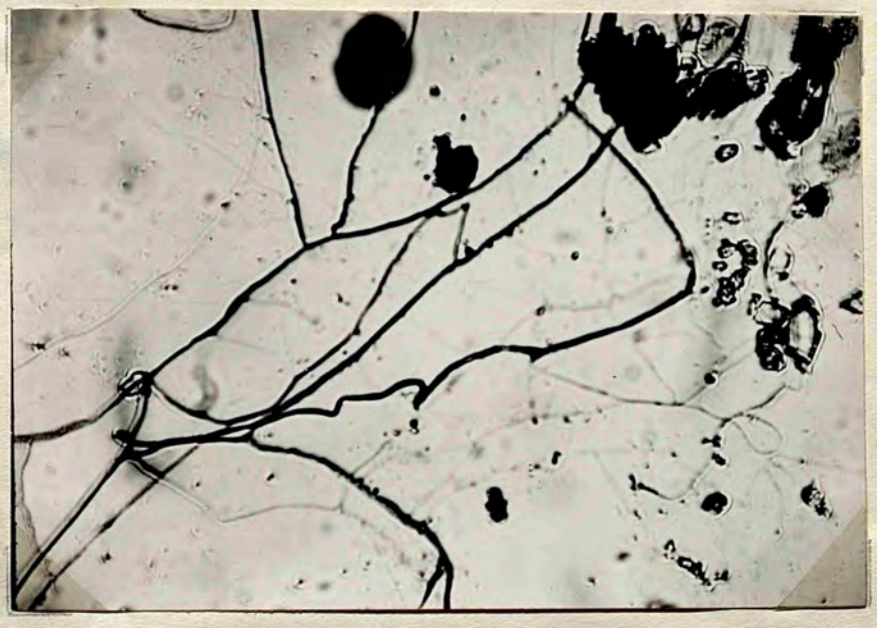


Plate 7. Typical appearance of septate, hyaline mycelium on Rossi-Cholodney slides, some heavily stained, others unstained. (X 160).

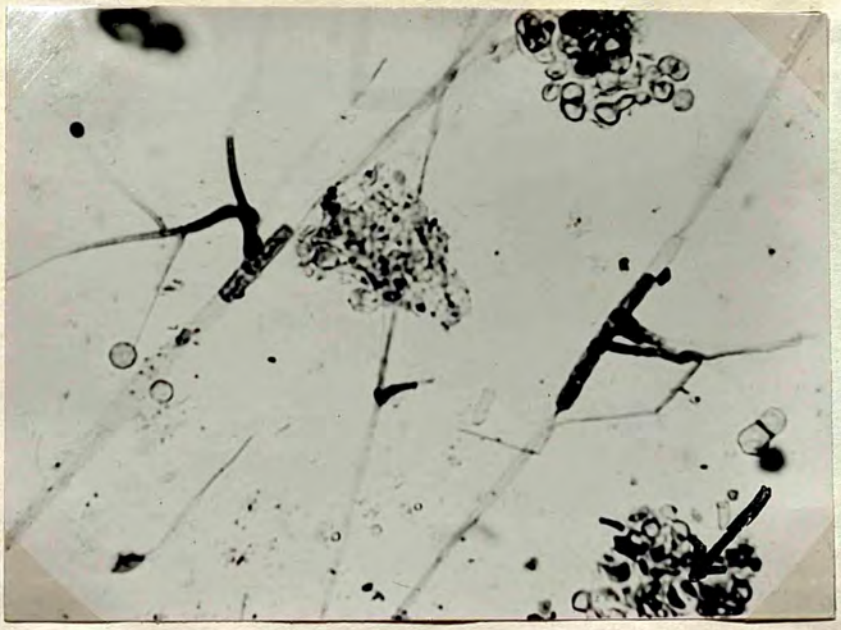


Plate 8. Mortierella marburgensis.
Typical appearance on soil slide preparations, sporangiophore and foot-cell deeply stained, parent hyphae unstained. Note the cluster of sporangiospores at bottom left and coccoid alga, embedded in detritus - top left. (×500).

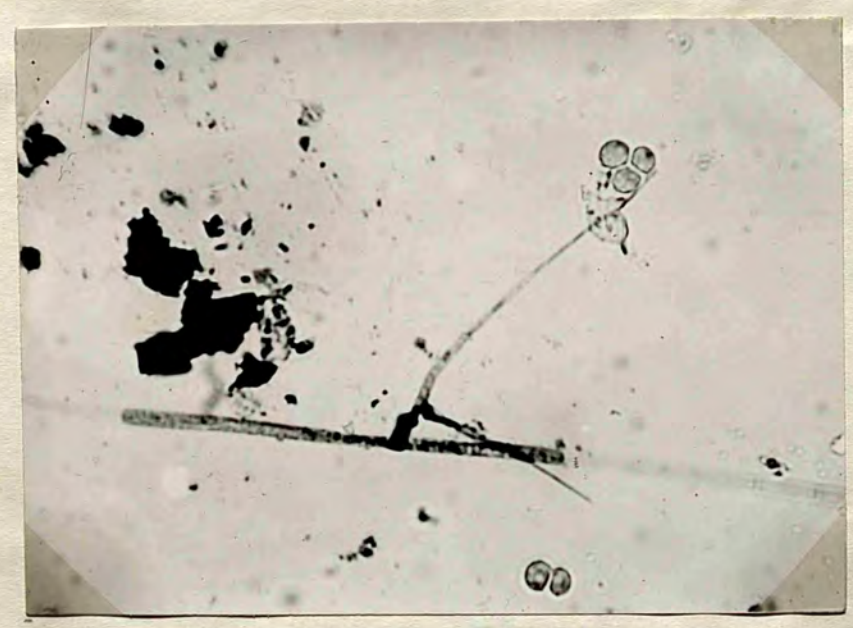


Plate 9. Single sporangiophore of Mortierella marburgensis, one two-spored sporangium is detached. Note the regular septation of mycelium which is devoid of protoplasm. (×500).

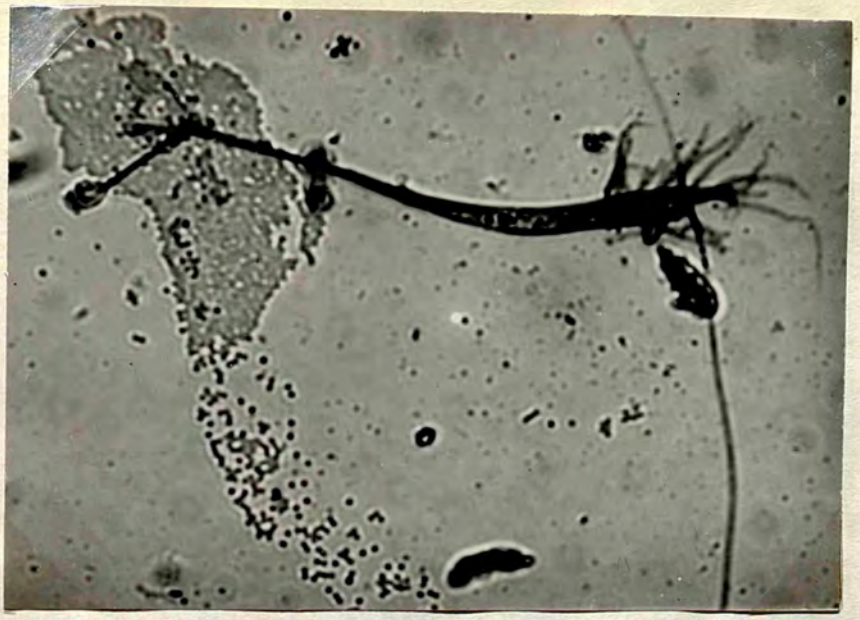


Plate 10. Sporangiophore of Mortierella parvispora with characteristic rhizoidal branching at foot. The three sporangia have dehisced and the spore-mass is fixed to the slide surface. (X 260).

