

An ecological survey of the soil
mycoflora of two forest communities
in Zululand.

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by

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CHAPTER 1

INTRODUCTION:

Although very little research has been done in South Africa on soil fungi, particularly soil microfungi, botanical observations of South African fungi date from the eighteenth century. A very extensive review of the early history of Mycology of this country is given by Dr. Ethel Doidge (1950).

The first record of any fungi collected in South Africa is that published in 1772 by Dr. Carl Pehr Thunberg. Although flowering plants were his main interest, he also collected a number of cryptogams, which included fourteen fungi. Amongst the fungi he collected were Agaricus muscarius, A. campestris, Boletus sanguineus, Clavaria capensis and Lycoperdon carcinomale. A considerable time elapsed before any further collections of South African fungi were made. In 1810 W.J. Burchell landed in Cape Town with the intention of undertaking an extensive collecting expedition into the interior of the country. His travels began in June, 1811. Altogether he covered approximately 4,500 miles and went as far North as Bechuanaland. He returned to the Cape in 1815. It is believed that during these travels Burchell collected at least 56 species of fungi. The specimens have, unfortunately, not been traced, although they are listed in his Catalogus Geographicus in the library of the Kew Herbarium.

In 1829, C.F. Ecklon and C. Zeyher formed a botanical partnership. They undertook many expeditions, but

these produced little of particular interest to the study of fungi. . Ecklon probably collected some fungi but they are difficult to trace. Later Zeyher became interested in the fungi of the district of Uitenhage. A systematic account of Zeyher's fungi was published in 1843. He listed 31 species.

J.F. Drège arrived at the Cape in 1826. He travelled extensively in the Cape Colony. He also accompanied an expedition into Natal and went as far as the Umgeni river. In his geographical analysis of South African flora, Drège mentions 103 collections of 64 species of fungi. Of these 66 specimens were collected between Port Elizabeth and Durban. This was probably the first record of fungi from Natal. During the years 1939 - 1940 a Swedish naturalist and hunter, J.A. Wahlberg, collected fungi in Natal and parts of the Cape. His specimens were examined by Elias Fries, who published an account of them in his 'Fungi Natalenses' in 1848. Of the forty species described, nearly all were Hymenomycetes.

During the nineteenth century several scientific expeditions were sent from Europe and America. A number of these expeditions made the Cape a port of call. During these short visits, many investigators undertook excursions into the interior. Expeditions which touched at the Cape during 1855 - 1875 made a definite contribution to the knowledge of cryptogamic plants. Names worth mentioning in this respect are: Charles Darwin (1833); Dr. Heinrich Wawra (1857, 1868, and 1872); Antony Tellingk (1857 - 1858); C.S. Wright (1883) and the Rev. A.E. Eaton (1874).

In the later years of the century, several persons with botanical interests became resident in South Africa and

continued with collections and studies of the plants of the areas in which they lived. Of these, John Medley Wood is one of the most important. Medley Wood arrived in Durban in 1852 and, after trading in Zululand, he took up stock farming at Inanda in Natal. It was in this neighbourhood that most of his fungi were collected. In 1879 a list of fungi which he collected was published in Grivillea by Cooke. In 1882 the latter author described 49 species from a total of 200 fungi collected by M. Wood. In 1899 P. Sydow published a list of 41 fungi from Natal, of which eight were described as new species.

At the beginning of the present century, a new chapter in the history of South African Mycology was begun. Illtyd Buller Pole-Evans was appointed to the post of mycologist in the Transvaal Department of Agriculture in 1905. During his first year in South Africa, Pole-Evans collected 200 specimens of fungi on native plants, which formed the nucleus of the mycological herbarium in Pretoria. In 1910 a separate Division of Plant Pathology and Mycology was formed for the investigation and control of plant diseases throughout the four provinces of the Union of South Africa. In 1913 Miss A.M. Bottomly joined the staff and took charge of the herbarium. During the twelve months after her appointment, 2,651 specimens were registered and by 1918 there were 11,369 fungi in the collection. At the end of 1945 the Cryptogamic Herbarium at Pretoria housed over 61,200 specimens, of which approximately 35,500 were fungi.

As mentioned at the beginning, very little research on South African soil fungi has been undertaken up to the present and I am not aware of any important publication on

the mycology of local soils. Cohen (1950), published a short paper on the influence of different burning and grazing treatments on soil fungi in the Transvaal. There are definitely no records available of the soil mycoflora of Zululand.

Doidge (1950), states ".....only a few specimens have come from the more tropical coast of Zululand and Mozambique, which would well repay exploration."

Recent work on soil mycology in the Department of Botany of the University of Potchefstroom has revealed that investigations of this nature could be very rewarding (Du Toit, 1967; Marasas et al. 1966; Papendorf, 1967 a and b; Papendorf and du Toit, 1967; Papendorf and von Arx, 1966). Scores of fungi, previously unrecorded in South Africa have been isolated. Of even greater significance is the fact that a considerable number proved to represent new genera and species. This shows that such investigations can add appreciably to our knowledge of the South African fungus flora.

The literature relating to soil micro-fungi is very extensive, and deals largely with the composition of the soil mycoflora; the ecological factors influencing the soil micro-population, including growth relations between micro-organisms and higher plants, and to that relating to biochemical breakdown of plant remains in natural soils and in artificial culture. Ever since the question of Waksman (1917): "Is there any fungous flora of the soil?" was answered in the affirmative, work has been directed towards answering fundamental questions concerning the composition of the fungous flora, the activity of particular members and the activity of the population as a whole.

The first account of soil microfungi was published by Adametz in 1886. Adametz made an investigation of the microfungi that developed on sterile culture media which had been inoculated with soil. He used soil samples collected from surface and sub-surface layers in sandy and loamy fields and isolated eleven species of fungi including Mucor, Hyphomycetes, and four yeast species. The first deliberate attempt to investigate the fungus population of the soil was the work undertaken by Oudemans and Koning (1902). They inoculated plates of wort agar or wort gelatin with aqueous suspensions obtained by pulverizing fragments of vegetable matter extracted from soil in a given volume of water, in a sterile crucible, and by diluting a portion of this suspension in sterile water. From the fungous cultures which developed on these plates they identified about forty species. Hagem (1908) investigated the Mucorales in Norwegian soils. He was the first person to use particles of soil to inoculate sterile media in plates. He also developed the method of subculturing. Jensen (1912), investigated soil fungi in the United States and reviewed all previous records of soil fungi. He presented a summary of all the available information concerning soil fungi up to 1912.

In 1917 Conn questioned whether or not fungi actually lived and grew in the soil, and suggested that isolations from soil might only reflect the presence of dormant spores which had settled on the soil as dust from the air, or had been washed down by rain. This was the first and most fundamental problem of soil mycology. Waksman (1916), had already demonstrated that by using particles of soil as inocula on agar plates fungous colonies developed on the plates within twenty-four hours after incubation at 30°.

Waksman further compared the rate of growth from inocula of hyphae and from inocula of spores of the same species, and demonstrated that the former produced the larger colonies in an initial limited period. From this observation, Waksman concluded that the earliest visible growth came from mycelium already present in the soil. The presence of mycelium in soils was proved beyond doubt by Conn (1922). He found that fungal mycelium was quite common on smears of soil dried and stained on a microscope slide. He confirmed his conclusions by examining samples obtained from a large variety of soil types. After thus establishing that living mycelia are present in soils and that at least some of the fungi isolated from soil occur there in an active state, the study of the fungous components of the soil developed rapidly. Various authors have given extensive reviews of the soil-inhabiting fungi (Waksman, 1916, 1917, 1944; Jensen, 1931; Chesters, 1949).

To facilitate the study of soil fungi, ecological grouping of the soil fungus flora has been attempted by various authors. Garrett (1956), classified soil fungi into two ecological groups, namely 1) soil-inhabiting fungi, including obligate soil saprophytes and unspecialized parasites and 2) root-inhabiting fungi, including specialized parasites and mycorrhizal fungi. Garrett (1950), gave the following definitions for the two groups of his classification. Soil inhabitants are organisms ".....characterized by ability to survive indefinitely as soil saprophytes" while "root-inhabiting fungi are characterized by an expanding parasite phase on the living host plant, and by a declining saprophytic phase after its death."

In his book "Micro-organisms in the soil", Burges (1958), gives the following provisional grouping of the organisms found in soil:

- (a) Obligate plant or animal parasites.
- (b) Parasites which are also soil invaders or soil inhabitants, and invaders of moribund tissue.
- (c) True soil organisms:
 - (i) Zymogenous organisms which carry out the initial stages of the decomposition of freshly added organic matter and
 - (ii) Autochthonous organisms which form a more stable group not noticeably affected by recent additions of organic matter.

Park (1957), suggested that so far as fungi are concerned a further group should be added to the zymogenous and autochthonous groups. This group, which he calls the exochthonous forms, is made up of soil invaders which are ill-adapted to continued vegetative activity in the soil substratum.

A more recent approach to the classification and ecology of soil fungi is through a survey of their substrate relationships. The substrate is the most important part of the soil habitat for any group of heterotrophic micro-organisms, including fungi. Competition between micro-organisms is first and foremost for substrates, just as competition between green plants is first and foremost for light (Garrett, 1963). Garrett (1951), proposed an ecological classification of the soil fungi based on the principal food

supply they utilize in the highly competitive environment of the soil. The true saprophytes range from the "sugar" fungi - which can only utilize relatively simple and easily decomposable organic matter, but not cellulose or lignin - through the cellulose to the lignin decomposers. According to Garrett (1956), there are two other important groups in the soil: the specialized root-inhabiting fungi, which include the mycorrhizal, the specialized and the unspecialized root-disease fungi, and the fungi which are predaceous either on other fungi or on other members of the soil population, including the soil fauna.

The sugar fungi are typically Phycomycetes, and since there is a great variety of different organisms in the soil which can use the same organic compounds for food, they are adapted to exploit these food supplies ahead of their competitors by a remarkably high growth rate, together with an equally rapid spore germination upon contact with a suitable substrate. Their primary source of food is injured, moribund or recently dead plant tissues.

The cellulose-decomposing fungi, which mainly belong to the Ascomycetes, Fungi Imperfecti and Basidiomycetes, come intermediately between the sugar fungi and the lignin fungi in their rate of growth.

The lignin fungi are species of the higher Basidiomycetes and are characterized by very slow growth rates. As there is little competition for their food supplies, slow growth is not an ecological disadvantage to these fungi. These fungi decompose lignins more easily if there is a reasonably high cellulose content associated with the lignins which is not too readily accessible to other micro-organisms.

According to Atkinson and Robinson (1955), additional nutritional factors which are of importance in the distribution of fungi in soils might form the basis for a more detailed ecological classification of soil fungi. A classification based on the nutritional factors of the soil would require much more detailed and specialized knowledge of the nutritional requirements and metabolic activities of the soil fungi. It is hoped that this knowledge will be gained as methods and techniques of observation become more refined.

Many aspects of soil-inhabiting fungi have been studied and a vast literature has accumulated. Quantitative studies on soil fungi have been carried out on cultivated soils (Chaud, 1937; Jensen, 1931 a and b, 1934; Singh, 1937), on forest soils (Ellis, 1940; Paine, 1927; Pistor, 1930), on soils with high salinity (Killian and Feher, 1935) and on soils with high organic matter content (Verona, 1934). The fungus flora of cultivated soils was compared with those of natural soils by Bisby, James and Timonin (1933), while the influence of different seasons on the soil mycoflora was investigated by Dixon (1928), and Ma (1933). Fungi have also been isolated from different horizons of a particular soil (Bisby, Timonin and James, 1935; Le Clerq and Smith, 1928; Todd, 1932). Part of this literature has a direct bearing on particular aspects of the present investigation and will therefore be referred to in the relevant chapters.

Ling-Young (1930), was the first to emphasize the importance of studies of the fungal flora associated with natural plant communities. Warcup's (1951), investigations in which he employed the soil-plate method, produced the first convincing evidence of the value of synecological studies in

such habitats. Most of the earlier work dealt with agricultural soils and, while yielding information of particular importance, has added little to our understanding of the general ecology of saprophytic soil fungi. Warcup was able to demonstrate fairly marked differences between fungal populations in five natural grassland soils. Investigations of heathlands have revealed a marked similarity between the fungus floras of acid podsoils in various localities (Jeffreys, Brian, Hemming and Lowe, 1953; Sewell, 1954; Mc Lennan and Ducker, 1954). A successful attempt at a detailed synthesis of the ecology of soil fungi and higher plants has also been made on a forest cortinum by Tresner, Backus and Curtis (1954). Investigations of this nature could provide a foundation for the micro-habitat studies advocated by Chesters (1949), and Garrett (1955).

Investigations of the micro-habitat like that associated with plant roots or with fragments of plant debris are an important aspect in the study of the ecology of soil fungi. The former micro-habitat has received much attention, but despite statements on the importance of the latter (Chesters, 1949, 1960; Garrett, 1955, 1957) very few reports on soil ecology make any mention of this habitat. Hudson and Webster (1958), investigated the succession of fungi bringing about the decomposition of couch grass, while Pugh (1958), noted the changes in the fungous flora of the decomposing leaves of Carex paniculata L. Pugh did not, however, study the fungi of the underlying mineral soil in order to determine the influence of the litter on the soil mycoflora. In an excellent publication, Witkamp (1960), describes the micro- and macrofungi on and in the litter of an oak forest at Zutphen in the Netherlands. He also includes a study of the underlying

mineral soil and was able to prove that the litter exerted a direct influence on the composition and activity of the mycoflora.

AIM:

The aim of the present investigation has been threefold: firstly, to obtain more factual information on the populations of soil microfungi of the subtropical coastal area of Zululand; secondly, to study the distribution of soil fungi in space and time and its relationship with a variety of soil environments; and thirdly, to compare the fungous population of the soil proper with that of the surface litter in order to establish the possible influence of the latter on the former.

Two sites were chosen for the purpose of this investigation, one being situated in a cultivated Eucalyptus-forest, and the other in a natural forest community. Although these two sites are in relatively close proximity, they differ considerably in a number of important aspects such as plant cover and soil type. A detailed description of these two sites is given in chapter 3.

CHAPTER 2

METHODS

INTRODUCTION:

Serious difficulties are encountered when ecological studies on soil fungi are undertaken. The method in which counts from soil dilutions are employed gives a fairly complete picture of the bacterial population of a soil, since each colony that develops on the plates is usually derived from a separate reproductive unit. The position is, however, quite different in such a highly differentiated structure as a fungus. The fruiting and spore-bearing structures whereby fungi are usually identified are neither regularly nor commonly seen on mycelia in situ. To be able to identify a fungus properly, it is usually essential to have it isolated and growing in pure culture under conditions where the characteristic fructifications and spores will be produced. In studies of the soil mycoflora two lines of investigation are usually followed:

(a) the examination of fungal mycelium by direct microscopical observation, and (b) the isolation and identification of fungi in culture from unknown propagules in the soil. Garrett (1956), summarized the complementary deficiencies of the direct observation and cultural methods by saying that with the cultural method "..... one identifies what one cannot see (i.e. in situ), whereas with the direct method one sees what one cannot identify".

1. Direct methods:

In the simplest form of the direct method a small quantity of soil is placed on a slide, a suitable stain is added and the slide is heated. The excess stain is removed and replaced by 50% glycerin. The soil particles are then

carefully spread out with a dissecting needle and examined microscopically. Conn (1922), was the first person to make use of this direct observation method. He dried and stained soil on microscope slides and found that mycelial fragments were quite commonly seen on these smears. The actual arrangement of the soil particles and micro-organisms as they occur in the natural, undisturbed soil is destroyed during the preparation of these smears. In the Rossi-Cholodny slide technique this disadvantage of soil-smears is eliminated. Rossi (1928), used clean glass microscope slides which he pressed against an exposed face of soil. He then fixed the organisms adhering to the glass slide by drying and gentle heating. This "contact picture" of the soil was then stained and examined microscopically. Cholodny (1930), improved this method by allowing the slides to remain in the soil for a number of days or weeks. Cholodny demonstrated that if a number of such slides were placed in position and removed one at a time at intervals of a few days or weeks, then the behaviour of the soil microflora during a certain period could be followed under the microscope. The Rossi-Cholodny slide technique has been used extensively. The most serious disadvantage of this method is that it gives only scanty information about the different species occurring in soil.

Jones and Mollison (1948), devised a method for the determination of the amount of fungous mycelium in the soil at any stage. They suspended a weighed amount of soil in melted but cooled agar. From this suspension they prepared films of known thickness with the aid of a haemocytometer slide. After drying, the films were stained and mounted on microscope slides for microscopic

examination. The total length of fungal hyphae was measured and expressed in terms of the original weight of soil.

A microscope with strong direct illumination (the Leitz "Ultrapak") was used by Kubiena (1938), for the examination of soil faces in situ. Kubiena also employed a resin for the impregnation of undisturbed blocks of soil. From these blocks sections were cut for examination under a microscope. Burges and Nicholas (1961), used hard-setting resins for soil impregnation. Sections were then ground from these impregnated soil blocks with the aid of geological grinders.

In an attempt to render the direct observation method quantitative, Thornton and Gray (1934), added a known quantity of indigo-particles to the soil and by establishing the relation between the number of erythrosin-stained micro-organisms and the indigo-particles, they were able to calculate the number of micro-organisms in the soil.

2. Cultural methods:

Various techniques have been developed for obtaining fungi in pure culture. This is particularly important for those fungi whose fruiting structures cannot be collected by hand.

One of the earliest and still most widely used cultural techniques is the dilution-plate method (Waksman and Fred, 1922). This method attempts to separate wanted individuals from one another and, as a corollary, to separate these individuals from contaminants. The method is described in detail by Johnson et al (1959). The dilution-plate

method suffers from several disadvantages and has been severely criticized. The most important disadvantage, and one which is at the same time common to all cultural methods, is that media are usually selective towards certain species and that no single medium is suitable for the development of more than only a small percentage of the total number of soil fungi. It has been shown by direct microscopic counts that about ten to twenty percent of the total number of species of living soil micro-organisms can be isolated by making use of dilution-plates and other cultural methods (Roth, 1965). It is assumed that this is due to the diverse nutritional requirements of many of the soil inhabitants.

The second disadvantage of the dilution-plate method arises directly from the procedure followed in the dilution of the original soil suspension. The heavier soil particles sink to the bottom of the shaking vessel before it can be pipetted off to make a dilution in fresh shake medium. The heavier soil particles contain the larger mineral particles, the soil crumbs that were not disaggregated by shaking, and the heavier organic fragments. The actual samples finally plated out are consequently suspensions of fine soil particles, bacterial and actinomycete cells, fungal spores and some hyphal fragments. From this it is quite evident that a heavily sporing soil species will be represented on the dilution-plate at a frequency out of all proportions to the volume of its active mycelium.

Many modifications of the basic dilution-plate method have been proposed and suggestions put forward for the standardisation of this technique (Waksman and Fred, 1922;

Waksman, 1922; Brierley, 1923; Brierley, Jewson and Brierley, 1927). The addition of various substances to media for the control of actinomycetes and bacteria have been suggested by some workers (Waksman, 1916; Tyner, 1944; Dawson and Dawson, 1947; Littman, 1947; Martin, 1950). Dilution plates are still used extensively in isolating fungi for soil floras or for other purposes, and for estimating the inactive component of the soil flora.

A method which has been widely used is the soil-plate method (Warcup, 1950). Small samples (0.005 - 0.015 g) of soil are incorporated in previously melted and cooled agar in Petri dishes. The dishes are manipulated before the setting of the agar so as to secure, as completely as possible, a dispersion of the soil particles. By incorporation of the soil with the agar, the method permits isolation of fungi that are normally rejected with the soil residue in the dilution-plate method. A disadvantage of the soil-plate is that inter-colony competition is more severe than on dilution-plates (Warcup, 1960).

Chesters (1940 and 1948), described a glass immersion tube having a number of small openings through which hyphae can grow and colonize the medium inside the tube. This method was designed for the isolation of active growing hyphae from the soil. According to Garrett (1963), it now seems extremely uncertain whether the method does discriminate between active mycelium and spores, because the spores of fast growing fungi germinate very rapidly when in contact with a substrate, and the growth soon overtakes the mycelia of more slowly growing fungi. This method tends to select fast-growing fungi.

Other methods based on the soil immersion tube method of Chesters have also been employed. In these methods use is made of plastic centrifuge tubes, wrapped in plastic insulation tape to keep the contents sterile before use (Mueller and Durrell, 1957); steel immersion tubes (Thornton, 1958) and capillary tubes (Mc Whitley, 1957).

La Touche (1948) developed a so-called slide-trap method. In this method two standard hanging-drop slides are clipped together in such a way that their cavities lie opposite one another enclosing a small quantity of suitable agar medium. The moisture of condensation which collects between the slides enables growth to take place until the agar is reached. This method is highly selective to rapidly growing species such as Trichoderma viride (Sewell 1956). Sewell (1956), developed a modification of the slide-trap method of La Touche. A shallow chamber of "Perspex", containing a suitable agar medium, is covered by a glass microscope slide and buried in the soil. According to the author this method gives essentially the same results as those obtained with the immersion tube method.

Thornton (1952), devised a method which is known as the screened immersion plate method. This method is an adaptation of the Rossi-Cholodny slide technique and permits the isolation of micro-organisms capable of growing from the soil onto agar media placed behind a perforated screen. A glass microscope-slide is coated with a thin layer of water agar, which is enclosed in a "Perspex" box with ten evenly spaced holes in the lid through which fungi can enter and colonize the adjacent

agar. This plate is buried in the soil and, after a suitable period, it is removed; the slide is examined under the microscope and the fungal colonies are subsequently sub-cultured on potato-dextrose agar. The results obtained by screened immersion plates showed close agreement with those of immersion tubes and Waksman's direct isolation method (Waksman, 1916), and differed from those of dilution-plates and soil-plates (Chesters and Thornton, 1956).

Warcup (1955, 1957), designed a method for the isolation of fungi occurring in soil as non-sporulating mycelia. This method is known as the hyphal isolation method. A soil crumb is allowed to become saturated with water and is then broken up with a strong jet of water. The heavier particles are allowed to precipitate and the finer particles removed by decanting the suspension. The process of washing is repeated, until a residue of the heavier particles only is retained. These residual particles are then spread out in a thin film of sterile water, and searched under the stereoscopic dissecting microscope for fungal hyphae, which are collected by means of a fine, sterile forceps and plated out on nutrient agar. Warcup (1957), found that the fungi obtained through hyphal isolation were species which were rare or absent on dilution plates. Some Basidiomycetes, which Chesters (1949), called "the missing link in soil mycology", were also isolated by this method.

Apart from these more general cultural methods, a wide range of selective methods has been used for the isolation of soil fungi. Of these the so-called

"baiting" techniques are the most important. In these methods, certain substrates are added to the soil to stimulate specific organisms or groups of organisms to colonize the particular substrate. These methods are especially valuable for the isolation of organisms present in very low numbers. There seems to be an infinite number of substrates that could be used. Examples of such substrates are carrot discs (Yarwood, 1946) for Thielavia basicola; rose stems for Chalaropsis thielavioides (Baker, 1953); potato tubers for Fusarium caeruleum (Mc Kee and Boyd, 1952); skin, wool or hairs for keratinophilic chytrids (Karling, 1946), and dermatophytes (Ajello, 1954); straws for root-infecting fungi (Warcup, 1959); hemp seed for Saprolegniaceae (Naumann, 1925; Thomasson, 1925) and pineapple shoots or roots for Phytophthora cinnamoni (Anderson, 1951).

In certain selective methods the soil is given a heat or chemical treatment prior to plating out. Warcup (1951, a), for instance, steamed soil for various periods before plating, and he succeeded in isolating greater numbers of ascomycetes by making use of this method. Soil fumigants such as chloropicrin, formalin and carbon disulphide act selectively at certain concentrations, destroying the propagules of most, but not all, of the soil fungi (Wensley, 1953; Warcup, 1952; Evans, 1955).

More recently, a method was devised by Parkinson and Williams (1961), for the isolation of fungi from soil microhabitats. The method is essentially a washing technique by which microhabitats of the soil can be

separated on a size basis, with the aid of a "Perspex" box with different sieve meshes, prior to plating. The authors claim that this method enabled them to remove most of the fungal spores from these organic soil propagules, and thus increase the number of isolates that are of mycelial origin. Heavily sporing fungi such as *Penicillia*, *Aspergilli* and *Trichoderma viride* which are usually isolated with a high percentage frequency from unwashed soil, were isolated much less frequently from washed soil.

CONCLUSION:

Several techniques have been developed for the study of the soil fungus flora, each with its own advantages and disadvantages. No single procedure, however, adequately describes the entire specific composition of the flora, nor does any one method accurately depict the mass of vegetative hyphae in the soil. All the methods devised for the direct observation of fungi in the soil suffer from the disadvantage that, although the organisms may be counted, it is seldom possible to identify them. It is usually only possible to place the observed organisms in morphological groups.

A wide range of cultural methods has been developed for the study of soil fungi. The two most common methods used for the isolation of these fungi are the dilution-plate and soil-plate methods. These methods yield a relatively broad spectrum of the micro-organisms present in the soil. Many modifications of these basic methods have been used, as well as methods designed for the isolation of specific fungi, known as "baiting" techniques.

In conclusion it may be said that no single method can claim to be the only one for solving the diverse problems relating to the isolation and study of soil fungi. Soil oomycetes, predaceous fungi, plant pathogens and higher Ascomycetes and particularly Basidiomycetes, the mycelium of which represent a large part of the soil micro-flora, require the use of special techniques. According to Garrett (1963), a complete knowledge of soil fungi and their activities can only be attained through the combined use of a number of complementary methods, all of which should include direct microscopic observation.

METHODS USED IN THIS INVESTIGATION:

The methods used in this investigation will be discussed under four headings, namely the collection of soil samples, the methods used for the isolation and recording of fungi from the mineral soil, the methods used in the isolation of leaf litter fungi and the procedures used in the identification of the fungi isolated.

1. Collection of soil samples:

(a) Surface soil samples:

The soil samples which were used for the investigation of the fungi in the surface layers of the soil horizon were collected in the following way. Sterile metal sampling tubes were used to remove soil cores. The tubes were constructed out of a two-inch (internal diameter) water pipe, cut in lengths of three inches.

One edge of a sampling tube was tapered so as to facilitate easy insertion into the soil. Each tube was plugged tightly with cotton wool on the side opposite to the tapered end, placed in a brown paper bag and sterilized in an oven. On each sampling occasion six soil cores of the top two inches of the soil were collected after the removal of the surface litter. The samples were taken over an area of about ten square yards and cores were taken at least three feet apart. The six sampling tubes, each with its core of soil, were placed in sterile brown paper bags and taken to the laboratory. In the laboratory the samples were mixed thoroughly and sieved. The composite sample was divided several times. Finally 25 grams (on an oven-dry basis) of the mixed composite samples were used for making the suspension.

(b) Profile samples:

The following sampling procedure was used for the investigation of the vertical distribution of fungi in the soil profile. In this instance the same method of sampling was used as the one used by Yung and Stenton (1964), and Warcup (1951, 1957). The soil samples were collected from one face of a profile pit by inserting sterile glass specimen tubes, with a length of six inches and an internal diameter of half an inch, into the soil at selected points. Samples were taken from the bottom of the pit upwards to avoid contamination of the soil face by material falling from the upper part of the profile. At each sampling spot the surface soil was first removed with

a sterilized spatula before insertion of the specimen tube. By revolving the specimen tube during insertion it was usually possible to extract a suitable core of soil. In one sampling area, where the soil was very coarse, particularly in the lower horizon, three or four specimen tubes were often needed to secure a suitable sample at a particular depth. The sampling was continued upward into the litter layer. Freshly fallen leaf litter, however, was collected with a sterile forceps and placed in sterilized Petri dishes. A second series of samples ~~were~~^{was} taken for the estimation of pH, and the moisture and organic content of the soil.

All soil samples collected by either of these two methods were plated within two hours of collection except in the case where dilution-plates were prepared. In the latter case the soil samples were stored in a refrigerator overnight and dilution-plates prepared the following day, after the moisture content of the soil had been determined.

The soil moisture content was determined by drying a soil sample of 5 to 7 grams in an electric oven at 105° for 12 hours.

Organic matter content was determined by ignition. The dry soil sample used for determining the soil moisture content was placed in a furnace at 450° for 12 hours and the loss of weight expressed as a percentage of the dry weight.

The pH was determined with a "Metrohm" pH-meter,

using a glass electrode, according to the method described by Piper (1942). The soil temperature was also recorded at each sampling, using a mercury soil thermometer.

2. Methods used in the isolation and recording of fungi from the mineral soil:

In all, four different methods were used in the isolation of the fungi from the mineral soil. Two of these methods were used constantly throughout this project for the investigation of various factors which influence soil fungi, such as the influence of temperature, soil moisture content, season and soil depth. These two methods, the dilution-plate and the soil-plate methods, will therefore be described in detail here. The other two methods, i.e. the modified soil-plate method and the soil steaming method will be described fully in the relevant chapters.

(a) Dilution-plate method:

A soil sample of 25 g, calculated on a dry-soil basis, was placed in a 1000 ml Erlenmeyer flask and 250 ml of sterile, distilled water was added to the soil. This gave an initial dilution of 1:10. The Erlenmeyer flask containing this primary soil suspension was shaken on a mechanical "wrist-action" shaker for 30 minutes at a moderate speed. Ten millilitres of this suspension were immediately pipetted into a 90 ml sterile water blank. This gave a dilution of 1:100. Ten millilitres of this dilution were transferred immediately to another sterile

water blank of 90 ml to give a dilution of 1:1000. Ten millilitres of this dilution were transferred to a 40 ml sterile water blank to give a final dilution of 1:5,000. In certain instances a dilution of 1:2,500 was used for soil samples from the lower horizons to compensate for the natural reduction in the number of fungi with increasing depth. This dilution was obtained by transferring 10 ml of the 1:1000 dilution to a 15 ml sterile water blank. Of the final dilution one ml samples were transferred aseptically into each of ten sterilized Petri dishes.

Fifteen millilitres of agar, cooled to just above the solidifying temperature, were poured into each Petri dish and the contents thoroughly mixed by gentle rotation and shaking of the dishes. The inoculated plates were incubated for 5 days at 26° and the colonies appearing on the plates were counted with the aid of a "Quibeck" colony counter. All transfers of the colonies were cultivated on potato dextrose agar (Riker and Riker, 1936), for subsequent study and identification. This medium gave good growth of the isolates.

The medium used for all dilution-plates was peptone dextrose agar with Rose Bengal and streptomycin (Martin, 1950). Preliminary experimentation revealed that the addition of Rose Bengal and streptomycin did not successfully suppress bacterial growth on all plates. With the subsequent addition of aureomycin at the rate of 5 ug/ml of medium, this problem was easily overcome. The composition and

preparation of the medium is detailed below.

Agar	20 g
K H ₂ PO ₄	1.0 g
Mg SO ₄ .7H ₂ O	0.5 g
Peptone	5.0 g
Dextrose	10.0 g
Distilled water	1 L
Rose Bengal	1:30,000
Streptomycin	30 ug/ml
Aureomycin	5 ug/ml

All the material except Rose Bengal and the two antibiotics were dissolved in the distilled water and slowly heated to boiling point. Rose Bengal was then added at the rate of 1 ml/100 ml of medium from a 1:300 stock solution of Rose Bengal. The medium was autoclaved at 15 lbs. pressure for fifteen minutes. The antibiotics were pipetted into the Petri dishes prior to the pouring of the plates.

(b) Soil-plate method:

The soil-plate method of Warcup (1950), was used extensively during this investigation. It was preferred to the soil dilution-plate method because of the well-known criticism that the latter method exaggerates the importance of heavily-sporing fungi.

A soil-plate was prepared by transferring approximately 5 mg of soil from the soil sample to a sterilized Petri dish into which a drop of sterile water had been pipetted beforehand. The soil inoculum was stirred thoroughly, with the aid of an

inoculating needle, in this drop of water. The addition of this small quantity of water facilitates the dispersion of the soil particles, especially when the soil is very dry. Fifteen millilitre of previously melted and cooled agar were then added and the soil particles dispersed evenly throughout the agar by rotation and gentle shaking of the dish.

Soil-plates were incubated at 26° for 5 to 10 days and examined daily for the recording and isolation of fungi. After ten days the plates were placed in a refrigerator for up to a month for observation and isolation of slow-growing fungi. For the examination of the plates use was made of a "Quibeck" colony counter as well as a wide-field stereoscopic microscope.

All soil plates were prepared from Czapek-Dox agar with yeast extract. The composition and preparation of this medium is detailed below.

Agar	15.0 g
NaNO_3	3.0 g
K_2HPO_4	1.0 g
$\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$	0.5 g
KCl	0.5 g
$\text{Fe SO}_4 \cdot 7\text{H}_2\text{O}$	0.01 g
Sucrose	30.0 g
Yeast extract ("Difco")	5.0 g
Distilled water	1.0 L
Rose Bengal	1:30,000
Streptomycin	30 ug/ml
Aureomycin	5 ug/ml

All the materials with the exception of the sucrose, Rose Bengal and the two antibiotics were dissolved in the distilled water and slowly heated to boiling point. The sucrose and Rose Bengal were then added and the medium was sterilized at 15 lbs. steam pressure for 15 minutes. The antibiotics were pipetted into the Petri dishes before the soil-plates were prepared.

With regard to the number of dilution-plates and soil-plates to be used for each soil sample, there seems to be disagreement among different investigators. In their comparison of various techniques for the isolation of soil fungi Chesters and Thornton (1956), for instance, used 8 soil-plates for each soil sample. Yung and Stenton (1964), investigating the ecology of Phycomycetes in Hong Kong, employed 6 dilution-plates when analysing each monthly soil sample, while Sewell (1959), used only 3 replicates per soil sample in his studies on Heathland soil fungi. In his investigations on the occurrence of fungi in a wheat field, Warcup (1957), used 9 dilution-plates and 6 soil-plates for each soil sample. The same worker used only three soil-plates per soil sample while investigating the ecology of soil fungi at Lakenheath Warren in 1951.

In this investigation 5 to 10 soil-plates and the same number of dilution-plates were prepared from each soil sample.

For the vertical distribution of the fungi in the soil profile and for the seasonal variation, the

numbers of fungi were assessed on a relative basis. Two assessments were made, viz occurrence (Warcup, 1951), and frequency of occurrence (Sewell, 1959). Occurrence is based on the presence or absence of a particular fungus on a batch of soil-plates or dilution-plates from a single soil sample. An example will illustrate this. If ten soil plates are prepared from a soil-sample, the occurrence of any particular fungus is determined by the number of plates on which this fungus occurs. Thus for this particular soil sample a maximum of ten records are possible. Frequency of occurrence is the percentage of plates on which a given fungus appears, either for the whole profile, for a particular depth, a particular month, or for all the plates prepared during the period of investigation. For example, at one site which was studied, 72 soil-plates were prepared from soil taken from the surface. The frequency of occurrence of any fungus in the surface soil is the percentage of 72 plates on which it occurred.

3. Methods used for isolating the litter fungi:

Most investigators have used washing techniques in their studies on the mycoflora of leaf litter and other plant débris on the soil. Parkinson and Kendrick (1960), used a modification of the serial-washing technique of Harley and Waid (1955), in their studies of the succession of fungi on the leaf litter of Pinus sylvestris. The litter was shaken in 10 changes of sterile 1% (w/v) aqueous solution of 'Teepol', followed by 10 changes of sterile water, prior to plating on 2% (v/v) malt agar.

They found this method satisfactory for freshly fallen leaf litter, but less effective for the older litter from the lower layers.

Witkamp (1960), used a method whereby only those fungi that grew actively on or in the litter material were isolated. He employed a technique using leaf-discs placed on sterile cover slips on the surface of agar. He thus created a separation between the leaf material and the agar substrate which prevented activation by nutrients of non-proliferating organisms. Moreover, his technique limited the number of bacterial colonies that developed.

Yung and Stenton (1964), also employed a serial washing technique for the study of Phycomycetes on freshly fallen litter. For the older, decomposed litter they used soil-plates without preliminary washing of the litter.

In this investigation three methods were used for the isolation of the fungus flora of leaf litter. The serial-washing technique of Parkinson and Kendrick, and the leaf-disc method of Witkamp were used for investigating the fungi of freshly fallen litter. From the decomposed litter of the lower layers soil-plates were prepared. These three methods will be described fully in the experimental section.

4. Identification of isolated fungi:

Most of the work concerned with the identification of

the fungi isolated during this investigation was carried out in the Botany Department of the University of Nottingham and in the Commonwealth Mycological Institute, Kew, England.

Standard mycological methods were employed in the transfer, cultivation and identification of the cultures. The majority of the cultures were incubated at 25° until they reached a suitable stage of development. The cultures were grown on potato-dextrose agar or on potato-carrot agar (see appendix) unless growth on these media was unsatisfactory, in which case other media, such as malt extract agar and soil-extract agar or natural substrates, such as sterilized grass débris, were used. *Penicillia* and *Aspergilli* were grown on Czapek-Dox agar.

CHAPTER 3

DESCRIPTION OF SITES STUDIED.

PORT DURNFORD GOVERNMENT PLANTATION (SITE 1):

Site 1 is situated approximately 12 miles South of Empangeni, Zululand, and 2 miles East of the main 'north-coast' road from Durban (see Figure 1). It is known as the Port Durnford Plantation of the South African Department of Forestry. The departmental reference to this part of the forest is D 2(a) Eucalyptus maculata.

1. Vegetation:

The vegetation at site 1 consists of a dense stand of "spotted" gum trees, Eucalyptus maculata Hook.f. The trees were planted in 1927 at 8 feet intervals. In the period 1938/39 the plantation was thinned to 120 stems per acre. A further thinning took place in 1940/41 to 95 stems per acre, while a final thinning in 1949/50 reduced the number of stems to 60 stems per acre.