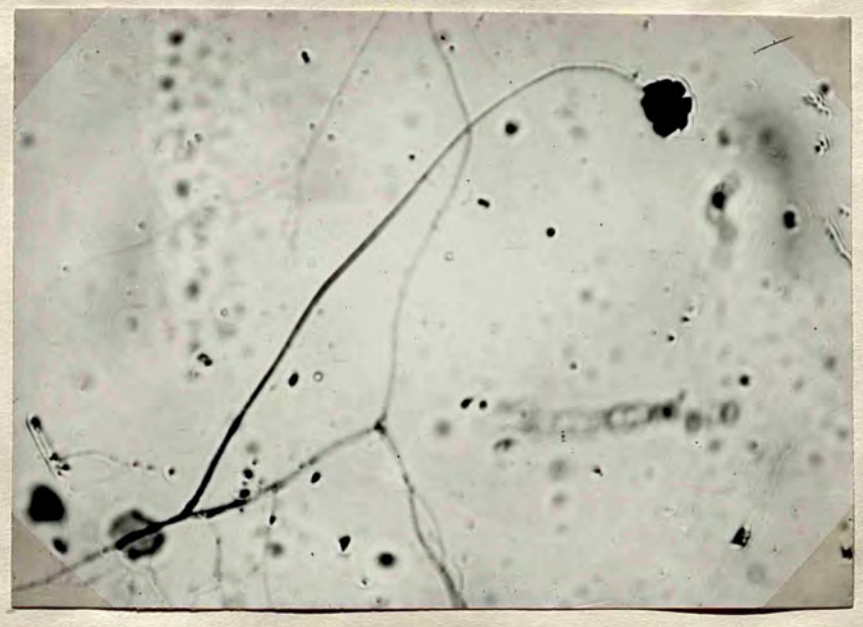


Plate II. Single sporangiophore of a Mortierella species. The dimensions of the spores and sporangiophore closely agree with those of M. zychae Linneman. (X.200)

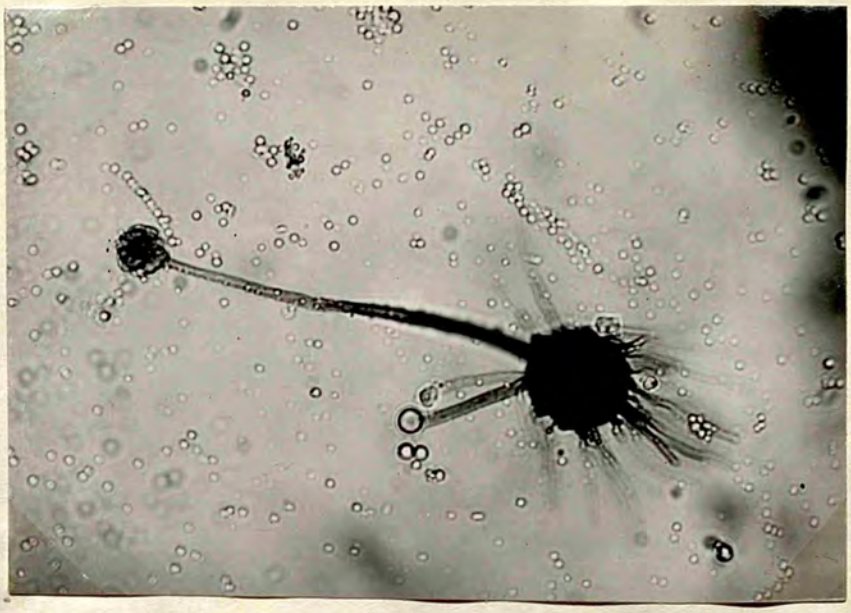


Plate 12. Unidentified Mortierella sp. Sporangiophores of this type, developing from large, globose bodies (approximately 30μ diameter) were not infrequently observed on soil slide preparations. (X400).

Plate 13. Fusidium sp. The mycelium of this fungus covered an area, on the slide surface, greater than 50 sq. mm., and was sporulating profusely. (X 500).

Plate 14. Mycelium of Penicillium sp. colonising insect (?) remains. One monoverticillate penicill is visible. (X700).

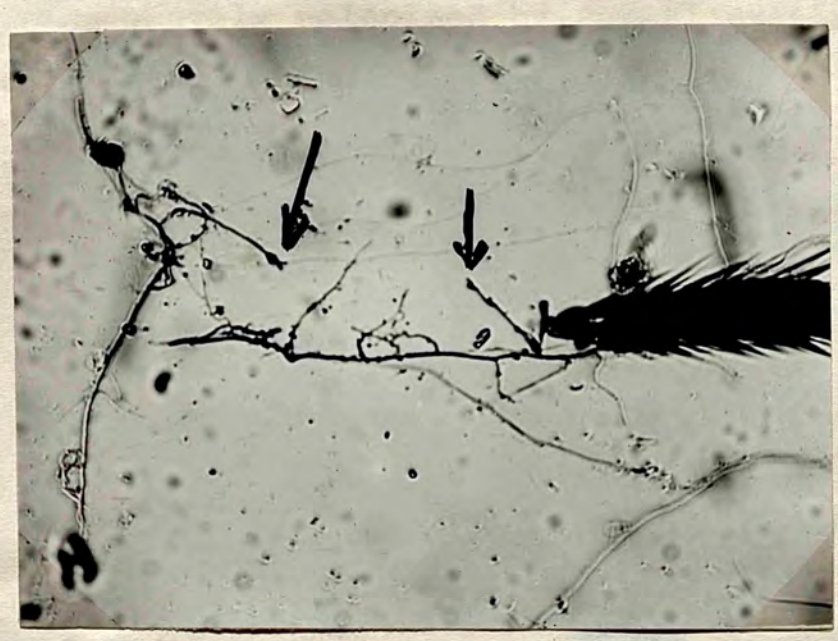


Plate 15. Upper section of insect (?) leg colonised by Penicillium mycelium. Two poorly developed penicills are indicated. (X 160).

Plate 16. Section of insect (?) leg with profuse development of hyaline mycelium. Almost without exception animal parts observed on soil slides were heavily colonised by fungal mycelia. (160 X.)

Plate 17. Part of the body of a nematode worm colonised by Penicillium spp. Three penicills are indicated, one is biverticillate (bottom left) and the remaining two are monoverticillate. The hyphae of these fungi ramify throughout, and invest, the whole body of the organism. (X 250).

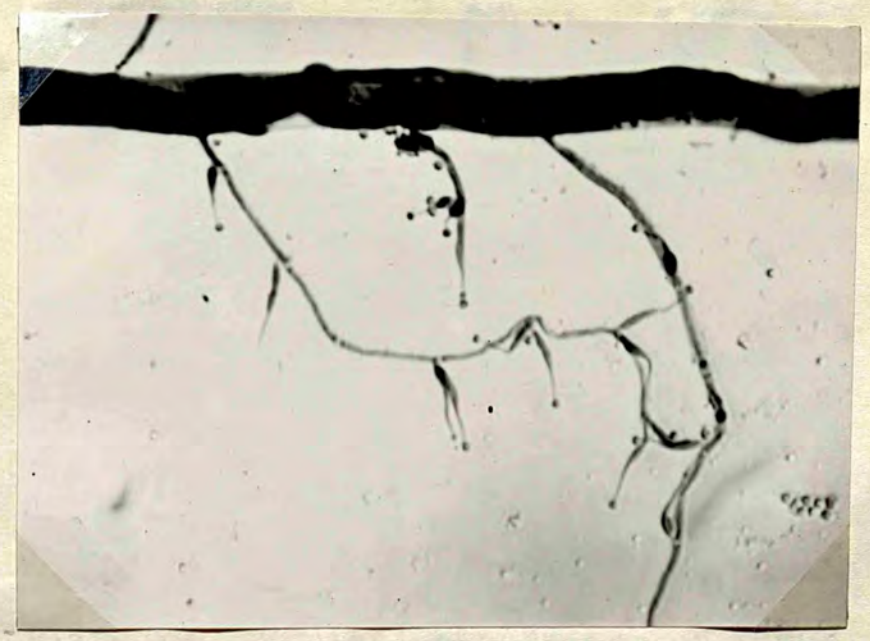


Plate 18. Mycelium of a phialosporic fungus colonising a nematode worm. The habit and dimensions of this fungus agree exactly with those of Beauveria bassiana (X.700)

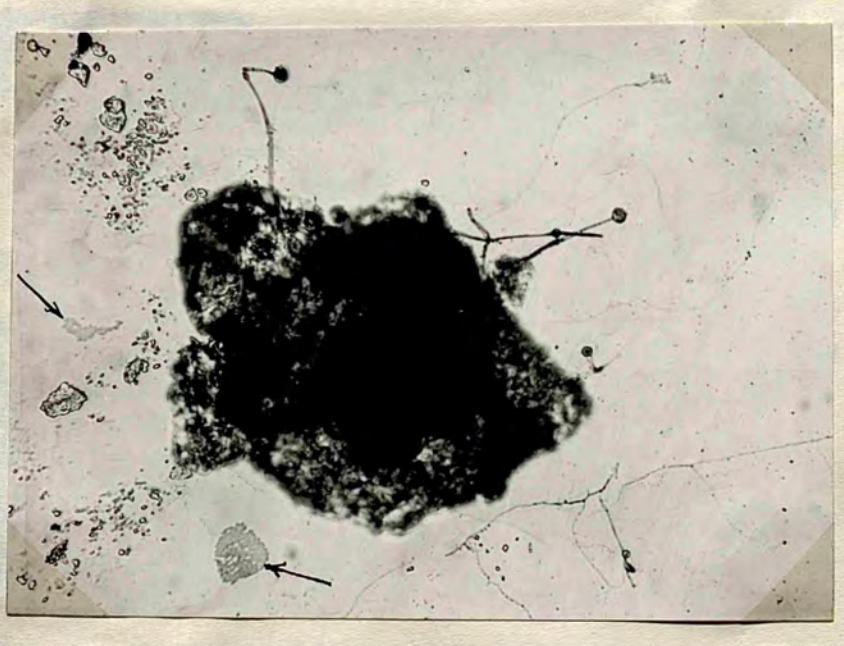


Plate 19. Mycelium and sporangiophores of Mucor ramannianus growing from a sand grain coated with fine fur-like humic material. Two masses of spores, probably of M. ramannianus, are indicated, (X 120)