At present the mean height of the gum trees is 116 feet while the mean diameter breast height (d.b.h.) is 19.3 inches. These old trees are between 30 and 40 feet apart with young gum trees scattered in between. The lush undergrowth is composed of some 42 species of trees, lianes, shrubs and herbs, covering approximately 70% of the area. The undergrowth of site 1 is dominated by Digitaria diversinervis (Nees) Stapf; Desmodium incanum DC.; Asystasia gangetica (L.) T. Anders.; Achyroopsis avicularis Hook.f.; Canthium ventosum (L.) S. Moore and Antidesma venosum E. Mey ex Tul. Other

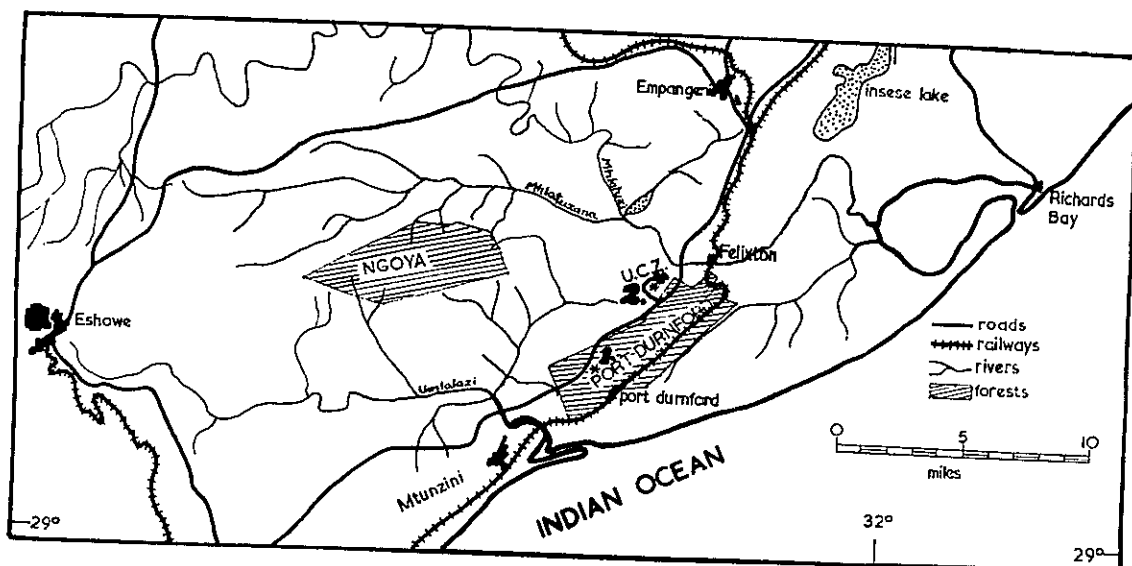


Figure 1: Map of the Mtunzini area of Zululand,
showing sites 1 and 2, marked *.

vascular plants occurring frequently as undergrowth in the plantation are listed in Table 1. See also Figure 2.

2. Litter:

A layer of two to three inches of plant débris permanently covers the forest floor. There is a considerable accumulation of litter reaching a depth of eight inches or more at the base of the trunks of the larger trees. This is partly due to an accumulation of bark from the tree trunks. The accumulated litter around a tree trunk can be seen in Figure 3. Two layers can be distinguished which correspond to those litter layers described by Hesselman (1926). These may be characterized as follows:

- (a) The upper, dry layer of freshly fallen leaves and other débris which is arranged very loosely. This layer consists of undecomposed litter. Few fungal hyphae can be seen on the leaves. This layer is designated as the A₀₀₀ layer or horizon.
- (b) Beneath the A₀₀₀ layer lies the A₀ or 'humus' layer composed of litter which, by active decomposition, has become reduced to small fragments frequently not more than 1 inch long and often matted together and held in clumps by intertwinning fungal mycelia. This layer lies immediately above the A₁ horizon of the soil proper. In the 'humus' layer humification prevails and the components of the débris are no longer recognizable as twigs, leaves or bark.

At site 1 no clear A₀₀ or "fermentation" layer

could be distinguished. In this description all the organic matter above the soil proper will be referred to as litter, while the soil directly below the litter will be called the mineral soil.

The forest soil at Port Durnford is a typical 'mor' soil. The Third International Congress for Soil Science gave the following definition for a mor soil: ".....organic matter practically unmixed with mineral soil, usually more or less matted or compacted. Transition to mineral soil always distinct."

To determine the composition of the litter, samples were collected from the Aooo layer at site 1 on June 25th. 1965. The composite samples were separated in different fractions namely Eucalyptus leaves, bark, twigs, fruit and litter from the undergrowth. The results of this analysis are given in Table 2. From this analysis it is clear that a fairly large portion of the litter consists of bark. Near the trunks of the older gum trees the bark was proportionately considerably higher.

No seasonal pattern in the shedding of the bark could be detected. This is also the case with the shedding of leaves. It seems that there is a continual shedding of old leaves throughout the year.

TABLE 1

LIST OF VASCULAR PLANTS FORMING PART OF
THE UNDERGROWTH OF THE FOREST AT SITE 1

Achyranthes aquatica R. Br.
Adenia gummifera (Harv.) Harms
Albizia adianthifolia (Schumach.) W.F. Wight
Aneilema cf. *A. aequinoctiale* (Beauv.) Kunth
Asparagus falcatus L.
Bridelia micrantha (Hochst.) Baill.
Coccinia palmata (Sond.) Cogn.
Cynanchum ellipticum (Harv.) R.A. Dyer
Cyperus albobstriatus Schrad.
Deinbollia oblongifolia (Sond.) Radlk.
Erythrina lysistemon Hutch.
Halleria lucida L.
Hewittia sublobata (L.f.) O. Kuntze
Mariscus gueinzii C.B. Cl.
Melia azederach L.
Panicum dregeanum Nees
Pellaea viridis (Forsk.) Prantl
Phymatodes scolopendria (Burm.) Ching
Rhus nebulosa Schonl.
Rhynchosia cf. *R. stenodon* Bak.f.
Scutia myrtina (Burm.f.) Kurz.
Senecio deltoideus Less.
S. mikanioides Otto
Setaria chevalieri Stapf ex Stapf et C.E. Hubb.
Sida pseudocordifolia Hochr.
Smilax kraussiana Meisn.

Sporobolus capensis (Willd.) Kunth

Stangeria eriopus (Kunze) Nash

Trichilia emetica Vahl

Trimeria rotundifolia (Hochst.) Gilg

Ziz^yiphus mucronata Willd.



Figure 2. Lush undergrowth in forest at site 1.



Figure 3. Accumulation of litter at the base
of a tree trunk.

TABLE 2.
COMPOSITION OF LITTER AT SITE 1.

LITTER FRACTION	DRY WEIGHT IN GRAMS	PERCENTAGE OF TOTAL
Eucalyptus leaves	60.23	26.32
Eucalyptus bark	89.62	39.15
Eucalyptus twigs	46.62	20.37
Eucalyptus fruit	19.62	8.57
Litter from undergrowth	12.81	5.59
TOTAL	228.91	100.00

3. The soil:

The soil of the Port Durnford plantation is of the Recent Sand (Red) type (Beater, 1957). The soil is rather featureless, with few aggregates. Most of the material is loose single-grained, becoming cemented and compacted when dry. No horizons could be detected in the soil profile. The soil is very deep. A road quarry about 50 yards from site 1 shows the depth of the soil and illustrates very clearly its featureless nature (Figure 4). A mechanical analysis of the soil yields a high percentage of sand fraction. The results of the mechanical analysis are given in Table 3.

For the chemical analysis of the soil, samples were collected on 15th June 1965. Four sets of samples were taken at various depths and each sample from a particular depth consisted of a well-mixed composite sample of ten samples weighing $\pm$ 100 grams each. Samples were collected from the following depths: A : 0 - 2 inches; B : 6 - 10 inches; C : 15 - 20 inches and D : 25 - 30 inches. In Table 4 the results of the chemical analysis of these soil samples are given.

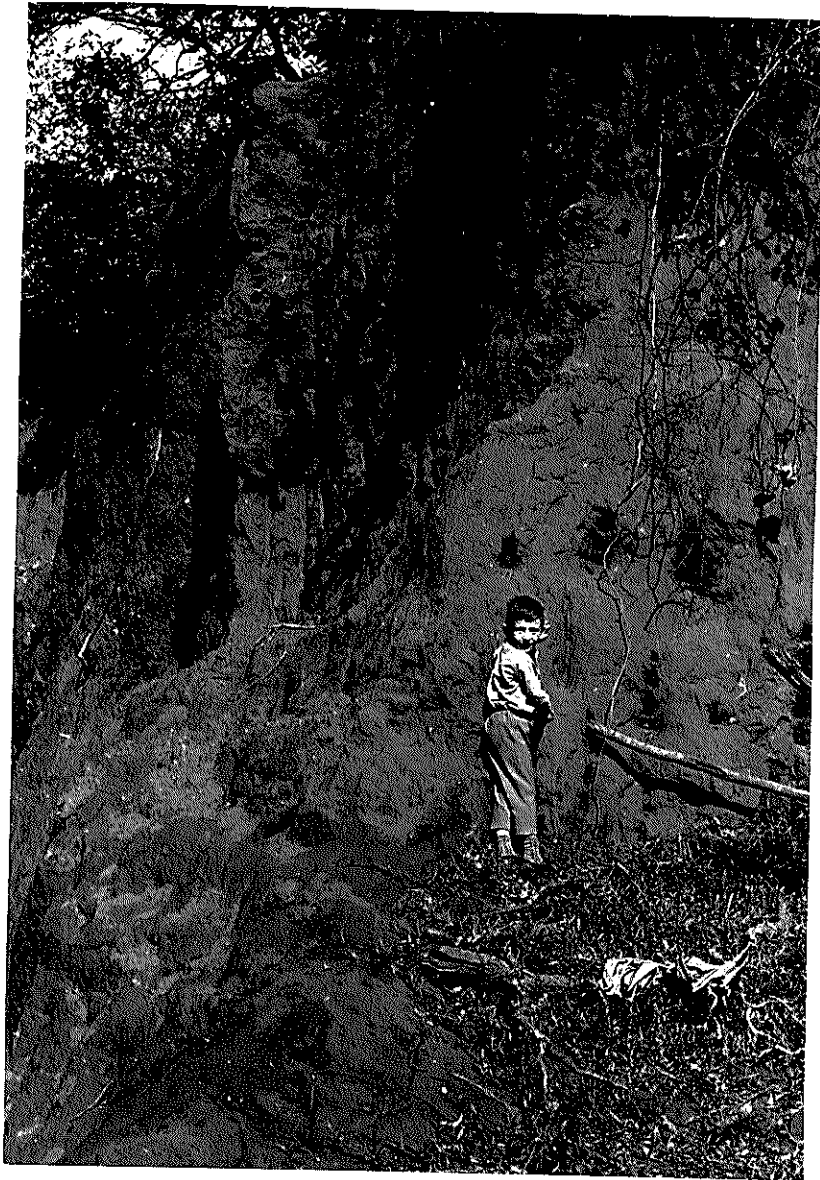


Figure 4. Road quarry at site 1 showing the very deep sandy soil. The featureless nature of the profile can also be appreciated.

TABLE 3.
MEAN MECHANICAL COMPOSITION OF RECENT
SANDS (RED) PROFILE. (BEATER, 1957)

MEANS OF HORIZONS IN INCHES.	0 - 9	9 - 28	28 - 72	AVERAGE
Coarse sand & gravel (10.0 - 0.2 mm)	65.6%	64.2%	57.0%	60.0%
Gravel (10.0 - 1.0 mm)	nil	nil	nil	nil
Fine sand (0.2 - 0.02 mm)	24.9%	27.2%	32.3%	30.0%
Silt (0.02 - 0.002 mm)	3.3%	2.5%	2.0%	2.3%
Clay (less than 0.002 mm)	6.2%	6.1%	8.7%	7.7%

TABLE 4.
CHEMICAL ANALYSIS OF SOIL FROM SITE 1.

SAMPLE	A	B	C	D
Depth in inches	0 - 2	6 - 10	15 - 20	25 - 30
N%	0.08	0.03	0.04	0.02
P ₂ O ₅ ppm.	3	3	4	4
K ₂ O ppm.	64	34	51	35
Organic matter %	2.17	0.98	1.05	0.63
pH	5.25	4.80	5.10	5.0
Mg ppm	76	16	46	55
Ca ppm	180	40	40	20
Na ppm	28	20	28	50

CAMPUS OF THE UNIVERSITY COLLEGE OF ZULULAND (SITE 2):

Site 2 is situated on the campus of the University College of Zululand (see Figure 1). It is a field of natural undisturbed vegetation with a relatively sharp slope to the south-east.

1. Vegetation:

The vegetation at site 2 consists of clumps of trees and shrubs in a somewhat open grassland as can be seen in Figures 5 and 6. At the actual sampling area the following trees are found: Canthium ventosum (L.) S. Moore; Ficus capense Thumb.; Maesa lanceolata Forsh.; Erythrina lysistemon Hutch.; Strelitzia nicolai Regel et Koern. and Halleria lucida L. The balance of the vegetation of the site and the immediate vicinity (one yard around the clump of trees) are listed in Table 5.

2. Litter:

Very little litter was found at site 2. The ground is densely overgrown with grass. No analysis or further studies of the litter fungous flora was made at this site.

3. The soil:

The soil at site 2 is a coarse, greyish or blackish, gritty loam derived from granite. It is of a crumbly structure and friable consistency. The colour ranges with depth from brownish to yellowish-grey merging in



Figure 5. Sampling area at site 2.

Note the dense stand of trees and shrubs.



Figure 6. Site 2 as seen from the University

College with site 1 (marked x) on the horizon.

Note the clumps of trees and shrubs in open
grassland.

TABLE 5

LIST OF PLANT SPECIES GROWING AT SITE 2

Acalypha petiolaris E. Mey.
Asparagus racemosus Willd.
Aspilia natalensis (Sond.) Wild
Athrixia phyllicoides DC.
Centella coriacea Nannfd.
Digitaria macroglossa Henr.
Diodia natalensis (Hochst.) Garc.
Eragrostis curvula (Schrad.) Nees.
Euclea crispa (Thunb.) Guerke var. *crispa*.
Hyparrhenia filipendula (Hochst.) Stapf.
Kraussia floribunda Harv.
Leonotis dysophylla Benth.
Phoenix reclinata Jacq.
Rhoicissus tridentata (L.f.) Wild et Drumm.
Rhynchosia sordida (E. Mey.) Schinz
Rubus rigidus Sm.
Senecio pterophorus DC.
Smilax kraussiana Meisn.
Stenotaphrum secundatum (Walt.) O. Kuntze
Themeda triandra Forsk.

irregular pockets and "fingers" into yellowish or orange-yellow material. Usually the lower part of the profile resembles well-weathered granite rather than soil. At the sampling area the soil is very shallow, varying from 6 to 15 inches from the surface to where the parent material starts. The shallow layer of top soil can be seen in Figure 7. The mechanical analysis of this granite soil profile is given in Table 6.

For the chemical analysis of the soil, samples were collected on June 16th, 1965. Three sets of samples were taken at various depths and each sample from a particular depth consisted of a well-mixed composite sample of ten samples weighing $\pm$ 100 grams each. The three sets of samples were collected at the following depths: A : 0 - 2 inches; B : 4 - 6 inches and C : 8 - 10 inches. The results of the chemical analysis of soil from site 2 is given in Table 7.

CLIMATIC CONDITIONS:

For the purpose of this investigation the climatic conditions of sites 1 and 2 are discussed simultaneously. As these two sites are only about five miles apart, no significant differences in climate, particularly macro-climate exist. The soil temperatures of the individual sites, as measured at the time of sampling, are given together with other results in the discussion of these sites.

1. Precipitation:

The mean annual rainfall at the Umhlatuzi Valley



Figure 7. Typical profile at site 2 showing the very shallow layer of actual top soil with the yellowish underlying parent material.

TABLE 6.

MEAN MECHANICAL COMPOSITION OF GRANITE SOIL PROFILE.

MEANS OF HORIZONS IN INCHES.	0-10	10-20	20-30	30-72	AVERAGE
Coarse sand and gravel (10.0 - 0.2 mm)	48.5%	55.3%	56.1%	56.6%	55.2%
Gravel (10.0 - 1.0 mm)	13.5%	20.5%	21.0%	22.0%	20.4%
Fine sand (0.2 - 0.02 mm)	18.5%	12.7%	13.7%	17.6%	16.5%
Silt (0.02 - 0.002 mm)	9.2%	7.2%	8.2%	12.3%	10.6%
Clay (less than 0.002 mm)	23.8%	24.8%	22.0%	13.5%	17.6%

TABLE 7.

CHEMICAL ANALYSIS OF SOIL FROM SITE 2.

SAMPLE DEPTH IN INCHES	A 0 - 2	B 4 - 6	C 8 - 10
N %	0.41	0.05	0.03
P ₂ O ₅ ppm	6	4	3
K ₂ O ppm	134	61	26
Organic matter	7.01	5.18	1.75
pH	5.45	5.35	5.70
Mg ppm	280	256	110
Ca ppm	620	580	260
Na ppm	47	46	32

Sugar Estate, Felixton (situated roughly four miles North of site 1) as measured over a period of 37 years (1924 - 1960), is 53.69 inches (136.37 cm). The minimum annual rainfall during this period was 31.61 inches in 1941, while in 1925 the maximum rainfall occurred, namely 91.46 inches. Table 8 gives the mean monthly average distribution of rainfall for Zululand for the period 1924 - 1960. From this table a clear seasonal pattern in the precipitation is evident. The lowest rainfall occurs during the winter from May to August while the rainfall increases to a maximum in the warm summer months of November to February. The actual precipitation during the period of sampling at the two sites, as measured at the Port Durnford Forest station, half a mile from site 1 is given in Table 9.

2. Temperature:

(a) Air temperature:

The mean annual screen temperature for Felixton (Station No 304/680) latitude $28^{\circ}50'$ S and $31^{\circ}53'$ E, with a height of 70 metres and situated approximately six miles North of site 1, is given in Table 10. These figures were abstracted from the records of the Weather Bureau, Pretoria and represent the period 1933 - 1940. The mean annual temperature at this station is 20.9° .

(b) Soil temperature:

In Tables 11 and 12 the mean monthly soil temperature and the mean annual soil temperatures on bare ground

and under sugar cane trash-cover are given respectively. These measurements were taken at Empangeni, which is about 10 - 12 miles North of sites 1 and 2. The mean annual soil temperature for bare ground is 29.1° while it is 21.1° for soil covered with a layer of sugar cane trash.

TABLE 8.

MEAN MONTHLY AVERAGE DISTRIBUTION OF RAINFALL FOR ZULULAND FOR THE PERIOD 1924-60.

MONTH	JAN.	FEB.	MAR.	APR.	MAY	JUN.	JUL.	AUG.	SEP.	OCT.	NOV.	DEC.
Mean (inches)	4.34	5.11	5.48	2.91	2.30	1.74	1.30	1.43	2.60	3.31	4.30	5.02
Mean (cm)	11.02	12.98	13.92	7.39	5.84	4.42	3.30	3.63	6.60	8.41	10.92	12.75

TABLE 9.

MONTHLY RAINFALL FOR THE PERIOD 1964/65 AS
MEASURED AT THE PORT DURNFORD FOREST STATION.

MONTH		TOTAL IN MM	NUMBER OF DAYS WITH 4 MM OR MORE	MAX. FOR 24 HOURS
July	1964	79.0	9	23.0
September		89.2	9	21.8
October		222.6	16	62.0
November		167.4	12	33.7
December		93.0	12	30.5
January	1965	86.6	7	34.0
February		71.4	10	28.0
March		83.2	9	26.0
April		129.7	6	69.5
May		94.5	8	40.0
June		194.2	7	62.1
July		54.2	6	23.0
August		171.0	6	48.5
September		162.3	11	62.0

TABLE 10.
MEAN MONTHLY SCREEN TEMPERATURES FOR FELIXTON. TEMPERATURE IN DEGREES CENTIGRADE.

JAN.	FEB.	MAR.	APR.	MAY	JUN.	JUL.	AUG.	SEP.	OCT.	NOV.	DEC.
24.3	24.2	23.4	21.6	19.4	17.3	16.9	18.3	19.6	21.2	22.3	23.2

TABLE 11.
MEAN MONTHLY SOIL TEMPERATURE ON BARE GROUND AND UNDER SUGAR CANE TRASH IN ZULULAND
SUGAR CANE AREAS IN DEGREES CENTIGRADE.

	JAN.	FEB.	MAR.	APR.	MAY	JUN.	JUL.	AUG.	SEPT.	OCT.	NOV.	DEC.
Bare	35.9	34.7	33.1	27.3	25.3	22.9	22.8	26.1	27.9	28.5	32.2	32.9
Cover	25.7	26.4	24.1	20.4	18.8	16.0	14.9	17.8	18.6	21.0	24.2	25.1

TABLE 12.
 MEAN ANNUAL SOIL TEMPERATURES ON BARE GROUND AND UNDER SUGAR CANE TRASH COVER AT
 EMPANGENTI IN DEGREES CENTIGRADE.

MAXIMUM		MINIMUM		MEAN		DEPTH 1"		DEPTH 4"		DEPTH 9"	
BARE	COVER	BARE	COVER	BARE	COVER	BARE	COVER	BARE	COVER	BARE	COVER
42.9	27.0	14.8	17.3	28.8	22.2	28.0	21.6	25.4	21.2	23.4	20.8

CHAPTER 4

FUNGI ISOLATED BY THE DILUTION-PLATE METHOD FROM SITE 1.

During the period September 1964 to September 1965, twelve monthly samples were collected from the forest floor at site 1. Samples were taken from the upper two inches of the soil profile, and from each monthly soil sample six dilution-plates were prepared. In Table 13 a list of all the fungi isolated from the dilution-plates, prepared from these soil samples, is listed. During the twelve months of sampling 72 dilution plates were prepared. The maximum occurrence of any fungus at site 1, therefore, is 72. Table 13 also gives the occurrence of these fungi on dilution-plates.

Representatives of three of the fungus classes were isolated. The number of fungi belonging to each class is as follows:

Phycomycetes	8
Ascomycetes	3
Fungi Imperfecti	31

By using the dilution-plate method it was established that the fungus floras of acid forest and heath soils are commonly characterized by the presence of Mucorales and species of the genera Penicillium, Aspergillus and Trichoderma (Cobb, 1932; Starc, 1941; Ellis, 1940; Rishbeth, 1951). This observation is in general confirmed by the results obtained in the present investigation. It will be noticed from Table 13 that, with the exception of the genera Cladosporium, Fusarium and Humicola, all the dominating species belong to the

TABLE 13.
LIST OF FUNGI ISOLATED FROM SITE 1
BY THE DILUTION-PLATES METHOD.

	OCCURRENCE (MAX. 72)
<u>PHYCOMYCETES</u>	
Absidia cylindrospora Hagem	40
Cunninghamella elegans Lendner	51
Gongronella butleri (Lendner) Peyronel and Dal Vesco	33
Mortierella isabellina (Oudemans) Zycha	5
Mucor silvaticus Hagem	4
M. strictus Hagem	3
M. vallesiacus Lendner	6
Rhizopus nigricans Ehrenberg	16
<u>ASCOMYCETES</u>	
Neocosmospora vasinfecta E.F. Smith	21
Pseudeurotium sp. UZ.85.	5
Thielavia terricola (Gilman & Abbot) Emmons var. minor (Rayss & Borut) Booth	4
<u>FUNGI IMPERFECTI</u>	
Alternaria ten ^u issina ^m (Fr.) Wiltsh.	9
Alternaria sp. UZ.84.	2
Aspergillus allahav ^b adii Mehrotra & Agnibotri	11
A. flavus Link	13

<i>A. japonicus</i> Saito	5
<i>Botryotrichum</i> sp. UZ.110.	1
<i>Candida albicans</i> (Robin) Berkhout	1
<i>Cladosporium oxysporum</i> Berk. & Cart.	50
<i>Cylindrocladium scoparium</i> Morgan	3
<i>Epicoccum nigrum</i> Link (1)	16
<i>Fusarium oxysporum</i> Schlecht. ex Fr.	70
<i>Humicola</i> sp. UZ.23.	61
<i>Humicola</i> sp. UZ.64.	38
<i>Lacellinopsis sacchari</i> Subramanian	2
<i>Paecilomyces elegans</i> (Corda) Mason et Hughes apud Hughes	3
<i>P. marquandii</i> (Masse) Hughes	2
<i>Penicillium casei</i> Loubière	49
<i>P. digitatum</i> Saccardo	5
<i>P. funiculosum</i> Thom	2
<i>P. multicolor</i> Grigorieva-Manoilova and Poradielova	7
<i>P. wilsoni</i> Bainier and Sartory	28
<i>P. soppi</i> Zaleski	2
<i>P. velutinum</i> van Beyma	31
<i>P. verruculosum</i> Peyronel	69
<i>P. citrinum</i> Thom	1
<i>Stysanus medius</i> Saccardo (<i>Doratomyces microsporus</i> (Sacc.) Morton & Smith)	3
<i>Trichoderma</i> sp. UZ.19.	72
<i>Trichoderma</i> sp. UZ.35.	41
<i>Trichosporon</i> sp. UZ.50.	21
Sterile colourless mycelium UZ.27.	38
Sterile colourless mycelium UZ.42.	5

Mucorales and the genera Penicillium, Aspergillus and Trichoderma. Species of these genera occurred frequently on most dilution-plates.

Members of the Mucorales predominating in the acid forest soil at site 1 include Absidia cylindrospora, Gongronella butleri and Cunninghamella elegans. Many investigators stress the fact that species of Mortierella are very common in forest soils (Chesters, 1948; Warcup, 1951; Bisby, Timonin and James, 1935). In the present investigation this observation did not hold true for the forest soil at site 1. Only one species of Mortierella, M. isabellina, was isolated and it did not have a high occurrence. The genus Mucor was represented by three species, M. silvaticus, M. strictus and M. vallesiacus, though they were not frequently found on dilution-plates. Members of this genus were, however, much more common on soil-plates prepared from the same soil samples (see later section).

Three Ascomycetes were isolated from dilution-plates of this site. Neocosmospora vasinfecta was found to be quite common. It occurred on dilution-plates throughout the period of investigation. Thielavia terricola and Pseudeurotium sp. occurred much less frequently.

No Basidiomycetes were isolated from site 1 by the dilution-plate method.

The majority of soil fungi isolated from site 1 belong to the Fungi Imperfecti. The genera Alternaria, Aspergillus, Humicola, Paecilomyces, Penicillium and Trichoderma were well represented. Many species of Penicillium have been recorded from acid forest soils (Bisby.

Timonin and James, 1935; Starc, 1941; Gilman, 1957). In this investigation the species P. verruculosum, P. velutinum and P. casei were found to be the dominating representatives of the genus.

The species Fusarium oxysporum occurred very frequently on many dilution-plates. Members of the genus Fusarium have previously been reported from forest soils (Niethammer, 1941; Bisby, Timonin and James, 1935; Starc, 1941).

Trichoderma species are very common in soils and few reports on soil fungi do not include one or more species in their floristic lists. In older literature constant reference is made to the species Trichoderma lignorum (Tode) Harz and T. Koningi Qud. (Starc, 1941; Jensen, 1931; Cobb, 1932; Ellis, 1940; Rishbeth, 1951). Bisby (1939), expressed the opinion that these forms are all representatives of a single monotypic species, Trichoderma viride Pers. ex Fries. In the present investigation two forms occurred regularly on most plates.

ECOLOGICAL OBSERVATIONS:

In an attempt to ascertain whether there was any seasonal variation in the fungus population of the forest soil at site 1, estimates of the total number of fungi in each monthly soil sample were made. Counts were made from dilution-plates (see page 56) and the number of colonies per gram of dry soil was calculated. The results of these investigations are given in Table 14, while they are represented graphically in Figure 8.

An analysis of these results show that there is little evidence of a well-established seasonal variation in the total

number of fungus colonies. The two warmest months of the year, i.e. January and February (see Table 10), gave the lowest count of fungus colonies. It must be pointed out, however, that during these months it was very dry in Zululand and that the moisture content of the soil was relatively low. If the rainfall figures for these two months (see Tables 9 and 14) are compared with those of the mean monthly precipitation for this area (see Table 8), it is evident that (whereas the months of January and February usually have the highest rainfall) they had an abnormally low rainfall during the period of sampling. The combined effect of low moisture content of the soil and of high soil temperature could account for these low counts. These results correspond with those obtained by Warcup (1957). He found that in a wheat-field soil the counts of fungus colonies were always lower during dry warm months. In the forest soil at site 1 there is a higher count for fungi during the late spring and early summer, from September to October.

From the results obtained during this investigation it seems that the influence of the seasonal factors on the number of colonies is not so close as that of the soil moisture content. This close correlation between soil moisture content and the number of colonies per gram of dry soil was noticed throughout this investigation. This correlation is clearly reflected on comparing the graph of the number of colonies with that of the soil moisture content (see Figure 8). From the available data it seems highly probable that the moisture content of the soil directly influenced the number of fungus colonies in the soil. During the period November 1964 to January/February 1965, there was a decrease in the soil moisture content. During this same

period there was a corresponding decrease in the number of colonies. This observation also holds true for the period March to May 1965. From April 1965 to June of the same year a gradual increase in the soil moisture content gave a corresponding increase in colony counts, while a decrease in soil moisture during August 1965 coincided with a marked decrease in colony numbers.

With regard to the rainfall, it is clear from the results that the moisture content of the soil was more or less directly influenced by precipitation. This can be seen in Figure 8.

There is no direct correlation between the soil temperature and the number of fungi. There is, however, some indication of the influence of temperature if the period from October 1964 to February 1965 is considered. During this period the soil temperature showed a tendency to drop and the number of colonies also decreased. But this also holds true for the soil moisture content during the corresponding period.

Conflicting evidence for a seasonal change in the soil micro-population was given by Dixon (1928), Brown and Benton (1938), Jensen (1934), and Timonin (1935). Changes in microbial numbers, demonstrated by Dixon appeared to bear some relation to variation in rainfall from month to month. This was confirmed by Jensen in that soil from low rainfall areas showed the lowest fungal numbers. Timonin, on the other hand, could come to no definite conclusion regarding the influence of soil moisture on the fungal population. Warcup (1951), found little evidence of a seasonal variation in the number of colonies of soil fungi in five Lakenheath Warren grassland soils.

Feher and Frank (1950), state that temperature and moisture are regulatory biofactors in the soil and that they operate in a complex manner. The product temperature x moisture gives R, where R is a 'regulative factor complex' representing the climatic factors which influence and govern the periodicity of soil life. If these components remain within their optimum limits they are physiologically favourable for growth. If one or both factors pass their optimal limits they act unfavourably. If this formula is applied to the results of the present investigation (see Table 14), it is quite evident that during the period January and February 1965 the regulative factor complex was above the optimal value, resulting in the low colony counts during this period.

Szilvinyi (1948) concluded that both soil temperature and soil moisture influence the numbers of soil micro-organisms and that fungi are primarily influenced by temperature. The results of this investigation do not agree with Szilvinyi's findings. The soil moisture content proved to be a more important ecological factor than the soil temperature. It must be borne in mind, however, that in Zululand no extremes of soil temperature exist. Very low soil temperature do not occur. Although high summer temperatures are recorded for Zululand, high soil temperatures are prevented as the soil at site 1 is almost always in the shade.

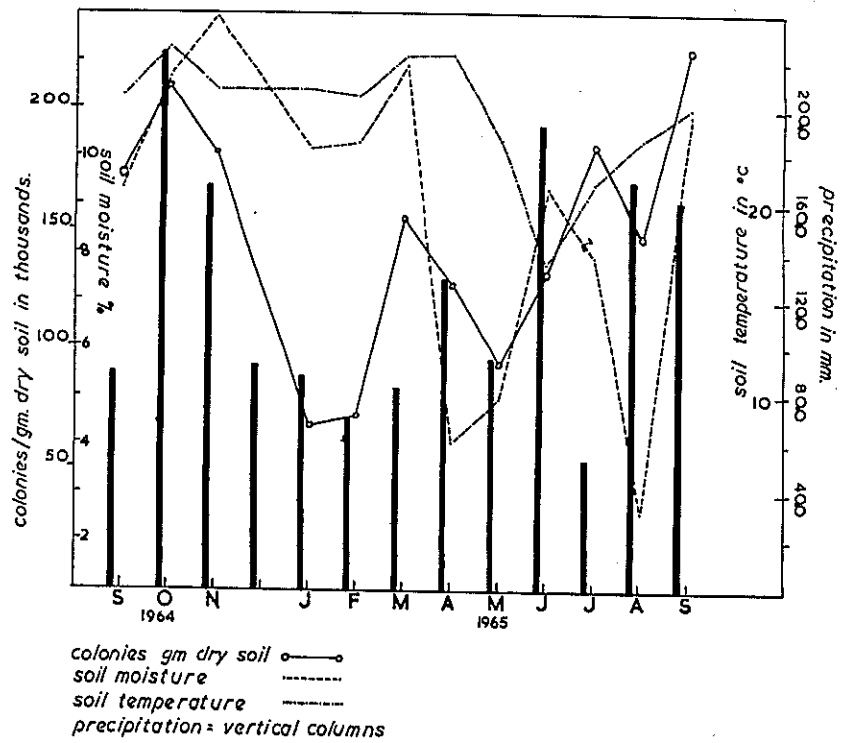


Figure 8. Seasonal variation of the fungus population at site 1 in relation to precipitation, soil moisture content and soil temperature.

TABLE 14.
NUMBER OF COLONIES PER GRAM OF DRY SOIL FROM
SOIL SAMPLES COLLECTED MONTHLY AT SITE 1.

DATE OF SAMPLING	SOIL TEMPE- RATURE AT 1" DEPTH	% SOIL MOIS- TURE (DRY SOIL)	RAIN- FALL FOR MONTH IN MM	AVERAGE NUMBER OF COLONIES PER GRAM OF DRY SOIL
18 September 1964	25.6	8.31	89.2	171,650
21 October 1964	28.3	10.67	222.6	210,850
16 November 1964	26.1	11.93	167.4	181,650
25 January 1965	26.2	9.19	86.6	67,500
23 February 1965	25.6	9.23	71.4	74,150
18 March 1965	27.8	10.88	83.2	154,150
19 April 1965	27.8	3.03	129.7	125,800
15 May 1965	23.3	3.90	94.5	92,500
14 June 1965	16.7	8.26	194.2	130,850
19 July 1965	21.1	6.95	54.2	183,500
22 August 1965	23.3	1.58	171.0	146,500
17 September 1965	25.0	9.92	162.3	224,000

CHAPTER 5

FUNGI ISOLATED BY THE SOIL-PLATE METHOD FROM SITE 1.

From September 1964 to September 1965 seventy-two soil-plates were prepared from soil samples collected from the forest floor at site 1. These soil samples were the same samples from which dilution-plates were prepared as described in the previous chapter. Six soil-plates were prepared from each monthly sample and all fungi appearing on these plates were recorded and subcultured. The fungi isolated from these soil-plates are listed in Table 15. The occurrence of each species is also given.

The soil mycoflora of the forest soil at site 1, as determined by the soil-plate method, consisted of the following numbers of species, representing three of the four major classes of fungi:

Phycomycetes	16
Ascomycetes	5
Fungi Imperfecti	47

During this investigation no Oomycetes or Basidiomycetes were recorded. The forest soil of site 1 is a dry, sandy soil probably unsuitable for the growth of oomycetous fungi. During the investigation several baiting techniques for the isolation of Pythium and other Oomycetes were employed. Such baits as boiled grass leaves, insect wings and pineapple leaves were used, but none of these fungi could be isolated from this site.

Phycomycetes were common in this soil. All

Phycomycetes isolated from soil-plates were members of the Mucorales and the following genera were represented: Absidia, Circinella, Cunninghamella, Gongronella, Mortierella, Mucor and Rhizopus. Previous investigators (Bisby, Timonin and James, 1935; Johann, 1932) have noted the fact that forest soils are generally rich in their mucoraceous-flora. Mucorales are so characteristic of certain soils that Johann (1932), distinguished various types of forest soils by the predominant mucoraceous fungi. He found that deciduous forests, which are rich in bases, contained a population consisting predominantly of the Mucor flavus type. Mixed forests, with acid soils, contained the Mucor ramannianus group while raw humus forests, with very acid soils, were characterized by a fungus flora of the Zygorhynchus moelleri type. In this investigation Mucor flavus was only observed on two soil-plates. The species M. silvaticus, M. lamprosporus and M. circinelloides were, however, very common. Soil dilution-plates also confirmed this observation. The three mucoraceous species Absidia cylindrospora, Gongronella butleri and Cunninghamella elegans appeared so regularly on the soil-plates of site 1 that these plates had a characteristic appearance.

The genus Mortierella was represented by two species, M. ramanniana and M. isabellina. As in the case of dilution-plates the members of this genus did not form an important constituent of the fungi isolated from this site. Other investigators have found the opposite, namely that this genus is usually dominant in acid forest soils. (Chesters, 1948; Warcup, 1951,a). No Zygosporangia were ever noticed on soil-plates containing members of the Zygomycetes. This is in contrast to the results of Warcup (1951, a), who often found the zygosporangia

TABLE 15

LIST OF FUNGI ISOLATED FROM SITE 1 BY
THE SOIL-PLATE METHOD.

	OCCURRENCE (MAX. 72)
<u>PHYCOMYCETES</u>	
Absidia cylindrospora Hagem	56
Circinella simplex van Tieghem	8
Cunninghamella echinulata Thaxter	2
C. elegans Lendner	45
Gongronella butleri (Lendner) Peyronel and Dale Vesco (1)	66
Mortierella isabellina (Oudemans) Zycha	16
M. ramanniana var. ramanniana (Moeller) Linneman	4
Mucor circinelloides van Tieghem	13
M. flavus Bainier	2
M. fragilis Bainier	7
M. lamprosporus Lendner	38
M. silvaticus Hagem	40
M. strictus Hagem	4
M. vallesiacus Lendner	14
Rhizopus nigricans Ehrenberg	21
Sterile Phycomycete UZ.29.	2
<u>ASCOMYCETES</u>	
Chaetosphaeria sp. UZ.53.	1
Neocosmospora vasinfecta E.F. Smith	17

	OCCURENCE (MAX. 72)
Penicillium asperum (Shear) Raper & Thom	4
Pseudeurotium sp. UZ.85.	3
Thielavia terricola (Gilman & Abbot)	
Emmons var. minor (Rays & Borut) Booth	8
<u>FUNGI IMPERFECTI</u>	
Alternaria tenuissima (Fr.) Wiltsh	6
Alternaria sp. UZ.84.	2
Aspergillus allahabadii Mehrotra & Agnibotri	19
A. flavus Link	5
A. japonicus Saito	6
Botryotrichum sp. UZ.110.	3
Candida albicans (Robin) Berkhout	2
Cladosporium oxysporum Berk. & Cart.	36
Cladosporium sp. UZ.41	6
Clathrosphaerina zalewskii van Beverw.	2
Cylindrocladium scoparium Morgan	1
Endomyces sp. UZ.46.	14
Epicoccum nigrum Link (1)	31
Fusarium semitectum Berk. & Rav.	2
F. avenaceum (Fr.) Saccardo	3
F. oxysporum Schlecht. ex Fr.	44
Fusarium sp. UZ.54.	1
Helminthosporium sp. UZ.72.	11
Humicola sp. UZ.23.	31
Humicola sp. UZ.64.	40
Humicola sp. UZ.67.	4

	OCCURRENCE (MAX. 72)
Lacellinopsis sacchari Subramanian	3
Monilia sp. UZ.28.	2
Paecilomyces elegans (Corda) Mason et Hughes apud Hughes	7
P. (?) elegans UZ.82.	4
P. marquandii (Masse) Hughes	3
Penicillium casei Loubière	42
P. digitatum saccardo	2
P. funiculosum Thom	3
P. humuli van Beyma	5
P. janczewskii Zaleski	6
Penicillium multicolor Grigorieva-Manoilova & Poradielova	9 36
P. olsoni Bainier & Sartory	36
P. soppi Zaleski	5
P. velutinum van Beijma	58
P. verruculosum Peyronel	66
P. pulvillorum Turfitt	2
Pestalotia sorbi Pat.	2
Rhinotrichum sp. UZ.33.	1
Stysanus medius Saccardo	12
Trichoderma sp. UZ.19.	70
Trichoderma sp. UZ.35.	38
Trichosporon sp. UZ.45.	3
Trichosporon sp. UZ.50.	12
Sterile colourless mycelium UZ.27.	6
Sterile colourless mycelium UZ.42.	8
Sterile dark mycelium UZ.25.	7

of heterothallic species on his soil-plates. Rhizopus nigricans was well represented on soil-plates but Circinella simplex appeared on only a small number of plates.