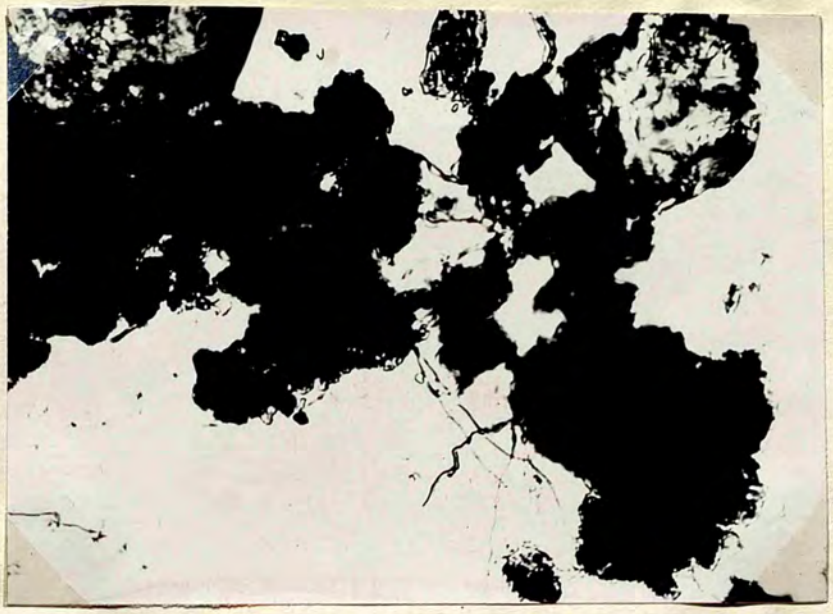


Plate 20. Typical appearance of humus particles with protruding hyphae of dark-coloured mycelia. Such mycelia, septate and brown, were never stained but were always clearly visible. (X 160)

Plate 21. Small knot of dark-coloured mycelium growing from a humus particle, hyphae emerging from and re-entering the particle in small loops. Note the thick-walled nature of these hyphae. (X700)

Plate 22. Sparse development of dark-coloured mycelium about skeletal root fragment. Hyphae can be observed growing along the root surface (indicated by arrows.) (X160)

Plate 23. Typical field of view observed on Rossi-Cholodney slides, showing root, humus and mycelial fragments. Some of the dark-coloured mycelial fragments are indicated. (X200).

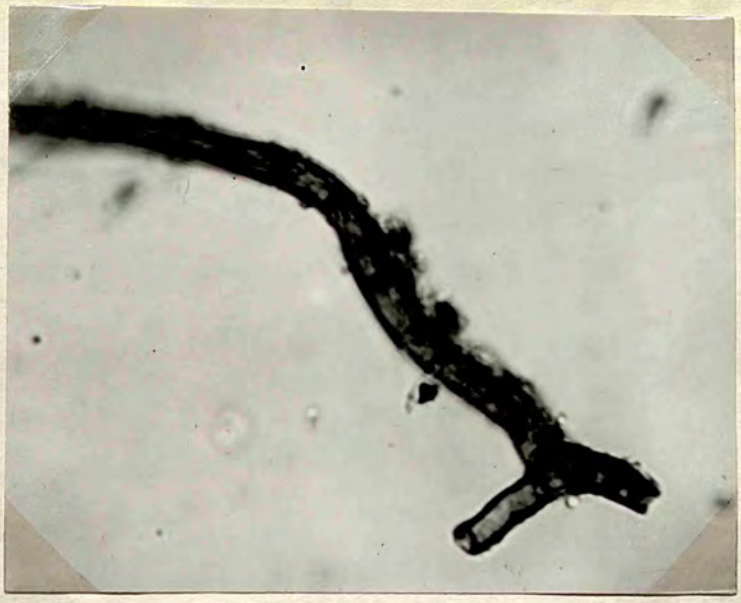


Plate 24. Dark coloured, mycelial fragment, greatly enlarged, of a type very commonly observed on Rossi-Cholodny slide preparations. The heavily thickened walls are clearly shown. (X 700).

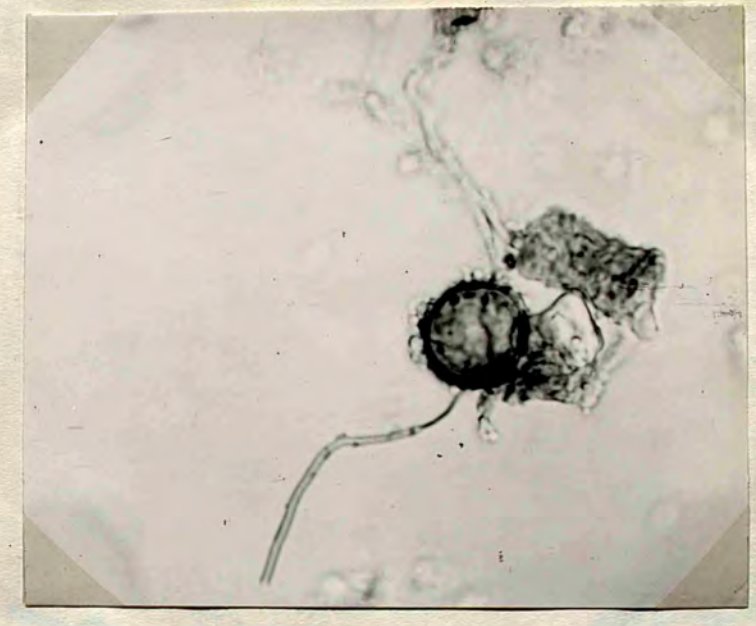


Plate 25. Thick walled, reddish-brown sclerotium of very frequent occurrence on soil slides (X 700).

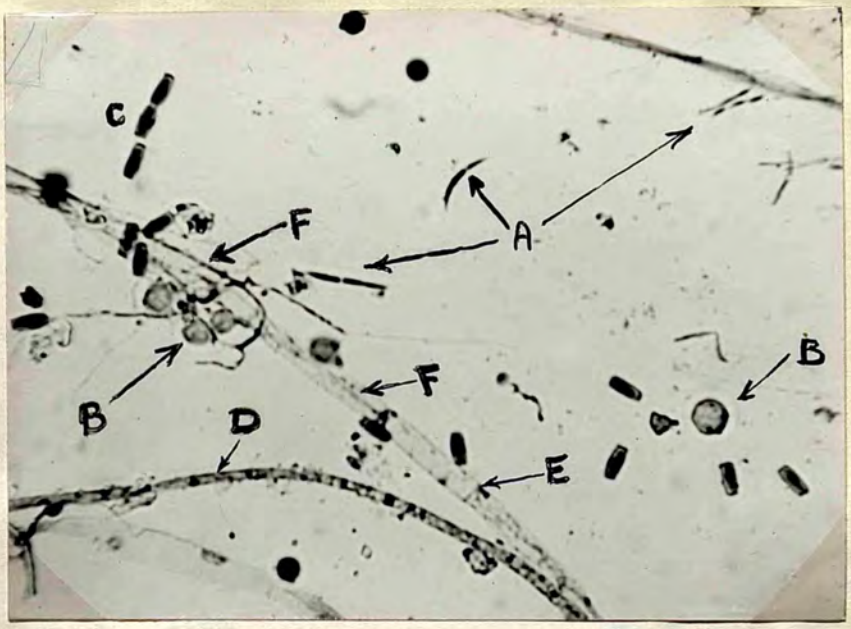


Plate 26. Field of view showing some commonly observed spore-types: A.Acicular, septate, hyaline spores. B.Spores of Mortierella marburgensis. C.Arthrospores of type produced by fungi of the Oosporeae; a portion of the parent mycelium is shown (D). E. Thin walled hypha of M. marburgensis - note formation of cross-wall at point of arrow. F.Fine hyphae spreading along mycelial filament of M.marburgensis - this type of association is of common occurrence, (X 500).

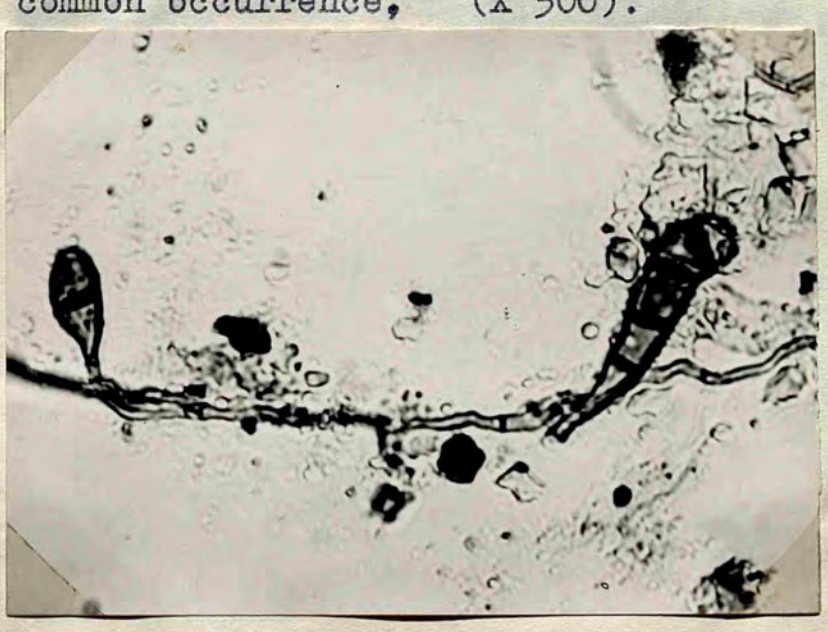


Plate 27. Two- and five-celled demetiacious spores common on soil slides. No fungi forming spores of this type were isolated. (X 700)

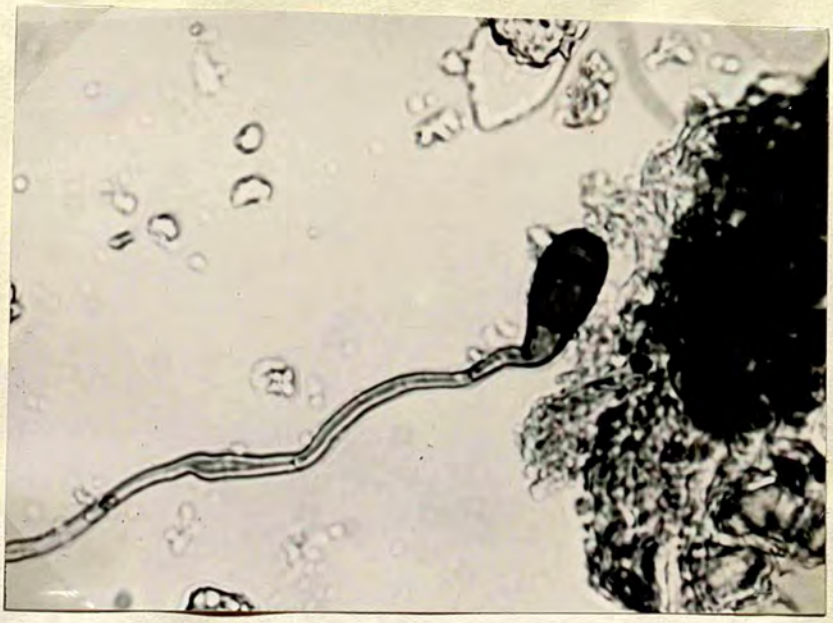


Plate 28. Demetiaceous spore similar to those shown in Plate 27. Note the great similarity between the hyphae in these two plates-thick walled, septate and very irregular in thickness. This type of mycelium was commonly observed but never isolated. (X 700)

Plate 29. Spores and sporing filaments of Streptomyces sp.. Actinomycetes were of rare occurrence on soil slides and only members of this genus were observed (X 1600)

Subsection (V1)a. Investigation of the Fungi Occurring on the Roots of Calluna vulgaris.

The investigations previously described have shown that the distributions of both fungi and the roots of Calluna were closely associated with the various horizons in podsolic soils. Since the work of many investigators has demonstrated that a correlation exists between the distribution of roots and micro-organisms; indeed, that the pattern of root distribution may be partially responsible for the distribution of micro-organisms (Harley, 1948). This present investigation was undertaken in an endeavour to determine whether the fungi present on the root surfaces were similar to those isolated from the soil, and whether the distribution of these fungi was most closely associated either with the type of root or with the soil horizon.

1. Materials and Methods.

(a) Types of Roots Sampled.

An account of the distribution of Calluna roots in the various soil horizons was given in Subsection (i), where three distinct systems (occurring in the A1, A2, and B1 horizons) were described.

Roots were sampled from the A1, A2 and B1 horizons; the amounts of root material in the lower horizons were very small, and collection of this material was not practicable. The A2 horizon was divided, at an arbitrarily chosen depth of 9 in. into upper and lower levels.

The root samples were classified according to their thickness so as to indicate roughly the root type and relative age. The range of roots of various diameters, sampled from four levels of the three horizons, is recorded in Table 30. Where certain types of root were not sampled the amount of material was too small for their collection to be practicable.

Table 30. Numbers of Root Fragments Sampled in Three Horizons.

	Horizon			
Root diameter (mm).	A1	A2(upper)	A2(lower)	B1.
(a) Suberised roots.				
Greater than 2.	100	100	-	-
1 - 2	100	100	100	-
0.5 - 1	100	100	100	-
Less than 0.5	100	100	100	100
(b) Unsuberised roots.				
Less than 0.5	100	-	-	100
Total:	500	400	300	200

All root samples from the A1 horizon, except those of diameter greater than 2 mm., were from the laterally spreading root system (see Subsection (i))

(b) Collection of Roots.

Roots from all horizons, except the B1, were removed directly from the soil, placed in sterilised containers and returned to the laboratory.

Roots from the B1 horizon were removed from soil samples in the laboratory: the firmly compacted nature of the soil,

and the fineness of the roots - none was of greater diameter than 0.5 mm. - made their removal in the field impossible. After returning pieces of (B1 horizon) soil to the laboratory they were placed on a sieve and washed under a water jet; this treatment broke down the soil structure and removed the soil particles to leave a thin mat of fine root material.

(c) Preparation and Plating of Root Material.

Root samples were rinsed in tap water, placed in sterilised petri-dishes, cut into short lengths (approximately 2 mm.) and then washed thoroughly in sterile water, using a washing device of the type described by Simmonds (1930)

Roots were washed, with five changes of sterile water, for a period of 30 minutes, and then were immediately transferred to Czapek-Dox solution agar (pH 4.5) plates, and incubated at room temperature. Five root fragments were placed in each petri-dish.

All roots were washed and plated on the day of collection.

2. Results.

(a) Species of Fungi Isolated.

The fungi which were isolated from roots are listed below. Where the identification is incomplete the collection number is given.

PHYCOMYCETES.

Absidia orchidis (Vuill.) Hagem.

Mortierella isabellina Oud.

M. marburgensis Linn.

M. parvispora Linn.

M. parvispora ? (A40)

M. zychae Linn.

Mucor hiemalis Wehmer.

M. piriformis Fischer.

M. ramannianus Möll.

FUNGI IMPERFECTI.

Sphaeropsidales.

Coniothyrium sp. (C33)

Coniothyrium sp. (C36)

Coniothyrium sp. (C37)

Phoma sp. (C2)

Phoma sp. (C22)

Moniliales.

Botrytis cinerea Pers.

Cladosporium herbarum Link ex Fr.

Gliobotrys sp. (D46)

? Monosporium sp. (F37)

Penicillium adametzi Zal.

P. citreo-viride.

P. lanosum.

P. namyslowskii Zal.

FUNGI IMPERFECTI.

Moniliales.

P. raistrickii series (E72)

P. spinulosum Thom.

P. terlikowskii Zal.

P. thomii Maire.

P. thomii series (E48)

P. viridicatum West.

Penicillium sp. (E62)

Penicillium sp. (E74)

Penicillium spp. (2)

Trichoderma viride Pers ex Fr.

Torula sp. (D48)

STERILE MYCELIA.

9 dark-coloured, septate, sterile mycelia: DS74-78, 80-82, 85.

Of the thirty-three spore-producing species of fungi isolated, twenty-three were Fungi Imperfecti, and ten were Zygomycetes. Ascomycetes, Basidiomycetes and Oomycetes were not represented. Penicillium, Mortierella and Mucor were the genera most commonly represented in isolations.

The species did not differ greatly from those isolated in other investigations, although eleven spore-producing species were isolated only from roots. Of these, four were species of the Sphaeropsidales - a group of fungi of which many species are parasites, but most saprophytes on stems, leaves and other plant tissues. (Ainsworth and Bisby, 1949). The other recorded

species of this group, Phoma sp. (C22), was isolated by the soil plate, immersion tube and slide-trap methods. Gliobotrys sp. (D46) and Torula sp. (D48) were isolated only from roots and were locally of frequent occurrence on them. The remaining species to be isolated only from roots were all Penicillia, only one of which was frequently isolated - P. viridicatum.

Of the sterile mycelial forms, only one was isolated by other methods - sterile mycelium, DS85: this mycelium was isolated with great constancy in soil plate investigations. The remaining eight sterile mycelia, although very similar to the 'DS' isolates of the soil plate investigation, in being dark-coloured, slowly-growing forms, all differed slightly from them and from one another.

(b) Distribution of Species on Roots in Three Horizons.

The occurrence of species of fungi on the various root types in four levels of the three horizons are recorded in Tables 31, 32 and 33.

From the results obtained there is no evidence that the occurrence of species was affected by the types of root which were sampled. The species of fungi, isolated from the roots in any one horizon, were mostly present on all root types, and those that were isolated from only one or two root types were always of very infrequent occurrence. To this statement there appears to be only one possible exception: the sterile mycelium, DS85, was never isolated from the largest root type,

representing the main or primary lateral root, but only from the roots of smaller diameter. Even between suberised and non-suberised roots (see results from A1 and B1 horizon roots) there was no noticeable difference in either the species isolated or their relative frequency of isolation.