With the possible exception of Neocosmospora vasinfecta, which appeared on seventeen plates during the period of investigation, none of the five Ascomycetes which were isolated from the Port Durnford forest soil showed a high frequency of occurrence. Neocosmospora vasinfecta formed very characteristic bright red, closely crowded perithecia on the soil-plates. An ascosporic species of Penicillium, P. asperum, was recorded on four plates.

As is the case with most, if not all, investigations of this nature, the bulk of the species listed were members of the Fungi Imperfecti. Species of Penicillium were very common and accounted for a considerable portion of the colonies found on soil-plates. Although many species of Penicillium have been recorded from forest soils, there is little resemblance between the species isolated. In this investigation, P. verruculosum and P. velutinum were the most abundant species while P. olsoni also had a high (50%) frequency of occurrence. The other Penicillia had a frequency of occurrence of less than fifteen percent.

Aspergilli were rare. Only three species were isolated of which Aspergillus allahabadii was the only one that appeared quite regularly on soil-plates. Species of Alternaria, Botryotrichum, Paecilomyces and Fusarium were isolated from soil-plates. However, they had a low occurrence with the exception of Fusarium oxysporum, which was abundant. The genus Humicola was well represented, both in number of species and in number of colonies.

Species of Trichoderma have been found to be consistently associated with acid soils (Jensen, 1931; Cobb, 1932; Svinnifvud, 1937; Niethammer, 1939; Starc, 1941; Ellis, 1940; Rishbeth, 1951). Okada (1938), found that species of Trichoderma are so characteristic of soils that he suggested employing them as an index of the fungus population and of its distribution. With soil-plates, the same two species of Trichoderma as those found with dilution-plates were recorded with great regularity from site 1.

A very interesting isolate, Lacellinopsis sacchari, was found on soil-plates from this site. The species is known only from herbarium material, and was found on leaves and culms of Andropogon sp., Borassus aethiopum, Oryzae sativa and Saccharum officinarum from Ghana, India and Jamaica (Ellis, 1957). This appears to be the first time this fungus has been isolated from soil and grown in pure culture. It is interesting to note that this fungus occurred on the leaves of S. officinarum in Ghana and that the forest at site 1 is adjacent to extensive sugar cane plantations. The presence of sugar cane in the vicinity could account for the presence of this fungus at site 1.

Several species of yeast-like fungi were encountered during this investigation, including Candida albicans and two Trichosporon species. A few cultures remained sterile on all media and could not be identified. They include cultures UZ.27 and UZ.42 which are colourless and culture UZ.25 which is dark-coloured.

A noteworthy fungus, which was recorded on only two soil-plates, is Clathrosphaerina zalewskii. According to van Beverwijk (1951), this is an aero-aquatic fungus,

usually found on decaying birch and other leaves floating on the surface of ponds and lakes. This is, to the author's knowledge, the first record of this fungus from soil. On ordinary culture media, such as potato dextrose agar and malt extract agar, abundant clathroid spores with their typical net-like appearance, are formed. Helicae spores were not observed in the cultures.

ECOLOGICAL OBSERVATIONS:

During this investigation an attempt was made to determine whether there was any seasonal incidence in the occurrence of specific fungi in the forest soil of site 1. The occurrence of abundant fungus species on six soil-plates, prepared from each monthly soil sample, was noted. In order to be designated 'abundant', a fungus should have a frequency of occurrence of fifty percent or more. In other words, for a particular fungus to qualify for 'abundant', it must have appeared on at least fifty percent of the seventy-two soil-plates prepared during the period of sampling. In this investigation, fourteen species qualified as being abundant and they are presented in Table 16 with their monthly occurrence on six soil-plates, their total occurrence for twelve monthly samples and their percentage frequency of occurrence. Among the most abundant species were five Phycomycetes, representing the genera Absidia, Cunninghamella, Gongronella and Mucor, four Penicillium species, two unidentified Trichoderma species, Cladosporium oxysporum, Fusarium oxysporum and one species of Humicola.

The seasonal distribution of these fourteen fungi are represented graphically in the histograms of Figure 9. It

must be pointed out that no data is available for December 1964 which accounts for the gap for this month in each histogram. In the histograms the soil moisture content is also given and it is represented by the narrow vertical columns in the two lower diagrams. In Figure 9 each fungus is represented by a numbered histogram and the numbers represent the following fungi:

- 1 : *Absidia cylindrospora*
- 2 : *Cladosporium oxysporum*
- 3 : *Cunninghamella elegans*
- 4 : *Fusarium oxysporum*
- 5 : *Gongronella butleri*
- 6 : *Humicola* sp. UZ.64
- 7 : *Mucor lamprosporus*
- 8 : *M. silvaticus*
- 9 : *Penicillium casei*
- 10 : *P. olsoni*
- 11 : *P. velutinum*
- 12 : *P. verruculosum*
- 13 : *Trichoderma* sp. UZ.19
- 14 : *Trichoderma* sp. UZ.35.

From these results the following conclusions may be drawn: Four of the fungi seemed to occur abundantly throughout the sampling period of one year, irrespective of changes in the moisture content of the soil, the soil temperature and other climatic factors. These are *Absidia cylindrospora*, *Gongronella butleri*, *Penicillium verruculosum* and *Trichoderma* sp. UZ.19. If the histogram of *Penicillium verruculosum* (Figure 9 : 12) is considered, it is evident that the occurrence during the summer period, i.e. November

to March, was lower than during almost the whole of the winter period. In July the occurrence of this fungus was, however, the same as that for the summer period. The other three fungi of the group showed a constantly high occurrence on soil-plates of the monthly soil samples.

Cunninghamella elegans, Cladosporium oxysporum, Mucor lamprosporus and Humicola sp. UZ.64 showed a definite seasonal variation, their occurrence being low during the warm summer months with an increase during autumn and reaching a maximum in late winter and early spring (July to September). No colonies of Cunninghamella elegans were recorded on soil-plates for January and February 1965. Colonies of Humicola species were absent on soil-plates prepared from the January soil sample.

If the seasonal occurrence of Trichoderma sp. UZ.35 is studied, as represented in Figure 9 : 14, it appears that this fungus had two periods of high activity, i.e. in autumn during March and April and again in spring from September to November.

The fungi Fusarium oxysporum, Mucor silvaticus, Penicillium casei and P. olsoni did not show any marked seasonal periodicity in their occurrence. It does appear, however, that Mucor silvaticus and Penicillium olsoni tend to have a higher activity during the spring period, from August to October. From their histograms, Figures 9 : 8 and 9 : 10 respectively, it is clear that the highest occurrence of the former fungus was during September to October, while that of the latter fungus was during July.

The marked influence which the moisture content of the

soil exerted on the seasonal distribution of the fungus population as a whole, as determined by the dilution-plate method, could not be proved in the case of the abundant species of the population. The combined effects of various factors seemingly influenced the seasonal distribution of the abundant fungi more than any single factor. As a whole, the data indicate that at site 1 the activity of particular, abundant fungi was lowest during the summer period. This shows agreement with the results obtained in the previous chapter with dilution-plates.

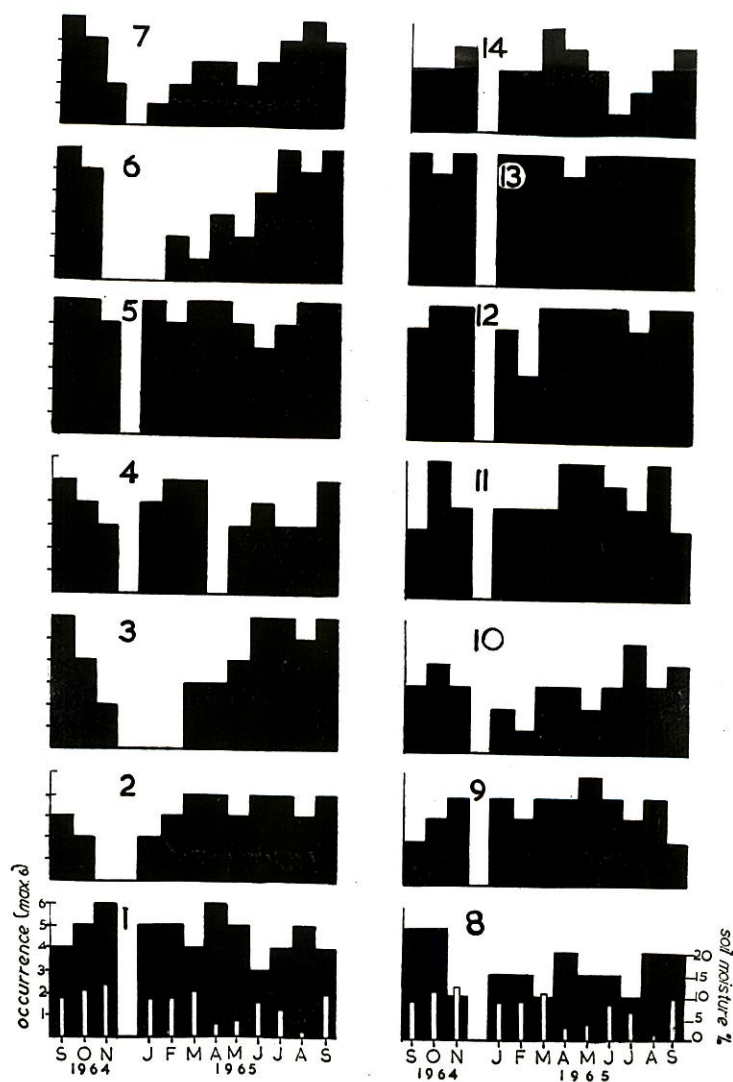


Figure 9. Seasonal distribution of fourteen abundant fungi at site 1. For explanation of the figure and key to the numbers see page (74) The narrow vertical columns in the two lower histograms refer to the percentage soil moisture.

TABLE 16.
SEASONAL DISTRIBUTION OF 'ABUNDANT' FUNGI ON SOIL-PLATES FROM SITE 1.

	OCCURRENCE (MAX. 72)	FREQUENCY OF OCCURRENCE	OCCURRENCE ON SIX SOIL-PLATES.											
			SEPT. 1964	OCT. 1964	NOV. 1964	JAN. 1965	FEB. 1965	MAR. 1965	APR. 1965	MAY 1965	JUN. 1965	JUL. 1965	AUG. 1965	SEPT. 1965
<i>Absidia cylindrospora</i>	56	78	4	5	6	5	5	4	6	5	3	4	5	4
<i>Cladosporium oxysporum</i>	36	50	3	2	-	2	3	4	4	3	4	4	3	4
<i>Cunninghamella elegans</i>	45	63	6	4	2	-	-	3	3	4	6	6	5	6
<i>Fusarium oxysporum</i>	44	61	5	4	3	4	6	5	5	-	3	3	3	5
<i>Gongronella butleri</i>	66	92	6	6	5	-	-	5	6	6	4	5	6	6
<i>Humicola</i> sp. UZ.64.	40	56	6	5	-	-	1	2	1	3	3	4	5	6
<i>Mucor lamprosporus</i>	38	53	5	4	2	1	2	2	3	2	2	3	5	4
<i>M. silvaticus</i>	40	56	5	5	2	3	3	3	2	4	3	4	4	4
<i>Penicillium casei</i>	42	58	2	3	4	4	4	3	4	4	5	3	4	2
<i>P. olsoni</i>	36	50	3	4	3	2	2	1	3	3	3	6	3	4
<i>P. velutinum</i>	58	81	3	6	4	4	4	4	6	6	6	6	6	3
<i>P. verruculosum</i>	66	92	5	6	6	6	5	3	6	6	6	5	6	6
<i>Trichoderma</i> sp. UZ.19.	70	97	6	5	6	6	6	6	6	6	6	6	6	6
<i>Trichoderma</i> sp. UZ.35.	38	53	3	3	4	3	3	5	4	3	1	2	3	4

CHAPTER 6

FUNGI ISOLATED FROM PARTIALLY STERILIZED SOIL FROM SITE 1.

While investigating fungus recolonization of soil partially sterilized by steam, Warcup (1951, b), noted that certain fungi were able to survive a mild application of this treatment. Ascomycetes, in particular, survived a short steam treatment, and many of the Ascomycetes isolated from these treated soil samples were not isolated before. Warcup concluded that these fungi possess spores which are tolerant of a short steam treatment. Many of them are also comparatively slow-growing organisms which are usually suppressed by fast growing fungi on soil-plates from untreated soil-samples.

At site 1 only five Ascomycetes were isolated using the dilution-plate and the soil-plate methods. In an attempt to discover whether any additional Ascomycetes could be isolated from this soil, the selective method of soil-steaming as described by Warcup (1951, b), was employed. The procedure followed in treating the soil-samples with steam was similar to that described by Johnson et al (1959).

Soil samples were collected from the top two inches of forest soil at site 1 on 11th May, 1965. Samples of soil, weighing 125 grams each, were placed in 500 ml glass beakers and the beakers placed in an autoclave. The autoclave was closed, but the steam valve was left open so that the temperature in the apparatus remained at 100°. Three periods were used for the steam treatment, i.e. two,

four and six minutes. After the treatment, the surface soil was removed to a depth of 1 cm, and ten soil-plates were prepared from the soil in each beaker. The results of this experiment are given in Table 17.

A total of twenty-five fungus species were isolated from the thirty soil-plates used in this experiment. Of these only four species were isolated from the soil samples which were steamed for six minutes. These are two species of Cladosporium, Lacellinopsis sacchari and a Peniophora species, being the only Basidiomycete isolated during this investigation.

Eight fungus species which were not recorded for untreated soil by either the dilution-plate or the soil-plate methods, were recorded from soil samples which were treated with steam for periods ranging from two to six minutes. These were Aspergillus fisheri, Ceuthospora sp. UZ.135, Cladosporium cladosporioides, two Humicola species, Peniophora sp. UZ.172, Penicillium aurantio-candidum and P. javanicum.

In this investigation the partial sterilization of soil prior to the preparation of soil-plates, the proportion of ascosporic to non-ascosporic species of fungi isolated, did not increase as greatly as Warcup claimed. Only two Ascomycetes were isolated by this method, viz. Aspergillus fisheri and Penicillium javanicum.

TABLE 17.

FUNGI ISOLATED FROM SOIL-PLATES PREPARED FROM
STEAM-TREATED SOIL SAMPLES FROM SITE 1.

	PERIOD OF STEAMING		
	2 MIN.	4 MIN.	6 MIN.
<i>Absidia cylindrospora</i>	+		
<i>Alternaria</i> sp. UZ.84.	+		
<i>Aspergillus fisheri</i> Wehmer	+	+	
<i>Ceuthospora</i> sp. UZ.135.		+	
<i>Cladosporium cladosporioides</i> (Frescn) De Vries		+	
<i>C. oxysporum</i>	+	+	+
<i>Cladosporium</i> sp. UZ.41.	+	+	+
<i>Cunninghamella elegans</i>	+		
<i>Fusarium avenaceum</i>	+		
<i>F. oxysporum</i>	+		
<i>Gongronella butleri</i>	+		
<i>Humicola</i> sp. UZ.39.	+	+	
<i>Humicola</i> sp. UZ.139.	+		
<i>Humicola</i> sp. UZ.153.		+	
<i>Lacellinopsis sacchari</i>			+
<i>Mucor strictus</i>	+		
<i>Penicillium asperum</i>		+	
<i>P. aurantio-candidum</i> Dierckx	+	+	
<i>P. citrinum</i>		+	
<i>P. javanicum</i> van Beijma		+	
<i>P. vermiculatum</i>	+	+	
<i>P. verruculosum</i>	+		
<i>Peniophora</i> sp. UZ.172.			+
<i>Trichoderma</i> sp. UZ.19.	+		
<i>Trichoderma</i> sp. UZ.35.	+		

CHAPTER 7

FUNGI ISOLATED FROM SITE 2 BY THE DILUTION-PLATE AND SOIL-PLATE METHODS.

At site 2, on the campus of the University College of Zululand, samples of the top two inches of soil were collected on three occasions. These samples were collected on the 21st April, 31st May and 14th June, 1965. From each of these samples ten dilution-plates and ten soil-plates were prepared. After a suitable incubation period (see chapter 2), counts were made of the number of colonies developing on each of the dilution-plates. All fungus colonies developing on both dilution-plates and soil-plates were subcultured on potato dextrose agar and their occurrence on these plates was recorded. The results of this investigation are given in Table 18.

The average number of colonies per gram of dry soil over the period April to June 1965, as determined by the dilution-plate method, was 127,250. During this period, the average soil moisture content was found to be 20.12% while the average soil temperature for this three-month period was 20.6°. During the corresponding period the following results were obtained from site 1: Average number of colonies per gram of dry soil - 116,383, average soil moisture content - 5.06% and average soil temperature - 22.6°. If the two sets of results are compared, the following conclusions may be drawn. At site 2, where the soil has a high clay content as can be seen from its mechanical analysis in Table 6, the soil moisture content was much higher than that of site 1. On the other hand, site 2 had

TABLE 18.

LIST OF FUNGI ISOLATED FROM SITE 2
BY TWO ISOLATION METHODS.