Some of the results show a discontinuity in the frequency of isolation of species - thus Trichoderma was isolated from 85% of the 1-2 mm. diameter roots in the A2 (lower) horizon; more than four times the frequency of isolation from other root types in this horizon. These variations are probably due, in part, to localisation of sampling, and hence may indicate the occurrence of local abundance, or 'flushes', of certain species on roots. Again other variations may be related to the effects of competition on root isolation plates.

While the root-type itself appeared not to affect the occurrence of species the effect of the soil horizon, in which the root-types were contained, was very marked. Thus species of Mortierella, Mucor (except M. ramannianus) and Absidia were strictly confined to roots of the A1 horizon; Mucor ramannianus was of sparse occurrence in the A1 horizon, but was abundantly isolated from roots of the A2 and B1 horizons. The sterile mycelial forms were of greater abundance on roots from the lower horizons. These patterns of distribution on roots agree very closely with those distributions previously described for the same fungi in the soil.

The distribution of Trichoderma viride, does not agree

Table 31. Numbers of Fungi / 100 fragments each of Five Root Types from the A1 Horizon.

List of Species.	Diameter of Roots (mm).				
	2	1-2	0.5-1	0.5	0.5 *
Absidia orchidis	-	2	1	.	.
Mortierella isabellina.	-	1	.	.	.
M. parvispora	30	52	24	13	10
M. marburgensis	6	11	3	16	2
M. zychae	2	.	.	.	.
Mucor hiemalis	.	1	.	.	.
M. piriformis	.	1	5	.	2
M. ramannianus	.	1	.	1	2
Phoma sp. (C22)	.	.	1	.	.
Botrytis cinerea	.	.	2	1	.
Cladiosporium herbarum	.	.	.	.	2
? Monosporium sp. (F37)	.	.	.	.	2
Penicillium adametzi	.	.	1	.	.
P. lanosum	.	1	1	2	.
P. namyslowskii.	.	.	9	5	.
P. spinulosum	8	2	5	10	20
P. thomii	.	.	1	.	.
P. thomii series (E48)	.	.	.	.	2
P. viridicatum	40	40	25	47	2
Penicillium sp. (E74)	.	2	1	.	.
Penicillium spp.	.	.	3	3	.
Trichoderma viride	74	67	56	53	62
Sterile mycelium, DS85	.	.	.	.	2
Other sterile mycelia	.	.	1	.	4
Total No. Isolates.	160	181	139	151	112

* Unsuberised roots.

Table 32. Numbers of Isolations of Fungi / 100 Fragments Each of Seven Root Types from the A2 Horizon.

	Upper Zone				Lower Zone		
Diameter of Roots (mm) :-	2	1-2	0.5-1	0.5	1-2	0.5-1	0.5
Mortierella parvispora ? (A40)	.	8	.	.	.	.	.
Mucor ramannianus	80	48	29	58	48	76	57
Coniothyrium sp.(C33)	1	9	.	2	.	.	.
Coniothyrium sp.(C36)	.	.	.	.	2	3	3
Coniothyrium sp.(C37)	.	1	.	.	.	.	.
Phoma sp. (C2)	.	3	21	.	.	.	.
Cladiosporium harbarum	.	2	.	.	.	.	.
Gliobotrys sp. (D46)	1	.	.	.	5	5	26
? Monosporium sp. (F37)	2	.	.	.	.	.	.
Penicillium adametzi	1	2	2	1	.	.	.
P. citreo-viride	.	1	1	3	.	.	.
P. namyslowskii	.	1	1	.	.	.	.
P. raistrickii series (E72)	.	1	.	.	.	.	.
P. spinulosum	2	.	.	.	1	.	1
P. terlikowskii	.	.	.	.	11	7	1
P. viridicatum	6	2	.	1	41	12	8
Penicillium sp. (E62)	.	.	.	.	.	1	.
Penicillium sp. (E74)	.	.	.	1	.	.	.
Penicillium spp.	.	.	.	.	.	1	1
Torula sp. (D48)	.	.	.	.	.	3	1
Trichoderma viride	3	19	41	3	85	20	7
Sterile mycelium, DS85	.	1	4	8	.	3	3
Other sterile mycelia	9	.	.	10	.	.	.
Total No. Isolates.	105	98	99	87	193	131	108

Table 33. Numbers of Isolations of Fungi / 100 Fragments to Each of Two Root Types from the B1 Horizon.

Diameter of roots:	0.5	0.5 *
Mucor ramannianus	37	67
Gliobotrys sp. (D46)	2	1
Penicillium lanosum	.	1
Penicillium spp.	.	3
Torula sp. (D48)	9	6
Trichoderma viride	.	2
Sterile mycelium, DS.85	37	13
Other sterile mycelia.	11	12
Total No. Isolations.	96	105

* Unsuberised roots.

with that obtained from soil investigations. This fungus was isolated frequently from roots of A1 and A2 horizons - indeed, from the lower part of the A2 horizon Trichoderma was isolated from 37% of the root fragments. Trichoderma viride was isolated only infrequently from the A2 horizon by the soil plate method, and very rarely by the slide-trap and immersion tube methods.

Species of Penicillium were most common on roots from the A1 horizon, but were recorded not infrequently from the A2 horizon. Over half of the total number of Penicillium

isolates were of P. viridicatum: a species not isolated by other methods. Only four Penicillium isolates were recorded from roots of the B1 horizon. The general distribution of species of this genus agrees with that obtained from soil plate investigations.

Species of the Sphaeropsidales - Coniothyrium spp. and Phoma spp. - were most commonly recorded on roots from the A2 horizon, as also were species of Fungi Imperfecti, other than those already mentioned. Whilst it is quite possible that these species were suppressed on 'root-plates' from the A1 horizon by the vigorously growing species of Trichoderma, Mortierella and Mucor, it remains that they were isolated from roots of the A2 horizon but not from the soil of this zone.

The results presented and discussed in this subsection are subject to considerable criticism owing to the limited nature of the experiments and to localisation of root sampling. All of the experiments were carried out during the months of October, November, December 1953, and January 1954.

Subsection (vi) b. 1. Investigation of the Fungus Flora of Rabbit Dung.

It is known that a highly specialised relationship exists between animals and the numerous fungi which grow on dung (Hawker, 1950) but little attention appears to have been given to the existence of recognised coprophilous fungi as active members of the soil flora.

Although this limited investigation may seem to be somewhat out of place in this thesis, the abundance of rabbit dung over the surface of the Burnt Hill soils led to the view that this dung might provide a food-base for some of the species of fungi isolated from the soil. The investigations of the fungi occurring in rabbit dung, resulting from this consideration, have strongly tended to support this view and their inclusion, therefore, is felt to be fully justified.

(a) Method of Isolation.

During the months of July and August, 1953, more than one hundred fresh pellets of rabbit dung were collected from well distributed areas of the Burnt Hill site.

The pellets were returned to the laboratory in sterilised containers, and, under a sterile hood, were cut into halves with a sterilised scalpel. A very small quantity of dung material, removed from the centre of each pellet, was plated in a manner similar to that used for soil samples in the preparation of soil plates: one plate was prepared from each pellet. Czapek-Dox solution agar was used as the isolating

medium, and all plates were incubated at room temperature.

(b). Results.

The fungi which were isolated from 'dung plates' are recorded in Table 34. The names of species not previously isolated are underlined.

Table 34. Percentage of Frequency of Isolation of Fungi from Rabbit Dung.

Species Isolated.	% age Frequency.
Trichoderma viride Pers. ex Fries.	45.3
Sporormia intermedia Auwers.	20.7
Gelasinospora cerealis Dowd.	18.8
G. retispora Cain.	17.0
Cladosporium herbarum Link ex Fr.	11.3
Coniothyrium sp. (C38)	11.3
Mucor piriformis Fischer	11.3
Pilairia anomala (Ces.) Schroter.	11.3
Penicillium namyslowskii Zal.	11.3
P. spinulosum Thom.	9.4
P. viridicatum Westling.	9.4
Mucor hiemalis Wehmer	5.6
Sordaria humana (Fuckel) Winter	5.6
Botrytis cinerea Pers.	3.8
Mucor saturninus Hagem.	3.8
Sordaria destruens (Shear) Hawker.	3.8
Gliocladium roseum (Link) Bain.	1.9
Mucor ramannianus Moll.	1.9
Mucor mucedo ? (A39)	1.9
Penicillium spp.	-

Of nine-teen determinable species only four were not previously isolated: two of these - Pilairia anomala and Sporormia intermedia - are well known coprophilous fungi,

and the former a highly specialised one. And of the remaining fifteen species, eleven were isolated by the immersion tube or (and) slide-trap method(s), and may be considered to be species capable of active mycelial growth in the soil.

It is of interest to note that the two species of Gelasinospora were among the commonest to be isolated, and it is, perhaps, dung which provides their standard substrate and from which they spread, by mycelial growth, into the neighbouring soil. If indeed the ascospores of these species are unable to germinate unless they have passed through the rabbit's gut, it would explain the fact that only one of these species was (very infrequently) isolated by the soil plate method. Ascospores of the two Gelasinospora species were never observed to germinate on the lids of petri-dishes containing them, whereas some of the spores of both Sordaria species rapidly germinated.