	SOIL-PLATE OCCURRENCE (MAX. 30)	DILUTION- PLATE OCCURRENCE (MAX. 30)
<u>PHYCOMYCETES</u>		
Cunninghamella echinulata Thaxter	5	
Gongronella butleri (Lendner) Peyronel (1)	13	17
Mortierella isabellina (Oudemans) Zycha	3	
Mortierella sp. UZ.166.	2	
Mucor lamprosporus Lendner	1	4
M. silvaticus Hagem	8	10
M. vallesiacus Lendner	1	
M. varians Povah	4	
Rhizopus oryzae Went & Gerlings		2
Zygorhynchus moelleri Vuillemin	1	5
<u>ASCOMYCETES</u>		
Chaetomium trilaterale Chivers	1	
Neocosmospora vasinfecta E.F. Smith	12	3
Penicillium asperum (Shear) Raper & Thom	4	2
P. javanicum van Beijma	13	18
P. vermiculatum	2	
<u>FUNGI IMPERFECTI</u>		
Alternaria sp. UZ.84.	2	
Aspergillus flavus Link	1	

<i>A. japonicus</i> Saito	2	5
<i>A. tubingensis</i> (Schröber) Mosseray	9	
<i>Bispora</i> sp. UZ.116.		1
<i>Botryotrichum</i> sp. UZ.110.	3	
<i>Candida albicans</i> (Robin) Berkhout	21	27
<i>Cephalosporium</i> sp. UZ.69.		4
<i>Cephalosporium</i> sp. UZ.121	1	
<i>Cladosporium oxysporum</i> Berk. and Cart.	5	2
<i>Epicoccum nigrum</i> Link (2)	2	
<i>Fumago</i> sp. UZ.95	2	
<i>Fusarium culmorum</i> (W.G. Smith) Saccardo	9	12
<i>F. moniliiforme</i> Sheld	1	3
<i>F. oxysporum</i> Schlecht ex Fries	10	7
<i>Humicola</i> sp. UZ.23.	15	3
<i>Humicola</i> sp. UZ.64.	12	2
<i>Humicola</i> sp. UZ.90.	2	
<i>Humicola</i> sp. UZ.151.		1
<i>Humicola</i> sp. UZ.161.	3	4
<i>Humicola</i> sp. UZ.162.	6	
<i>Humicola</i> sp. UZ.167.	1	
<i>Monilia</i> sp. UZ.108.	1	
<i>Paecilomyces elegans</i> (Corda) Mason et Hughes apud Hughes	14	9
<i>Penicillium citrinum</i> Thom	12	16
<i>P. jenseni</i> Zaleski	14	3
<i>P. ochro-chloron</i> Biourge	4	2
<i>P. olsoni</i> Bainier and Sartory	16	27
<i>P. soppi</i> Zaleski	4	9
<i>P. simplicissimum</i> (Oudemans) Thom	4	4

P. steckii Zaleski	2	
P. velutinum van Beijma		8
P. verruculosum Peyronel	1	3
Phialophora sp. UZ.165.	1	
Pestalotia pauciseta Saccardo	2	
Sclerophoma sp. UZ.118.		3
Scopulariopsis brumptii Salvanet-Duval		4
Septonema sp. UZ.130.		3
Trichoderma sp. UZ.19.	21	9
Trichoderma sp. UZ.35.	7	12
Trichoderma sp. UZ.61.	3	
Trichoderma sp. UZ.115.		2
Trichoderma sp. UZ.122.		3
Trichoderma sp. UZ.125.		1
Trichosporon ^r sp. UZ.45.	15	
Trichosporon sp. UZ.50.	6	
Verticillium sp. UZ.14.	9	4
Sterile dark mycelium. UZ.150.		3
Sterile colourless mycelium. UZ.42.	6	
Sterile colourless mycelium. UZ.164.	9	
TOTAL NUMBER OF SPECIES ISOLATED	53	39

a lower soil temperature (than site 1), during this period. If the earlier remarks on the influence of soil moisture content on the number of colonies developing on dilution-plates are borne in mind, it is not unexpected to find that the soil samples from site 2 yielded a higher colony number than those from site 1.

Although the soil of site 2 gave a higher number of colonies per gram of dry soil than site 1, fewer species of fungi were isolated from the former site. The fungus flora of this site was found to be composed of the following numbers of species representing each of the major classes of the fungi.

Phycomycetes	10
Ascomycetes	5
Fungi Imperfecti	50

Of the ten species of Phycomycetes isolated, none represented the Oomycetes. They all belonged to the Zygomycetes. The following genera were represented: Cunninghamella, Gongronella, Mortierella, Mucor, Rhizopus and Zygorhynchus. The genus Mucor proved to be the most abundant Phycomycete. Mucor silvaticus was the most common species and it was recorded on both soil-plates and dilution-plates. Three other species of Mucor, M. lamprosporus, M. vallesiacus and M. varians were also isolated from site 2, although these three species did not appear on the isolation plates regularly. Many investigators have found that the genus Mortierella was very common in the soils which they investigated. Bisby, James and Timonin (1933) found species of this genus to be the dominant members of the Phycomycete flora of Manitoba soils. Warcup (1951)

came to the same conclusions regarding his studies on the fungus flora of Breckland soils, while Stenton (1953) found Mortierella minutissima van Tieghem the most abundant species in the alkaline, peaty soils of Wicken Fen. At site 2 only two Mortierella species were isolated. Both these fungi had a low occurrence and they were only isolated from soil-plates. Warcup (1957) noted that many fungi, including Mortierella, were more abundant on soil-plates than on dilution-plates.

The most abundant phycomycetous species at site 2 was Gongronella butleri (isolate 2). It occurred with great regularity on both soil-plates and dilution plates, having an average frequency of occurrence of 50% on these plates. Although the author believes that this isolate represents a strain different from G. butleri (isolate 1) which was isolated from site 1, these two isolates have in common the fact that they are the fungi that occur most abundantly at their respective sites. Yung and Stenton (1964), found in their study of the phycomycete-flora of Hong Kong soils that Mucor, particularly M. subtilissimus Oudemans, and Gongronella butleri were the fungi which occurred most commonly and abundantly. They also found that soil samples from certain of the sites which they investigated yielded no isolates of Mortierella. In these two respects the results of the present investigation are in accordance with those obtained by these two authors.

Five species of Ascomycetes were recorded for site 2. Of these, an ascosporic species of Penicillium, P. javanicum, was the most important. It occurred with great regularity on dilution-plates and soil-plates prepared from the surface

soil. A member of the Hypocreales, Neocosmospora vasinfecta, was also very common, particularly on soil-plates.

Chaetomium trilaterale was recorded from only a single soil-plate. Two more ascosporic Penicillia were recorded, viz. P. asperum and P. vermiculatum, but they were quite rare on isolation-plates.

No Basidiomycetes were recorded from site 2. During the period of investigating this site the author found no basidiocarps growing above ground.

Fungi Imperfecti represented the majority of colonies which developed on the isolation-plates of this site. Penicillium undoubtedly constituted the most important genus, both in number of species and in occurrence. In soils largely under grass cover most of the species of Penicillium were found in acid rather than alkaline soils. Jensen (1931), found that Pencillium species were common in acid soils. In acid Wareham Heath soils Penicillia were found to be the most important constituent of the fungus flora (Brian, Hemming and Mc Gowan, 1945). These results were also confirmed by Warcup in 1951(a). At site 2 the species P. citrinum, P. jenseni and P. olsoni were the most common. Paecilomyces elegans also had a high frequency of occurrence on isolation-plates.

Although three species of Aspergillus, A. flavus, A. japonicus and A. tubingensis, were isolated from site 2, they had a low occurrence and represented an insignificant component of this sites mycoflora.

The genera Candida, Cladosporium, Fusarium, Humicola, Trichoderma, Trichosporon and Verticillium were quite common

and occurred regularly on the isolation-plates. Niethammer (1941), found Fusarium species to occur in the presence of species of Mucor and Zygorhynchus in the soil. This was also the case at site 2 where, apart from the latter two Phycomycetes, three species of Fusarium, F. culmorum, F. moniliforme and F. oxysporum occurred. F. culmorum and F. oxysporum had a relatively high occurrence on both soil-plates and dilution-plates. F. moniliforme was rare.

The genus Humicola formed an important constituent of the fungus population of site 2, both in the number of species and in the number of colonies developing on isolation plates. The Humicola species were particularly abundant on soil-plates and very few soil-plates did not actually contain at least one colony of this genus. Two isolates of the yeast-like genus Trichosporon were recorded regularly on soil-plates, while the yeast Candida albicans was one of the fungi to appear most frequently on soil-plates and dilution-plates of the natural soil of this site. Cladosporium was represented by only one species, namely Cladosporium oxysporum.

The genus Trichoderma was one of the most common Moniliales of this soil. Seven different isolates, which show definite morphological and cultural differences, were isolated. The species UZ.19 and UZ.35 were the most abundant. These two species were also found to be very abundant in the forest soil of site 1 and must be regarded as important soil fungi of the warm Zululand climate. Many investigators, in many parts of the world, agree that species of Trichoderma represent an important part of the soil microflora. Sewell (1959), could demonstrate by direct

observation of soil by the Rossi-Cholodny slide technique, that Trichoderma hyphae were present in the soil. He found that this fungus grew vigorously in the soil, particularly in the surface soil, and on root surfaces.

Trichoderma can thus be regarded as a true soil-inhabiting fungus.

If the number of species isolated by the two isolation-methods are compared it is noticed that a larger number of species were isolated by the soil-plate method than by the dilution-plate method. Fifty-three species were isolated using soil-plates while only thirty-nine species were isolated from dilution-plates. This is in contrast to the results obtained by Warcup (1957), in Australian wheat-field soil.

CHAPTER 8

THE VERTICAL DISTRIBUTION OF FUNGI AT SITES 1 AND 2.

It has been found that fungi are most numerous in the surface layers of the soil and that their numbers usually decrease with increasing soil depth. This is particularly the case in a soil which is fairly uniform in texture and composition. The phenomenon that the number of fungi decrease with depth is, however, not always the case. In soils which have well-developed horizons, these may influence both the number of individuals and the species of fungi present (Burges, 1958). In order to determine the vertical distribution of fungi in the two soils studied, the following investigations were performed. At each site a profile pit was dug and soil samples were collected from one face of this pit by the use of glass specimen tubes. The procedure of this sampling method is described in detail in Chapter 2.

1. Site 1:

The vertical distribution of fungi at this site was investigated along two lines, i.e. by the dilution-plate method and by a modified soil-plate method.

(a) Dilution-plate method:

On 5th May, 1965, profile soil samples were collected from the forest soil at site 1. Samples were taken at depths of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28 inches below the surface. From each of these soil-samples

five dilution-plates were prepared, and after they were incubated for a suitable period, the fungus colonies that developed on the plates were counted. A second series of soil samples were taken for the determination of the soil-moisture content, the pH and the organic matter content. The results of this investigation are tabulated in Table 19. On the day of sampling very small differences in the soil temperature were determined at the different soil levels down to 28 inches. The average soil temperature was 22.1°.

Preliminary experimentation revealed that very few colonies developed on dilution-plates from soil-samples at levels deeper than 9 inches. To compensate for this reduction in the number of fungi, a lower dilution of the soil suspension was used in preparing dilution-plates from these soil horizons. The results of this investigation are presented graphically in Figure 10.

The results obtained clearly indicate that there is a marked decrease in the number of colonies with increasing soil depth at site 1. The phenomenon that the number of colonies of fungi generally decrease rapidly with increasing depth, has been demonstrated by previous investigators (Waksman, 1932; Warcup, 1951, a, 1957; Stenton, 1953; Sewell, 1959; Yung and Stenton, 1964). From the present results it can be seen that the decrease in the number of colonies takes place very rapidly from the surface to a depth of 8 to 10 inches (see Figure 10). At

depths greater than 10 inches the decrease in colony numbers is not so sharp. In fact, a slight increase in colony numbers between 12 and 14 inches and between 20 and 24 inches of soil depth was noticed.

The reason why the number of colonies decreased with depth in the very homogenous soil profile of site 1 is not obvious. The soil temperature as an edaphic factor in determining this vertical distribution of the fungus population may be excluded. It was found that this factor remains quite constant in the different soil layers.

While there was a rapid decrease in soil moisture and organic matter content in the first 8 to 10 inches of soil, accompanied by a rapid decrease in colony numbers, this correlation does not hold true for the whole profile. For example, from a depth of 6 to 8 inches and also from 18 to 22 inches, there was an increase in the organic matter content. But in these layers the constant decrease in fungal numbers continued, evidently not influenced by the increased organic matter content. Obviously the carbon content of this soil alone cannot account for this decrease of colony numbers in the soil profile. This conclusion does not agree with the results of Gray and Mc Master (1933), and Gray and Taylor (1935), who found that the activity of micro-organisms depends upon the organic matter content in the various soil horizons. A major role was also assigned to the organic matter content of the soil in determining the distribution of soil-organisms by Timonin (1935).

TABLE 19.

NUMBER OF COLONIES PER GRAM OF DRY SOIL AT VARIOUS DEPTHS OF SAMPLING AT SITE 1. (SOIL TEMP. 22.2°).

DILUTION FACTOR	DEPTH OF SAMPLE IN INCHES	SOIL MOISTURE CONTENT PERCENTAGE	pH GLASS-ELECTRODE	% ORGANIC MATTER (LOSS ON IGNITION)	TOTAL NO. OF COLONIES ON FIVE PLATES.	AVERAGE NUMBER OF COLONIES PER PLATE	COLONIES PER GRAM OF DRY SOIL
1:5000	1	7.9	5.86	2.88	121	24.2	121,000
"	2	7.6	5.68	1.90	93	18.6	93,000
"	3	6.7	5.54	1.83	56	11.2	56,000
"	4	6.0	5.33	1.47	47	9.4	47,000
"	5	6.5	5.30	1.22	39	7.8	39,000
"	6	6.0	5.26	0.98	35	7.0	35,000
"	7	5.1	5.22	1.10	30	6.0	30,000
"	8	4.3	5.23	1.18	25	5.0	25,000
1:2500	9	4.5	5.21	1.11	43	8.6	21,500
"	10	4.1	5.21	0.83	41	8.2	20,500
"	12	4.1	5.16	1.08	39	7.8	19,500
"	14	3.1	5.15	0.99	43	8.6	21,500
"	16	2.9	5.14	0.96	40	8.0	20,000
"	18	2.7	5.16	0.89	23	4.6	11,500
"	20	3.1	5.17	1.05	10	2.0	5,000
"	22	3.2	5.17	1.19	16	3.2	8,000
"	24	3.1	5.20	0.81	19	3.8	9,500
"	26	3.4	5.50	0.89	12	2.4	6,000
"	28	3.5	5.45	0.80	7	1.2	3,000

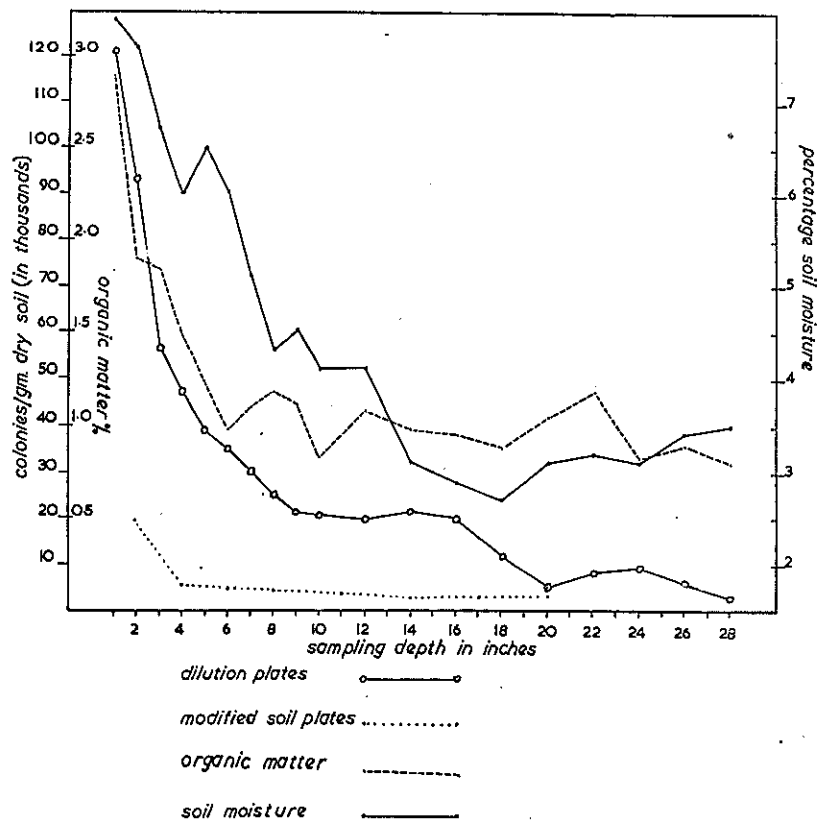


Figure 10: Vertical distribution of the fungus population at site 1 in relation to organic matter and moisture content of the soil profile.

The same author showed, however, that other factors may be involved. He found that numbers of micro-organisms in peat decreased with depth although there was a high humus content throughout the profile. Brown (1958), found that there is a general tendency for fungus populations to be poorer in the deeper layers of the soil even if other factors are the same. She also found that in dune soils a sub-surface increase in organic matter content of the soil could be correlated with an increase in mycelial content. This was not the case at site 1. Although there was an increase in organic-matter content in some sub-surface soil layers, for example between 18 and 22 inches, no increase in the colony numbers took place in these soil layers. This is in accordance with observations by Timonin (1935), and Stenton (1953). They found that micro-organisms decreased with increasing soil-depth, despite high concentrations of organic matter.

From a depth of 18 inches and deeper there is a more or less constant increase in soil moisture content, and although there is not a rapid decrease in fungus numbers below 18 inches, the tendency for the numbers to decrease is quite clear from the results. The results obtained at site 1 indicate that the water content of the soil does not have a direct influence on the vertical distribution of the fungi.

Table 19 shows that there is a general decrease in the pH of the soil to a depth of 16 inches, after which a slight increase again takes place. All these

results make it quite obvious that the decrease in number of fungal units with increasing depth cannot be attributed to a single factor alone. It is probable that the combined effect of a number of factors, such as soil-moisture content, soil-temperature, carbon content and probably some other factors all act together to bring about this vertical decrease in numbers. Waid (1960), lists the following non-biological factors which may influence the growth and mycelium production of fungi in soil:

- (i) Temperature
- (ii) Moisture
- (iii) Carbon dioxide and oxygen concentration
- (iv) Size of soil-pore spaces
- (v) Durability of fungal mycelium
- (vi) Interaction between soil fungi and soil fauna.

There is yet another factor which may be concerned in the determination of the vertical distribution of fungi in sandy soils, such as that of site 1. Burges (1950), obtained data which suggested that the downward movement of fungal spores in the soil, particularly those spores with wettable, mucilaginous coats, may determine the depth at which such fungi might be isolated. He was able to demonstrate a correlation between spore-type and the depth at which a fungus occurred in the soil. Hepple (1960), disagreed with the theory of Burges that spores are washed downwards through the profile. She concluded that the conditions needed to bring about movement of spores in the soil are very specialized and are likely

to occur only exceptionally in nature.

(b) Modified soil-plate method:

Because of the well-known criticism that the dilution-plate method exaggerates the importance of heavily sporing fungi, an attempt was made to estimate the vertical distribution of the soil population at site 1 by means of the soil-plate method. In view of the fact that the soil-plate method, as originally described by Warcup (1950), is not a quantitative method, the following modification was employed.

Profile soil samples were collected in the usual manner (see Chapter 2). but instead of large glass sampling tubes special, small glass sampling tubes were used. These sampling tubes were $2\frac{1}{2}$ inches long with an internal diameter of $\frac{3}{8}$ inch. They were plugged tightly with non-absorbent cotton wool and kept in small polythene bags to reduce drying out of the soil samples. After a soil sample had been removed from the face of a profile pit, the sampling tube was carefully cleaned and accurately weighed. Approximately twenty milligrams of soil ~~was~~^{were} transferred aseptically from the sampling tube to an Erlenmeyer flask containing 100 ml of sterile, melted, but cooled Czapek-Dox with yeast extract agar. Beforehand, the correct amount of Rose Bengal, Streptomycin and Aureomycin was added to this medium. The flask, containing the soil suspension was shaken gently by hand for five minutes and then the agar was poured into ten sterile Petri dishes. While pouring the plates, it was necessary to shake

the agar continually to keep the soil particles evenly dispersed. A little experience was required to distribute the 100 ml of medium evenly between the ten plates. The plates were incubated at 26° for 5 days after which all fungus colonies growing on all ten plates were counted.

After the removal of an unoculum from a sampling tube, the tube was again weighed. The tube, with the remaining soil was dried at 105° for 12 hours. After cooling in a desiccator the tube was again weighed. From these determinations the moisture content of the soil, the weight of the inoculum, and the dry weight of the inoculum could be calculated. The average amount of soil (the inoculum) could easily be calculated then, and from this, the number of fungal colonies per gram of dry soil.

Soil samples for this investigation were collected on 22 nd. April, 1965 from the forest soil at site 1. The special glass sampling tubes were inserted in one face of a profile pit at depths of 2, 4, 6, 8, 11, 14, 17 and 20 inches below the surface in order to extract a soil sample. The moisture content and the inoculum weight of each profile sample is given in Table 20 while the number of colonies per gram of dry soil at the various depths of sampling is given in Table 21 and presented graphically in Figure 10.

Although the counts obtained with these soil-plates were considerably lower than those with dilution-plates, the results obtained by the modified soil-plate method reflect the same decrease in fungal

TABLE 20

MOISTURE CONTENT AND INOCULUM WEIGHT
FOR MODIFIED SOIL-PLATES FROM SOIL
SAMPLES COLLECTED AT SITE 1.

DEPTH OF SAMPLE IN INCHES	MOISTURE CONTENT PERCENTAGE	INOCULUM WEIGHT IN MG	WEIGHT OF INOCULUM IN MG AFTER CORRECTION FOR MOISTURE CONTENT
2	5.685	5.40	5.10
4	15.134	13.90	12.07
6	33.348	18.10	13.57
8	14.374	16.55	14.31
11	7.109	11.55	10.78
14	9.784	17.40	15.84
17	11.098	29.80	26.22
20	12.349	33.00	29.37

TABLE 21.

NUMBER OF COLONIES PER GRAM OF DRY SOIL AT VARIOUS DEPTHS OF
SAMPLING AT SITE 1 AS DETERMINED BY MODIFIED SOIL-PLATES.

SAMPLING DEPTH IN INCHES	REPLICATES										TOTAL NUMBER OF COLONIES	AVERAGE NUMBER OF COLONIES	COLONIES PER GRAM OF DRY SOIL
	1	2	3	4	5	6	7	8	9	10			
2	11	10	11	11	10	9	12	10	9	7	100	10.0	19,608
4	6	8	8	8	6	7	5	5	6	4	63	6.3	5,219
6	4	6	6	7	8	8	9	7	6	5	66	6.6	4,864
8	5	7	8	6	6	8	5	9	7	6	67	6.7	4,682
11	6	2	5	3	2	2	6	3	6	6	41	4.1	3,803
14	5	3	4	3	5	3	4	4	3	5	39	3.9	2,462
17	9	8	7	7	6	6	5	6	8	6	68	6.8	2,593
20	12	9	9	7	7	11	12	12	8	8	95	9.5	3,235

units with increasing depth. There is a rapid decrease from two to four inches, while the decrease in colony numbers is much less from four to twenty inches in depth. A slight increase from a depth of fourteen inches to twenty inches might, in this case, be attributed to a marked increase of soil moisture at this level, as can be seen from table 20.

The reason why the number of colonies per gram of dry soil was so much lower with the modified soil-plate method than with the dilution-plate method, should be sought in the procedure followed to obtain the dilution of the soil suspension. In the dilution-plate method the soil inoculum is shaken for 30 minutes in water, while in the modified soil-plate method the soil inoculum is shaken for only five minutes in melted agar. A much better dispersion of soil particles is thus obtained by the first method and fungus propagules are broken up more completely. Another reason for this low count might be that a certain amount of the soil inoculum is retained in the flash containing the agar when pouring the plates. In the author's view this method is neither very reliable, nor is it successful.

2. Site 2:

On the 6th June, 1966, soil samples were collected at depths of 1, 3, 5, 7, 9, 11, 13, 15 and 17 inches below the surface of the soil at site 2. In order to determine the vertical distribution of the fungus population, ten dilution-plates were prepared from each

TABLE 22.

NUMBER OF COLONIES PER GRAM OF DRY SOIL AT VARIOUS DEPTHS OF
SAMPLING AT SITE 2. (SOIL TEMP. 20.0°)
(DILUTION FACTOR 1:5000).

DEPTH OF SAMPLE IN INCHES	SOIL MOISTURE CONTENT PERCENTAGE	pH GLASS- ELECTRODE	% ORGANIC MATTER (LOSS ON IGNITION)	TOTAL NO. OF COLONIES ON TEN PLATES	AVERAGE NUMBER OF COLONIES PER PLATE	COLONIES PER GRAM OF DRY SOIL
1	25.58	6.10	9.20	330	33.0	165,000
3	24.00	6.10	8.58	298	29.8	149,000
5	21.62	6.07	7.61	274	27.4	137,000
7	16.47	6.32	6.02	241	24.1	120,000
9	16.02	6.45	5.23	194	19.4	97,000
11	9.60	6.12	2.48	88	8.8	44,000
13	17.29	5.95	4.92	54	5.4	27,000
15	20.86	5.70	6.24	101	10.1	50,500
17	23.98	5.75	6.81	217	21.7	108,500

profile sample and from these plates the number of colonies per gram of dry soil was determined. Because of the very rocky nature of the lower levels of this soil, sampling could not be carried out at depths greater than 17 inches. The results of this investigation are given in Table 22. A second series of soil samples were taken for the determination of the soil-moisture content, the pH and the organic matter content. The results of these determination are also given in the table. The average soil temperature for the profile on the day of sampling was 20.0° . In the author's view differences in temperature between different soil layers were so slight that this factor need not be taken into account.

The results of this investigation, which are graphically represented in Figure 11, show clearly that there was a relatively constant decrease in the number of fungus colonies from the surface soil layer down to a depth of thirteen inches. It is interesting to note that in the profile of site 2 there was not such a very sharp initial decrease in the colony numbers in the first few inches of soil as that found at site 1.

A very marked increase in the number of fungus colonies was recorded in the layers between thirteen and seventeen inches. Although this phenomenon might seem strange, a possible explanation might be found if the soil-moisture content and the organic-matter content of this profile is taken into account. The value of both these factors decreases considerably from the soil surface to a depth of eleven inches (see also Table 7, chapter 3). From a depth of eleven inches to a depth of seventeen inches, an increase in the moisture content and the organic matter

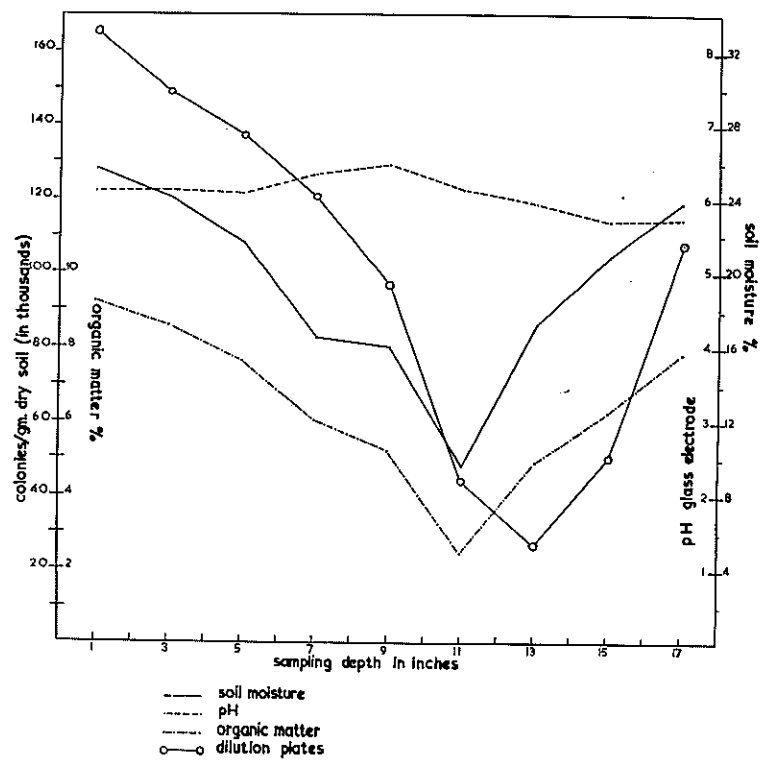


Figure 11: Vertical distribution of the fungus population at site 2 in relation to soil moisture content, pH and organic matter content of the soil profile.

content of this soil took place. In the case of the soil population of this forest soil the increase in numbers may be attributed to the high organic matter content of the soil. This increase in the number of colonies as a result of an increase of the carbon content in the subsurface soil layers is in accordance with the results obtained by Timonin (1935), in some Canadian podzol profiles. The results of this investigation also support the conclusions of Gray and Taylor (1935), that the activities of micro-organisms in a soil can be correlated with the carbon content of the various soil horizons. The results obtained from site 2 differ from those of site 1 in this respect. At site 1 an increase in organic matter content of the soil horizon was not accompanied by a corresponding increase in colony numbers.

No correlation between the acidity of the soil horizon and the colony counts could be demonstrated. It should be emphasized, however, that the differences in the pH-values for the various horizons of the profile were insignificant.

Burges (1958), found that, in certain East Anglican soils with a uniform profile of coarse sand, the number of organisms diminished markedly within a few centimetres of the surface and continues to decrease with depth. The results obtained at site 1 with the present investigation are in agreement with the results obtained by Burges. The soil of site 1 has the same characteristics as that of the East Anglican soil and there is a marked decrease in colony numbers with increasing soil depth.

Burges also found that in soils which have well-

defined horizons there often is a marked increase in the number of colonies in the lower soil horizons. Site 2 has a well-marked A and C horizon (see chapter 3), and the results obtained in this investigation agree with those obtained by Burges.

CHAPTER 9

THE VERTICAL DISTRIBUTION OF THE MORE COMMON SPECIES OF FUNGI AT SITES 1 AND 2.

In order to determine the vertical distribution of the more common species of soil fungi in the two Zululand soils, the following investigation was carried out.

1. Site 1:

Soil samples were collected on 19th July, 1965 from the forest soil at site 1. Samples were taken at 1, 2, 4, 6, 8, 10, 12, 16, 21, 25 and 30 inches below the surface. From each soil sample ten soil-plates were prepared. After a suitable period of incubation the plates were examined under a dissecting microscope. All fungi which could not be recognized were subcultured on potato dextrose agar for subsequent identification. A fungus received a positive record for every soil-plate on which it appeared. The results of these isolations are given in Table 23.

From the results it is evident that the occurrence of individual fungus species decreased with increasing depth. These fungi were abundant in the top six inches of the soil profile, while their occurrence fell off at depths greater than six inches. From the results in Table 23 and in Figure 12, it can be seen that there was not only a decrease in the number of colonies with increasing depth but also a decrease in the number of fungal species in the deeper soil layers. At a depth

TABLE 23

OCCURRENCE (MAX. 10) OF FUNGI ON SOIL-PLATES
PREPARED FROM SOIL SAMPLES COLLECTED AT
VARIOUS DEPTHS AT SITE 1.

SAMPLING DEPTH IN INCHES	OCCURRENCE ON 10 SOIL-PLATES										
	1	2	4	6	8	10	12	16	21	25	30
<i>Absidia cylindrospora</i>	8	5	3	5	6	2	-	-	-	-	-
<i>Alternaria</i> sp.	2	1	-	1	-	1	1	-	1	3	-
<i>Alternaria tenuissima</i>	2	1	4	7	2	-	3	2	1	-	1
<i>Aspergillus allahabadii</i>	5	2	-	3	-	3	-	3	1	3	2
<i>Cephalosporium</i> sp. UZ.69.	-	-	-	-	-	-	-	-	3	-	-
<i>Cladosporium oxysporum</i>	5	6	4	3	3	4	5	3	3	2	3
<i>Cladosporium</i> sp. UZ.41.	3	-	1	-	-	4	-	-	-	-	-
<i>Cunninghamella elegans</i>	4	-	-	-	1	-	1	-	-	-	-
<i>Epicoccum nigrum</i> (1)	4	2	-	-	-	-	-	-	-	-	-
<i>Fusarium oxysporum</i>	9	8	8	4	6	2	-	-	-	-	-
<i>Fusarium</i> sp. UZ.54.	1	-	1	-	-	1	-	-	-	-	-
<i>Gongronella butleri</i>	10	6	9	10	8	8	5	2	2	-	-
<i>Helminthosporium</i> sp. UZ.72.	1	-	-	-	1	3	1	1	2	-	-
<i>Humicola</i> sp. UZ.23.	1	1	-	1	-	-	-	-	-	-	-
<i>Humicola</i> sp. UZ.64.	6	-	2	4	-	-	-	-	-	-	-
<i>Lacellinopsis sacchari</i>	1	-	-	1	-	-	-	-	-	-	-
<i>Mucor lamprosporus</i>	4	-	-	1	-	-	-	-	-	-	-
<i>Mucor silvaticus</i>	7	3	-	-	-	-	-	-	-	-	-
<i>Mucor vallesiacus</i>	2	-	1	1	-	-	-	-	-	-	-
<i>Neocosmospora vasinfecta</i>	-	-	-	-	-	1	1	4	-	-	-
<i>Penicillium casei</i>	5	4	3	3	-	4	5	7	8	9	6
<i>P. olsoni</i>	3	3	-	3	1	-	-	-	-	-	-
<i>P. soppi</i>	1	-	-	-	-	-	1	-	-	-	-

P. velutinum	6	6	3	8	5	6	-	-	-	-	-
P. verruculosum	7	5	4	1	-	-	-	-	-	-	-
Psendeurotium sp. UZ.85.	-	-	-	-	-	-	-	-	-	1	-
Sterile colourless mycelium UZ.27.	1	-	-	1	-	-	-	2	1	-	-
Sterile colourless mycelium UZ.43.	-	-	1	-	-	-	-	-	1	-	-
Thielavia terricola	-	1	-	-	1	-	-	-	-	2	1
Trichoderma sp. UZ.19.	8	8	7	10	8	2	-	1	-	-	-
Trichoderma sp. UZ.35.	2	3	3	6	3	-	2	3	1	-	-
Trichosporon sp. UZ.45.	-	1	-	-	-	1	1	-	-	-	-
TOTAL NUMBER OF SPECIES	26	18	15	19	12	14	11	10	11	6	5

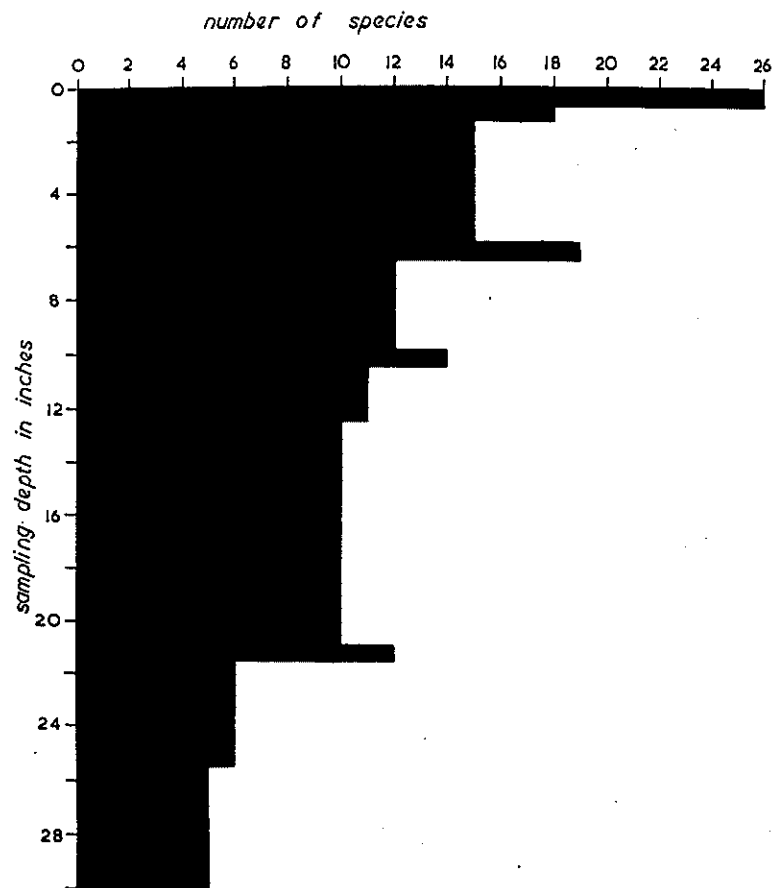


Figure 12. Variation in the numbers of species of fungi with increasing depth at site 1.

of one inch, twenty-six species of fungi were recorded on the soil-plates, while only five species were recorded on plates from the deepest soil sample, i.e. a depth of thirty inches. A similar decrease in the number of species has been recorded by various authors (Warcup, 1951; Sewell, 1959; Yung and Stenton, 1964).

In Figure 13 histograms are presented of some of the abundant fungi from site 1. The histogram numbers refer to the following fungi:

- 1 : *Absidia cylindrospora*
- 2 : *Alternaria tenuissima*
- 3 : *Cladosporium oxysporum*
- 4 : *Penicillium velutinum*
- 5 : *P. casei*
- 6 : *Gongronella butleri*
- 7 : *Penicillium verruculosum*
- 8 : *Fusarium oxysporum*
- 9 : *Trichoderma* sp. UZ.19.

The available data show that in general most of those species which were abundant in the surface soil of site 1, were also common in the subsurface layers. In other words, many of the abundant fungi had a wide vertical range in the soil. This fact has also been reported by Tresner, Backus and Curtis in 1945, and by Warcup in 1957. A few species which occurred abundantly in the surface soil layers were, however, not recorded in the subsurface layers. They are *Aspergillus allahabadii*, *Humicola* sp. UZ.23, *Mortierella isabellina*, *Mucor vallesiacus*, *Neocosmospora vasinfecta*, *Rhizopus nigricans*, *Stysanus medius* and *Trichosporon* sp. UZ.50. These fungi are probably limited

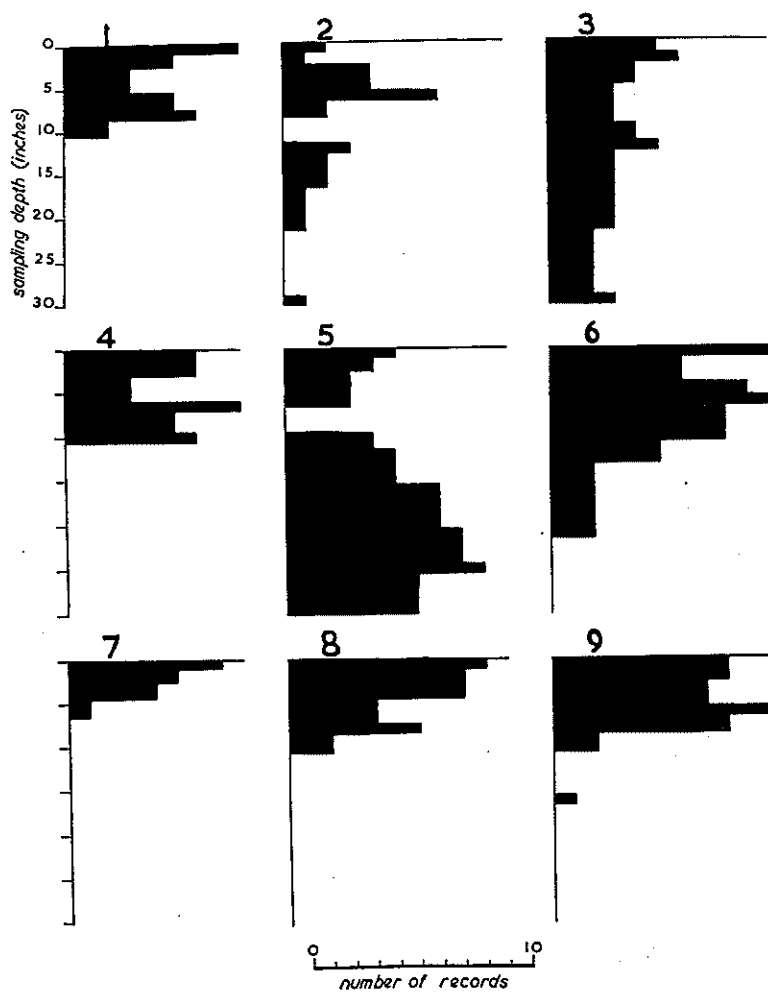


Figure 13. Vertical distribution of abundant fungi at site 1. For explanation of the figure and key to the numbers see page 112.

to the upper layers of the soil profile where suitable substrates are, presumably, most abundant.