The vigorously growing Trichoderma viride was the most commonly isolated species; it would be of interest to determine whether this fungus colonised the dung from the soil, or was able to submit, unharmed, to the digestive processes within the rabbit's gut.

All species of Mucor which were isolated from the soilwere also isolated from dung, although the relative frequencies of isolation from these two sources, whilst not strictly comparable, were widely divergent. Mucor spp. are among the primary colonisers of dung (Smith, 1946)

Several species of Penicillium were isolated of which four were frequently isolated from the soil and root surfaces.

Thus the fungus flora of rabbit dung was extremely similar to that of the soil: some part of the coprophilous flora appeared to be derived from the soil flora, and it seems possible that the dung itself provided a base for the active spread, into the soil, of certain more or less specialised coprophilous species.

It would be of great interest to determine whether this similarity of the fungal floras of dung and soil is to be found generally. Chesters (1949) has stated that the fungi colonising aerial parts of plants, before their deposition at the soil surface, considerably affect the soil flora and the activities of the soil flora; that some of these aerial saprophytes may become active soil inhabitants is conceivable: some may be introduced into the soil as spores from the aerial mycelium, some as active mycelia on discarded dead plant shoots and some after passage through the gut of an herbivorous animal which has fed on these shoots. Such a close relationship between the aerial saprophytic flora and the soil flora within a plant community would be expected to occur more markedly where non-deciduous plants (as Calluna) rather than deciduous ones were present. Although confirmation of such a relationship would entail detailed investigation of the fungi occurring on the aerial plant systems as well as investigations of the soil flora, this restricted examination

of the dung flora does lend confirmatory evidence to this view.

Further, the investigation of specific habitats, in association with general isolation studies, provides a very useful means of determining, at least in part, the spatial relationships and activities of certain members of the soil flora. This aspect of the investigation is discussed in the final summary.

PART III

General Discussion.

A survey of the results obtained during the investigations of the fungi occurring in Calluna-heathland soils.

General Discussion.

Three recently-described methods were employed in an investigation of the fungi occurring in a very limited area of Calluna-heathland soil. These methods of isolation were augmented by microscopical examination of the soil and its contained fungus flora.

More than ninety spore-producing species of fungi and twenty-nine sterile mycelia were isolated. The percentage composition of the fungus flora of Burnt Hill soils as demonstrated by these three methods is recorded in Table 35.

Table 35. Percentage Compositions of the Fungus Flora of Burnt Hill soils as demonstrated by differing Methods of Isolation.

Method of isolation:	Immersion Tube.	Slide-Trap.	Soil Plate.
Moniliaceae (- Penicillia)	17.2	21.7	12.3
Demetiaceae.	6.9	-	18.4
Sphaeropsidales.	3.4	13.0	4.6
Ascomycetes.	13.8	-	4.6
Mortierella spp.	24.1	34.7	7.7
Mucor spp.	20.7	13.0	1.5
Penicillium spp.	3.4	4.3	23.0
Sterile mycelia.	10.3	13.0	27.7

The dominant position occupied by certain species and groups of fungi varied according to the method employed. While both of the methods which isolate active growing mycelia indicated the flora to be dominated by species of Mortierella,

the Penicillia were the most common species to be isolated by the soil plate method. The latter (Penicillium) species were isolated by the 'active methods' (immersion tube and slide-trap) very rarely and only when competition from faster growing species was absent. Microscopical examinations revealed that Mortierella species were the commonest fungi to grow over the surfaces of submerged slides, and that Penicillia developed locally and in particular where a specific nutrient substrate was available. The high sporing capacity of the Penicillia was probably responsible for their frequency of occurrence on soil plates - even when they were only locally active in the soil: Chesters (1948) has suggested that vigorous sporulation in the soil causes early cessation of vegetative growth.

It is a surprising feature of these investigations that certain fungi, shown to exist in the soil as active mycelia and frequently isolated by the 'active methods' (i.e. species of Mortierella and Mucor), were very infrequently isolated by the soil plate method. It is possible that a fungus growing in the soil but not sporulating would fail to develop on soil plates when only small fragments of its mycelium were plated - these torn and isolated fragments being incapable of regeneration. Such an explanation may account for the rarity of Mucor spp. on soil plates, but the microscopical investigations have shown that Mortierella spp., in fact, did sporulate vigorously on the slide surface whilst it was immersed in the soil. It is possible that many of these

spores were non-viable or were incapable of competing on soil plates, but it must be borne in mind that these fungi may have grown better on a continuous solid surface (as that provided by the immersion tube, slide-trap and soil-slide respectively) than in between soil particles (Russell, 1950). Hence they would have been isolated frequently by the active methods, and observed frequently on Rossi-Cholodney slides, whilst in fact their development in soil did not justify the importance accorded them.

The growth rate and ability of a fungus to colonise agar media appeared to be most important factors in controlling the fungi isolated by all methods. Slowly growing fungi were very seldom isolated by the 'active methods' and, although isolated not infrequently by the soil plate method, they only developed in the absence of faster growing species. The results indicated that the modified slide trap was capable of isolating a greater range, including more slowly growing species, than the immersion tube method, but this range was clearly very limited.

The soil plate method, while permitting the isolation of a very great range of species, allowed no distinction to be made between active mycelia and inactive stages. It was suggested, however, that the ecological concept of constancy possibly provided a basis for such a distinction - the constantly occurring species being those most likely to exist in the soil as active mycelia. This theoretical consideration was well supported by the use of complimentary methods.

Of the species listed as being of high constancy, and therefore probably active, thirteen were isolated by 'active' methods, and only seven were not: these seven were all very slowly growing species and one of these, Trichobotrys sp. (D27), was observed to be growing in the soil by the Rossi-Cholodney slide method.

The dark-coloured hyphymycetes relatively commonly isolated by the soil-plate method were never isolated by the 'active' methods. All grew slowly on agar media and this factor alone would account for these results, but from the soil slide observations it was apparent that many dark-coloured hyphomycetes were confined, in their growth, to the soil humus fragments: such a restriction to their immediate medium may have largely controlled their isolation (Chesters 1948).

From the results of observation and isolation it appeared that the darker-coloured hyphymycetes while poorly represented in isolations were of great abundance in the soil. Burges (1939) in classifying the true soil fungi into "sugar fungi" and "humus fungi", remarked that most of the genera and species usually listed as true soil fungi (cf. Waksman, 1927) were in fact "sugar fungi" - mostly rapidly growing forms. And Chesters (1949) suggested that investigations of the space relationship of mycelium in the soil, with reference to organic debris, might lead to a concept of local and wide colonisers, and -- "discover to us that the inhabitant flora consists of many more members which are constant in the soil, but which,

heretofore, have not been able to compete at isolation with the wide colonisers such as species of Mucor and their allies". The groups of fungi to which these investigators refer are somewhat similar (though by no means identical), the majority of wide colonisers being "sugar fungi" and many "humus fungi" being local colonisers. The concept of local and wide colonisers is one of great value in fungal ecological studies. The methods of isolation employed in this investigation - and indeed all methods so far described -- readily permit the isolation of wide colonisers, while the local colonisers are either completely unrepresented or represented only in the absence of the former. From the microscopical examination of the soil microflora it was clear that the majority of dark-coloured mycelial forms were dissimilar to any of the species of fungi isolated, and that this important and numerous group of humus-inhabiting fungi were largely unrepresented among the isolates. It is a paradoxical situation that these local colonisers - many of which were humus-inhabiting forms and perhaps were more truly soil fungi than the widely spreading and wide-spread sugar fungi - were so poorly represented.

The presence on Rossi-Cholodny slides of localised masses of spores of types not produced (in culture) by any of the fungi isolated demonstrated further that many species of fungi, present and active in the soil, were not represented among the isolates.

Apart from that yielded by the soil slide method very

little information was obtained concerning the substrate relationships and growth in the soil of the fungi isolated. The soil slide method indicated that Mortierella spp. and certain unidentifiable hyaline, septate mycelia were capable of rapid and extensive growth in the soil, particularly in soil spaces (and perhaps, more especially, over smooth surfaces). And the soil plate method indicated that certain species were present, either as spores or mycelia, in the soil itself - as opposed to their occurrence on living plant root surfaces. The investigations of fungi occurring in the various horizons demonstrated that certain species and genera of fungi were more or less restricted to definite soil horizons.

In order to augment this information the fungi occurring in two well defined habitats were investigated: the surfaces of Calluna roots and rabbit dung. The results obtained from these investigations, in combination with those of the soil isolation methods, were of considerable interest. A complete analysis of the fungi isolated by all methods is presented in Table 36, where the numbers of species of the most prominent groups and genera of fungi, isolated by the various methods and combinations of methods are displayed.

Of nine Mortierella species all but one were isolated by the 'active' methods (most of these by both the immersion tube and slide trap methods). The one remaining species, Mortierella humilis, although isolated only by the soil plate method, was observed to grow vigorously over immersed slides. Thus it is concluded that all Mortierella spp. were active in

Table 36. Numbers of Species from the dominant Groups and Genera of Fungi isolated by various Methods and combinations of Methods, from Burnt Hill Soils.

Method of Isolation.	Abbrev.																
Immersion tube and/or slide trap.	A.M.	AM	AM	AM	AM	AM	AM	AM	AM	.	.	.	.	.	.	.
Soil Plate.	S.P.	.	.	SP	SP	SP	SP	.	.	.	.	SP	SP	SP	SP	.
Root plate.	R	.	.	.	.	R	R	R	R	R	R	R	R	.	.	.
Dung plate.	D	.	D	.	D	D	.	.	D	.	D	D	.	.	D	D
	Total No. Species.															
Mortierella spp.	9	3	.	.	.	.	4	1	.	.	.	.	.	1	.	.
Mucor spp.	6	1	2	.	.	1	.	.	2	.	.	.	.	.	.	.
Spp. of Ascomycetes	7	.	3	.	1	.	.	.	.	.	.	.	.	2	.	1
Spp. of Sphaeropsidales.	9	1	.	1	.	.	1	.	.	4	.	.	.	1	.	1
Penicillium spp.	20	.	.	.	.	1	.	.	.	4	1	1	5	8	.	.
Spp. of Moniliaceae (- Penicillia)	11	1	.	3	.	1	1	.	1	.	.	.	.	3	.	1
Spp. of Demetiaceae.	16	2	.	.	.	.	.	.	.	2	.	1	.	11	.	.
Sterile mycelia, 'DS' forms.	26	.	.	.	.	.	.	.	.	8	.	.	1	17	.	.
Total		8	5	4	1	3	6	1	3	18	1	2	6	43	0	3

the soil and, from their frequent occurrence on roots, that these species grew most vigorously over smooth continuous surfaces in the soil. Such a growth habit would explain the infrequent isolation of Mortierella spp. by the soil plate method and also the frequency of their isolation by immersion tubes and slides traps. It was suggested above that the immersion of solid objects into the soil might act directly (in providing a smooth continuous surface for growth) or indirectly (by changing soil conditions) and thus bring about more luxuriant and rapid growth and consequently increased isolation of these species. Several other Mortierella spp. were observed on soil slides but were not isolated. No Mortierella species was isolated from dung.

In contrast to the Mortierella, species of Mucor were all isolated from dung (except one which was isolated only once by the immersion tube method). Also all were isolated by active methods but only one species, M. ramannianus, was isolated by the soil plate method. While it is uncertain, and probably doubtful, that these Mucor spp. are true coprophilous fungi - requiring subjection to animal digestive secretions for completion of their life cycle - these fungi are known to be among the primary colonisers of dung (Smith, 1946), and it is possible that the growth of these species in the surface soils was stimulated by the presence of dung: Mucor hiemalis and M. saturninus were among the few species for which localised activity was demonstrated by the immersion tube 'nesting' design. The reason for their lack of isolation by

the plating method is possibly a reflection of the vegetative spread of these fungi without sporulation in the soil. Unlike species of Mortierella they appeared not to be stimulated by immersed slides and it is perhaps inherent in the soil plating procedure that the factor determining their lack of isolation is to be found. The preparation of soil plates involved the use of very small quantities of soil and it is possible that small, torn, mycelial fragments of these species, when plated, were incapable of regeneration. Warcup (1951) observed that, on soil plates, "most fungal colonies were found to develop from humus particles (sic) or spores rather than from pieces of mycelium".

In their occurrence the Ascomycete species were somewhat similar to the Mucors. Five of the seven species isolated occurred on dung: two, isolated only by the soil plate method, were species of Thielavia, and one species - Sporormia intermedia - was isolated only from dung although it was isolated by the soil plate method from another heathland soil, Newborough Warren. Pyrenomycetous fungi have frequently been recorded as true coprophilous fungi (Hawker, 1950) and it is suggested that the occurrence of these Ascomycete species in surface soils was perhaps directly related to the presence of dung, which provided a food base and from which their mycelia spread into the soil. So far as can be determined the two species of Gelasinospora, which were the commonest Ascomycetes to be isolated from a variety of heathland soils, have not previously been recorded as soil fungi.

More than half of the total number of species of Sphaeropsidales were isolated from root surfaces - four of these (five) species being isolated from no source other than root plates. Three species were isolated by 'active' methods. Thus it appears that fungi of this group were largely confined to their immediate habitat and that their mycelial development in the soil was limited.

No dark-coloured (DS) sterile mycelia and very few of the demetiaceous fungi were isolated by the 'active' methods - all being isolated either from soil or root plates, while in contrast most of the hyaline hyphomycetes (the moniliaceous fungi and the sterile mycelia, C18, C21 and C34) were isolated by either the immersion tube or slide-trap methods, or both. Thus the results of observation and isolation were, in this case, in close agreement, for in soil slide preparations hyaline mycelia were observed to grow vigorously over the slide surfaces, and the darker-coloured hyphomycetes to be slowly growing and largely restricted to their immediate medium.

It is a striking feature of the results (see Table 36) that such a large number of species were isolated by the soil plate method alone; this is probably largely due to the great number of soil plates which were employed, and to the fact that many more soil levels were sampled by this method - levels where competition between fungi was limited - thus facilitating the development and isolation of many more slowly growing species. Almost certainly this number would have been

reduced had further specialised habitats been investigated: in particular the various components of the soil organic matter (see Chesters 1948). The combination of general soil isolation methods, although discriminating but little between the various microhabitats, and of specific habitat investigations, combined with microscopical observation, clearly provides a means of obtaining more detailed information of the relationships between soil organisms than has hitherto been possible.

In all isolation methods competition between species was a factor which largely determined the results - emphasising the importance of certain wide colonising species, while minimising, or even neglecting, the local colonisers. This factor dominated others which may have been inherent in the methods: thus, for example, the immersion of tubes or slides into the soil brings about changed soil conditions - which may not naturally occur - and enhances the development of certain species. It is possible that these changed conditions may even initiate such development (see Dobbs & Hinson, 1953). At all times the results of isolation (and observation) studies needed to be examined critically and with utmost caution. The use of complementary methods was of great help in indicating the major selective factors inherent in each of the methods employed.

The results from all methods of isolation agreed in demonstrating that the number of species of fungi decreased rapidly with increase in soil depth. The degree of this rate of

decrease was, however, variable. While all showed the A1 horizon to be rich in species and the A2 to be poorer, the 'active' methods demonstrated a marked reduction in the abundance of mycelia below the first inch of soil - a distribution pattern not demonstrated by the soil plate method. It appeared that, in fact, the distribution patterns demonstrated by the plating method were due to the occurrence of spores rather than mycelia, and that the results from plating methods were further subject to error where the spores were those of rapidly growing species. While many hyaline mycelia were observed to develop on soil slides immersed in the uppermost inch of soil, very few were observed beneath this level. Even within the humus-rich A1 horizon mycelial activity was observed to be confined largely to the uppermost inch of soil and below a depth of 6 in. the soil was mostly barren of hyaline mycelia. Timonin (1935) has shown that the numbers of micro-organisms in peat decrease with depth despite a high humus content.

The influence of the competition between species in modifying results, not only of the species present, but also of the vertical and lateral distribution, was considerable. Thus Mucor ramannianus was isolated by the plating method from lower levels of the A1 horizon in association with a large number of the more slowly growing species - yet this fungus was observed to be almost the only mycelial form present by microscopical examination. Many similar examples were found which indicated that the range over which a fungus was

isolated from the soil not only did not completely coincide with its activity, but was quite divorced from it. A most marked example of such distribution patterns was demonstrated by Trichobotrys sp. (D27); this fungus was isolated with great constancy from the A2 horizon, but only on two occasions from the A1 whilst it was observed to be active only in the A1 horizon. Again an "affected" distribution pattern was observed for the very large group of dark-coloured, slowly growing, sterile mycelial (DS) fungi, where soil plate isolations indicated in increase of occurrence with depth which was the reverse of the distribution observed microscopically. It is clear from these results that the competition factor determining the occurrence of species on soil plates is quite different from that determining their growth and activity in the soil. While the soil provides a multiplicity of habitats - the agar medium provides only one and only those fungi most suited to it will develop and be isolated. The fungi which do develop are not necessarily dominant or even active in the soil sampled.

All results obtained by the plating method concerning the vertical distribution of species require to be interpreted with caution, particularly perhaps in the soils of the A2 horizons - for it was in soils of this type (more than 90% sand) that Burges (1950) demonstrated a rapid downward movement of spores. It must be pointed out, however, that both Mucor ramannianus and ? Monosporium sp. (F37), which, by the plating method, were shown to predominate the A2 horizon soils, were both isolated

by 'active' methods from this horizon. But the large numbers of colonies occurring with such perfect constancy on plates prepared from A2 horizon soils was clearly mainly due to the high sporing capcity of these fungi rather than their vigorous mycelial activity. (cf. H.L. Jensen, 1931). Fortunately the use of several methods did not always produce such conflicting evidence and many of the commonest species of fungi (including Mortierella spp., Trichoderma viride, Absidia orchidis and Ascomycete species) were isolated largely from the uppermost inch of the A1 horizon by all methods.

Investigations of the fungus flora of the illuviated horizons and parent subsoils were restricted mainly to the plating method, by which Mucor ramannianus was frequently isolated from the B1 horizon and more rarely from the B2 and C horizons. Many sterile mycelia were also isolated from these levels. In one experiment immersion tubes were buried in the B1 horizon soil but after three weeks they remained uninfected. Rossi's (1935, 1936) soil crushing method* was also employed in a microscopical examination of these soils, but apart from very few dark mycelial fragments no other mycelial development was observed. Because of the almost sterile nature of these soils - in particular the B2 and C horizons, they were tested for the possible presence of toxic substances* by the method

* These two investigations were not described in the experimental section.

described by Rayner & Neilson Jones (1944): the growth of none of the test fungi (Trichoderma viride, Mucor ramannianus, Beauveria bassiana, Trichobotrys sp. (D27) and Penicillium spp.) was inhibited or reduced by the presence of B or C horizon soils within the agar film. In view of the fact that Calluna roots very rarely entered the B2 horizon, it is considered that the mechanical composition and very firmly concreted nature of the B2 horizon soils prevented their penetration by roots or fungi (either as active mycelia or spores).

The distribution of fungi on root surfaces in the A1 and more superficial levels of the A2 horizons was in close agreement with their distribution in the soil. Absidia orchidis, Mortierella spp. and Mucor spp. (other than M. ramannianus) were confined to the roots from the uppermost soil levels, and Mucor ramannianus was very abundant on roots in the A2 and B1 horizons.

The distribution of fungi on root surfaces could be more exactly related to the occurrence of the roots in the soil horizons than to the root type, although the results indicated that Trichoderma viride was more abundant on root surfaces than it was as a free living soil fungus in the A2 horizon. It is perhaps largely by their mycelial growth over root surfaces that certain fungi enter the deeper - in this case 'arid' regions - of the soil.

The soil plate and immersion tube methods were employed

each month for ten and sixteen months respectively in investigations of the fungus flora of Burnt Hill soils. The results obtained from the soil plate study supplied no evidence of seasonal fluctuation in the occurrence of soil fungi. Very pronounced seasonal variation was demonstrated by the immersion tube isolations.

With the exception of Meredith's (1940) investigation of the seasonal occurrence of soil Pythia, earlier workers have related only variation in numbers of fungi to the seasons. The results of these investigations - largely based on the evidence provided by bacteriological methods of isolation - have been conflicting, and while 'numbers of fungi' may be related to the periodic sporulation of species it does not necessarily follow that sporulation is exclusive of active mycelial growth (Garrett, 1952). The dilution plate method, permitting no distinction between the active and dormant phases of the fungal cycle in the soil, is not suitable for the exhibition of seasonal variation in the growth of soil fungi (Singh, 1937). Similar criticisms can be made of the soil plate method, and Warcup (1951) found that the variation in fungal populations from samples collected a few yards apart on the same date was as great as that recorded from samples taken over a period of time. Such criticisms do not apply to the immersion tube method which isolates only those fungi existing in the soil in an active mycelial phase (Chesters, 1949).

The results obtained by the immersion tube method

demonstrated a seasonal variation in the isolation of nearly all species. Trichoderma viride completely dominated the isolates during the summer months and its occurrence was very closely related to the prevailing temperature: during the colder winter months it was not isolated. This seasonal variation of Trichoderma was noticed even in the limited slide-trap experiments.

In contrast to the occurrence of Trichoderma, species of Mortierella and Mucor were isolated most abundantly during the colder months. It is suggested, however, that the seasonal variation of these genera was but a reflection of the activity of Trichoderma, which prevented their isolation during the periods of its most vigorous growth. Species of Mortierella were observed to grow extensively over buried slides during periods of the year when these fungi were not isolated by the immersion tube method.

In view of the seasonal variation demonstrated for species of Pythium by Meredith (1940), and of Trichoderma in this present investigation, it seems most probable that many other soil fungi may be affected in a similar manner. Such seasonal variations, however, can only be shown when methods suitable for their demonstration are designed. As succession and zonation are established concepts in both higher plant and fungal ecology, it is easily conceivable that seasonal societies of soil fungi will eventually be distinguished, just as they are in higher plants.

Since the study of the investigation of the fungi in Burnt Hill soils by the plating method demonstrated that seasonal variation did not affect the results, it was permissible to compare the fungus floras, investigated by the soil plate method at various times during two years, of several heathlands in England and Wales. (See Warcup 1951). But in the light of the Burnt Hill investigations it is apparent that many results of these soil plate studies are susceptible to considerable criticism: the method has been shown to favour the isolation of fungi which possess a large sporing capacity, and such species were shown to dominate the floras of the majority of heathland soils; also it was demonstrated that competition between species on soil plates was an important factor in determining which species were isolated. At the same time it must be admitted that, while perhaps according undue importance to certain groups of fungi, the soil plate permitted the isolation of a wider range of species than any other method. From Burnt Hill soils more than two thirds of the total number of species isolated by five methods were represented in the soil plate isolations.

The vertical distributions of fungi in the various heathland soils need to be examined with care, for such distributions have been shown to be dependent, in part at least, on the competition factor on soil plates. And the occurrence of those species whose lateral distributions were shown to be related to their distribution in depth again require to be examined with care.

Throughout the soil plate investigations, however, it was apparent that the fungus floras of widely separated heathland soils showed a fairly high degree of constancy, and since it has been demonstrated, in soil plate investigations, that constancy of occurrence was closely related to active mycelial development, it must be concluded that the active fungus floras of these Calluna-heathland soils were, in part, similar. It is these fungi, of high constancy, which charecterise the habitat and conversely are determined by the habitat.

Warcup (1951) has demonstrated that two distinct populations of soil fungi exist in acid and alkaline grassland soils respectively, and that between the two extreme types of habitat there was a marked graduation of species. While such a distinction was possible by the use of a single 'mass isolation' method, certainly finer distinctions could be obtained by the replicated and methodical use of a variety of methods, as recommended by Chesters (1948). Probably only by such detailed studies can the various fungal communities, occurring in differing soil types and in association with differing higher plant communities, be recognised. And only when such fungal societies are recognised can a fuller understanding of the inter-relationships existing in the soil complex be obtained.

As Watt (1947) concluded his presidential address to the Ecological Society by quoting T.S. Eliot's words concerning Shakespeare's works, so this present thesis may also be concluded: "We must know all of it in order to know any of it".

PART III

SUMMARY.

SUMMARY.

1. The fungi occurring in thirteen Calluna-heathland soils distributed over England and Wales were examined by the soil plate method: one limited area of heathland was investigated in greater detail by several recently described methods.

2. Many of the species of fungi isolated varied according to the method of isolation; species with a large sporing capacity were isolated abundantly by the plating method, and Phycomycete species were isolated most abundantly by the immersion tube and slide-trap methods. Throughout the investigation no Oomycete species were isolated.

3. Microscopical examination by the Rossi-Cholodny slide technique revealed the presence of many species of fungi which were not isolated, these species were mainly humus-inhabiting, darker-coloured hyphomycetes.

4. Many fungi isolated by the plating method were of constant occurrence in soils beneath Calluna vegetation. The occurrence of other species could, in some cases, be related to variations in the habitat, although - as a result of competition on soil plates - the distribution in area of some species was considered to be related to their distribution in depth.

5. The numbers of species decreased with depth - the majority of species were most common in the surface, humus-rich horizons. The presence of organic material was not the

sole factor determining vertical distribution. A few species were isolated most frequently, or only, from the leached soil horizons.

6. The vertical distribution of fungi observed in the soil by the soil slide method agreed generally with that demonstrated by isolation methods, but showed that the majority of active mycelia were confined to the uppermost inch of soil.

7. Evidence suggested that the illuviated horizons limited the vertical distribution of many spore-producing species; deeper soil levels were often found to be colonised solely by a miscellany of sterile mycelial forms.

8. The species of fungi occurring on root surfaces were mostly similar to those in the soil. Some species appeared to penetrate more deeply into the soil on root surfaces than as freely growing soil organisms.

9. The majority of species isolated from rabbit dung were also isolated from the soil as actively growing mycelia. It was suggested that, in some cases, the dung provided a food-base from which mycelia spread into the soil.

10. Marked seasonal variation in the activities of several species of fungi was demonstrated by the immersion tube method, but it was considered that this apparent variation was partly due to the real variation of one vigorously growing species - Trichoderma viride. The activity of Trichoderma in the soil was shown to be very closely related to the prevailing temperature. No seasonal variation was demonstrated by the soil plate method.

11. A modification of La Touche's (1949) slide-trap method of isolation was described: the results obtained by this method agreed closely with those obtained by the immersion tube method. The modified slide-trap was considered to be a simple and useful method for the isolation of actively growing mycelia from the soil.

12. The simultaneous use of several methods of studying the soil fungus flora supplied a means of ecological investigation not (at present) possible by any single method. The results obtained by single isolation methods, when considered alone, provided an incomplete picture of the fungus flora which was often misleading. Each method was shown to be selective of certain groups and species of fungi, and only by comparison could each be adequately criticised and adjudged.

13. From the investigations it was demonstrated that "mass isolation" studies, in combination with investigations of individual habitats, provided a rewarding method of approach to some of the problems of soil fungal ecology.

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