From the results given in Table 23 and Figure 13 it is evident that most of the individual species of fungi show the same pattern of decrease in numbers with increasing depth as that found in the fungus community as a whole. Exceptions to this generalization, however, were Penicillium velutinum, P. casei and Trichoderma sp. UZ.19, which were common in the sub-surface soil layers, and actually had a higher occurrence on soil-plates prepared from the deeper soil samples than on those from the surface samples.

The vertical distribution of Penicillium casei is noteworthy. This fungus appeared very commonly on soil-plates from most soil layers of the profile, reaching a maximum occurrence at a depth of 25 inches. This species was also very abundant on dilution-plates and modified soil-plates of soil samples from these profile samples, and it accounted for the majority of colonies on these plates.

Most of the species isolated from soil-plates decreased rapidly at depths of more than six inches. Alternaria tenuissima and Cladosporium oxysporum, however, occurred fairly commonly throughout the profile.

2. Site 2:

Soil samples were collected on 16th June, 1966 from the face of a profile pit at site 2 at the following depths: 1, 3, 5, 7, 9, 11, 13, 15 and 17 inches. From each soil sample ten soil-plates were prepared and the occurrence of fungi was recorded on these plates.

TABLE 24

OCCURRENCE (MAX : 10) OF FUNGI ON SOIL-PLATES
PREPARED FROM SOIL SAMPLES COLLECTED AT
VARIOUS DEPTHS AT SITE 2.

	OCCURRENCE ON 10 SOIL-PLATES									
DEPTH OF SAMPLE IN INCHES	1	3	5	7	9	11	13	15	17	
Alternaria sp. UZ.84.	2	2	4	2	5	3	1	2	1	
Aspergillus tubingensis	-	-	-	1	-	1	1	2	1	
Candida albicans	-	-	-	1	-	1	1	3	1	
Cladosporium oxysporum	6	7	6	4	4	2	-	3	3	
Fusarium culmorum	3	1	3	1	1	-	-	-	1	
Fusarium oxysporum	-	-	1	-	-	-	-	-	-	
Gongronella butleri	3	2	-	-	-	-	-	-	1	
Humicola sp. UZ.23.	3	2	1	1	1	-	-	-	2	
Humicola sp. UZ.64.	2	-	1	-	-	-	-	-	-	
Humicola sp. UZ.162.	1	-	-	-	-	-	-	-	-	
Mucor silvaticus	2	2	1	2	1	-	1	-	-	
M. vallesiacus	1									
Neocosmospora vasinfecta	2	3								
Paecilomyces elegans	3	-	2	-	-	-	-	-	-	
Penicillium asperum	-	-	-	1	-	-	-	-	-	
P. citrinum	6	8	5	-	2	-	-	-	1	
P. javanicum	6	4	3	4	2	1	-	-	1	
P. jenseni	1	-	1	2	1	-	-	-	1	
P. olsoni	10	7	8	7	10	10	9	10	10	
P. steckii	1	1	-	1	-	-	-	-	-	
Pestalotia pauciseta	1	-	-	-	-	-	-	-	-	

Trichoderma sp. UZ.19.	8	9	5	4	5	5	4	2	2
Trichoderma sp. UZ.35.	10	10	7	10	8	6	6	4	6
Trichoderma sp. UZ.61.	1	1	-	-	-	-	-	-	1
Trichosporon sp. UZ.45.	2	4	1	-	-	2	-	-	1
Verticillium sp. UZ.14.	-	2	-	-	1	-	-	-	-
TOTAL NUMBER OF SPECIES	21	16	15	14	12	9	7	7	15

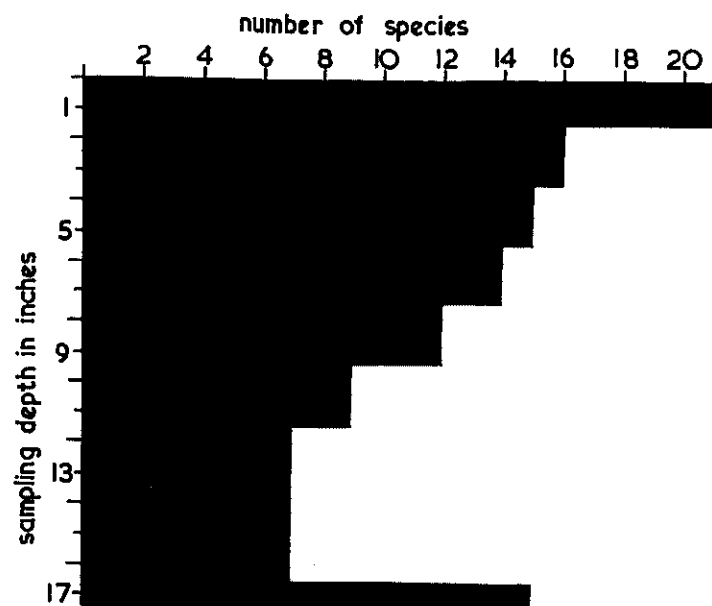


Figure 14. Variation in the numbers of species of fungi with increasing depth at site 2.

The occurrence of twenty-six of the more common fungi of this site is given in Table 24.

With reference to the number of fungus species at various levels of the soil horizon, a sharp decrease is evident in the first fifteen inches of the profile (see Figure 14). A surprising phenomenon is, however, a very pronounced increase in the number of species at a depth of seventeen inches, where the top-soil layer and the underlying granite layer meet. This increase in species numbers also corresponds to a similar increase in the numbers of colonies, as discussed in the previous chapter. The same increase in the number of species at a depth near seventeen inches was also noted on dilution-plates prepared for the estimation of colony numbers. No satisfactory explanation can be given for this increased activity in the mycoflora at this horizon. It is true that there is a considerably higher organic matter content in this layer than in the overlying soil layers. This higher carbon concentration could account for the increase in colony numbers and species numbers at a depth of seventeen inches.

Some of the more abundant fungi at site 2 show an interesting distribution through the soil profile. The vertical distribution of six of these fungi is presented graphically in the histograms in Figure 15. The numbers of the histograms refer to the following fungi:

- 1 : *Alternaria* sp. UZ.84.
- 2 : *Cladosporium oxysporum*
- 3 : *Penicillium javanicum*
- 4 : *P. olsoni*

5 : *Trichoderma* sp. UZ.19.

6 : *Trichoderma* sp. UZ.35.

With increasing soil depth four of these species showed a marked decrease in their occurrence. They are Cladosporium oxysporum (see Figure 15 : 2), Penicillium javanicum (see Figure 15 : 3), and the two Trichoderma species, UZ.19 (see Figure 15 : 5) and UZ.35 (see Figure 15 : 6). Trichoderma sp. UZ.35 was very common on soil-plates of the whole soil profile and, although there was a decrease in the occurrence of this fungus with increasing soil depth, this decrease was very slight. It still had a relatively high occurrence even at the lowest sampling depths. The number of recordings for Trichoderma sp. UZ.19 declined rapidly with increasing depth. Similar results were also obtained for this species at site 1, where the lowest depth from which it was isolated was sixteen inches (see Table 23). Sewell (1959), found that Trichoderma viride occurred on every soil-plate prepared from the first inch, but decreased rapidly in its occurrence with increasing depth in Calluna-heath soils.

The vertical distribution of Alternaria sp. UZ.84 (see Figure 15 : 1) in the soil profile is very interesting. This fungus was more active in the subsurface soil layers with a maximum occurrence at a depth of nine to eleven inches. This same phenomenon was noted at site 1 for another Alternaria sp., viz. A. tenuissima, which had a higher occurrence in the subsurface soil layer, with a maximum occurrence at a depth of six inches.

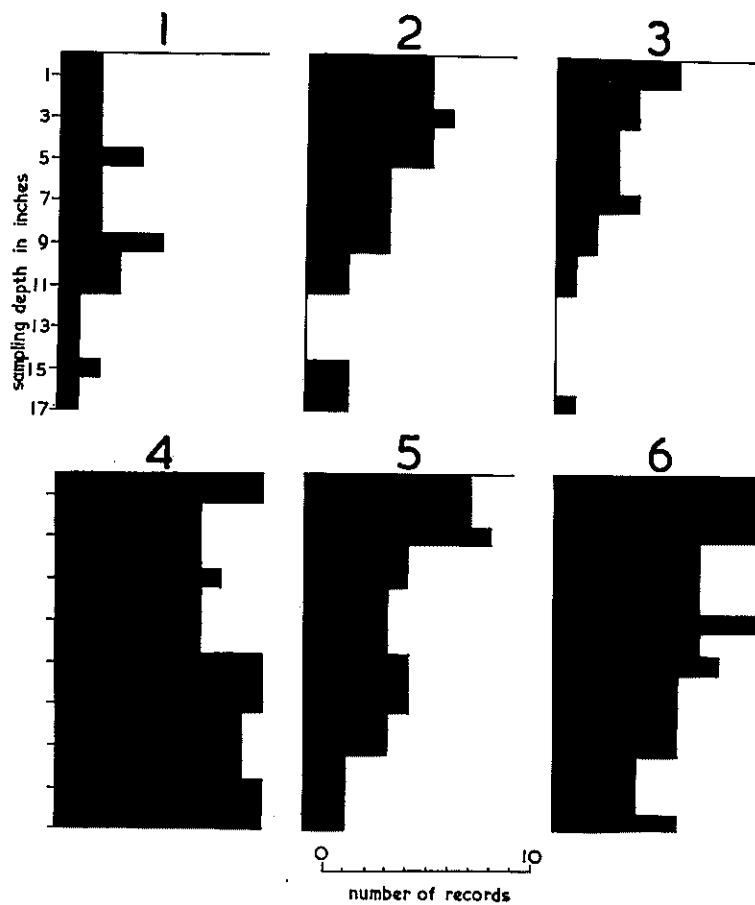


Figure 15. Vertical distribution of abundant fungi at site 2. For explanation of the figure and key to the numbers see pages 118 and 119.

Penicillium olsoni occurred abundantly throughout the soil profile. This species was particularly abundant in the lower soil horizons at a depth varying from nine to seventeen inches. It occurred so abundantly on dilution-plates prepared from soil samples of the lower soil horizons, that the colonies of this species represented the majority of colonies that developed on these isolation plates.

In this investigation at site 2, no indication of any influence of such factors as pH, soil moisture content and organic matter content on the vertical distribution of some of the abundant fungi could be found. These factors, particularly the latter two, had a strong influence on the fungus population of this site as a whole, but evidently not on the individual fungi which were investigated.

It is difficult to evaluate the data on the vertical distribution of fungi in the soil profile. According to Warcup (1951), it is probable that many of the fungi occurring commonly in the lower soil horizons are slow growing types and may be present in the surface regions, but as a result of close competition they fail to grow on the crowded isolation-plates prepared from the surface soils. Sewell (1959), came to the same conclusion. He also found that the vertical distribution as revealed by soil-plates could, with a few exceptions, be related to the growth rates of the particular fungi on soil-plates and that the competition of rapidly growing species greatly influenced the isolation of those with lower growth rates.

CHAPTER 10

FUNGI ISOLATED FROM LEAF LITTER OF EUCALYPTUS MACULATA AT SITE 1.

In his presidential address before the British Mycological Society, Chesters (1949), stressed the importance of including a study of the fungus flora of the surface litter in any attempt at studying the microbial ecology of a particular soil. He said: "I am certain that soil investigations in future must commence by examining this surface litter and must work downwards into the lower horizons." In 1960, the same author went further and pointed out the importance of litter to the economy of natural soils: "It is a layer of sheet compost contributing, by its microbiological decay, minerals and soluble organic compounds to the water descending from the surface into the soil. Physically it interposes a surface mulch between the A₁ horizon and the atmosphere - a not invaluable contribution."

Up to the present, few microbiological investigators of soil paid much attention to the surface litter. Ward (1952), and Kendrick (1958), studied the association of fungi occurring in coniferous litter. Pugh (1954), studied the fungi developing on Carex paniculata leaves. Witkamp (1960), studied the seasonal variation of the fungus flora on the litter of an oak forest. More recently, Macauley and Thrower (1966), investigated the succession of fungi in leaf litter of Eucalyptus regnans F. Muell. in Australia. Unfortunately the latter two authors did not investigate the mycoflora of the underlying mineral soil. As a matter of fact, with the exception of Witkamp, not one of the above

mentioned investigators did investigate the soil mycoflora of the sites which they studied.

In this investigation an attempt was made to study the fungus flora of the two litter layers, i.e. Aooo and Ao (see chapter 3) in the Port Durnford forest, and to compare and correlate the fungous flora of the litter with that found in the underlying mineral soil. Three methods were used for the isolation of the mycoflora of the litter. For the analysis of the mycoflora of the Aooo horizon, i.e. freshly fallen gum tree litter, the serial washing technique of Parkinson and Kendrick (1960), and the leaf disc method of Witkamp (1960), were used. For the study of the Ao horizon, i.e. the well-decomposed litter, the soil-plate method was used.

1. Serial washing of litter from horizon Aooo:

Freshly fallen gum tree leaves were collected with sterile forceps and placed in sterile, stoppered glass jars. In the laboratory, the leaves were cut into strips of about 15 mm x 5 mm and placed in a sterile flask. The leaf strips were then subjected to a serial washing in order to remove unwanted spores and other contaminants adhering to the leaf material. They were shaken in ten changes of sterile 1% (V/V) aqueous solution of "Teepol", and then shaken in ten changes of sterile, distilled water. The leaf strips were then plated out on Czapek-Dox agar to which yeast extract had been added, as with soil-plates. The usual anti-bacterial agents, i.e. Rose Bengal, Streptomycin and Aureomycin, were also added to this agar for the

suppression of bacteria. Three leaf strips were placed on each plate and distributed evenly on the surface of the agar. After an incubation period of five days at 26°, all the colonies that developed on these plates were isolated and subcultured on potato dextrose agar. During August, 1965, three batches of leaf-litter were collected from site 1 and from each batch ten plates, with three leaf strips each, were prepared. The fungi isolated from this leaf litter by the serial washing technique are given in Table 25.

2. Leaf disc method for litter from horizon Aooo:

Freshly fallen gum tree leaves were collected as described above. In the laboratory, leaf discs, with a diameter of 5.5 mm, were punched from unwashed leaves with the aid of a sterile cork borer. The leaf discs were placed on sterile cover slips, with a diameter of 25 mm, on the surface of potato dextrose agar plates. The leaf discs did not come into contact with the agar medium and only hyphae growing from the discs could colonize the medium. Bacterial contamination was limited and the addition of antibacterial agents to the potato dextrose agar proved to be unnecessary. The plates were incubated at 26°, and examined periodically until they were either overgrown, or until no more new colonies developed. To prevent desiccation of the leaf discs on the coverslips, a drop of sterile water was administered, with the aid of a hypodermic syringe, to each disc every second day. The colonies that developed were isolated and subcultured on potato dextrose agar. During the period June to September, 1965,

four batches of leaf litter were collected. From each batch six plates, with three leaf discs on each plate, were prepared. The results of these isolations are given in Table 25.

3. Soil-plate method for litter from horizon Ao ('humus layer'):

Well-decomposed litter from the humus layer at site 1 was collected with the aid of a sterilized steel spatula. After the surface litter was scraped away carefully, the underlying litter was picked up with the spatula and placed in sterile Petri dishes. In the laboratory the litter was pulverised with the aid of a sterile steel spatula. Soil-plates were then prepared from this pulverised litter-material. After an incubation period of five days at 26°, all colonies developing on these plates were isolated and subcultured on potato dextrose agar. During the period June to September 1965, the humus litter layer at site 1 was sampled four times and from each litter sample ten soil-plates were prepared. The results of these isolations are given in Table 25.

The mycoflora of the forest litter of site 1 consisted of the following numbers of species, representing three of the major classes of fungi:

Phycomycetes	10
Ascomycetes	5
Fungi Imperfecti	30

No Basidiomycetes were isolated.

TABLE 25.

FUNGI ISOLATED FROM THE FOREST LITTER FROM
SITE 1 USING THREE ISOLATION TECHNIQUES.

HORIZON	Aooo			Ao
	ISOLATION METHOD	SERIAL WASHING	LEAF DISC	SOIL PLATES
<u>PHYCOMYCETES</u>				
Absidia cylindrospora Hagem		+	+	+
Cunninghamella elegans Thaxter		+	+	
Gongronella butleri (Lendner) Peyronel & Dal Vesco (1)		+		+
Mortierella isabellina (Oudemans) Zycha		+		
M. ramanniana var. ramanniana (Moeller) Linneman				+
Mucor circinelloides van Tieghem		+		+
M. flavus Bainier				+
M. fragilis Bainier				+
M. silvaticus Hagem			+	
M. strictus Hagem		+		+
<u>ASCOMYCETES</u>				
Chaetomium sp. UZ.97.			+	
Chaetosphaeria sp. UZ.53.				+
Melanospora sp. UZ.99.			+	
Penicillium asperum (Shear) Raper & Thom		+		
Thielavia terricola (Gilman & Abbot) var. minor (Rays & Borut) Booth			+	

<u>FUNGI IMPERFECTI</u>			
Aspergillus cervinus Massee ememd. Neill		+	
A. flavus Link	+		
A. japonicus Saito	+		+
Aureobasidium sp. UZ.98.		+	
Cephalosporium sp. UZ.69.			+
Cladosporium oxysporum Berk. & Cart.	+	+	+
Fusarium avenaceum (Fr.) Saccardo			+
F. oxysporum Schlecht. ex Fr.	+		+
F. semitectum Berk. & Rav.			+
Fusarium sp. UZ.54.			+
Helminthosporium sp. UZ.72.	+		
Monilia sp. UZ.28.	+		
Penicillium casei Loubière			+
P. funiculosum Thom	+		+
P. humuli van Beijma		+	+
P. multicolor Grigorieva-Manoilova & Poradielova	+		+
P. olsoni Bainier & Sartory	+		
P. pulvillorum Turfitt	+		
P. soppi Zaleski		+	+
P. velutinum van Beijma	+		+
P. verruculosum Peyronel	+	+	+
Pestalotia sorbi Pat.	+		
Phoma sp. UZ.94.			+
Trichoderma sp. UZ.19.	+		+
Trichoderma sp. UZ.35.			+
Trichoderma sp. UZ.61.			+

Sclerotium sp. UZ.155.		+	
Sphaeroraema sp. UZ.58.		+	
Sterile colourless mycelium UZ.127.	+		
Sterile colourless mycelium UZ.164.		+	
TOTAL NUMBER OF SPECIES FOR EACH METHOD	22	15	26
TOTAL NUMBER OF SPECIES FOR EACH HORIZON	Aooo:33		Ao:26

The total number of species isolated from each litter zone show no significant difference. Twenty-six species were isolated from the A₀ horizon while thirty-three were isolated from the A₀₀₀ horizon. The distribution of the individual species in these horizons, however, differed widely. Only fourteen of the forty-five species isolated from the litter occurred in both the litter zones (see Table 25). The ascomycetes were more abundant in the fresh (A₀₀₀) litter than in the A₀ litter layer, where only one ascomycetous species was isolated, namely a species of Chaetosphaeria. The Fungi Imperfecti appeared to be evenly distributed throughout the litter profile. This confirms the results obtained by Ward (1952), on coniferous forest litter. With regard to the Phycomycetes, Ward found that members of the Mucorales were more abundant in very highly decomposed than in fresh litter. In this investigation six mucoraceous species were isolated from the A₀₀₀ horizon, seven from the A₀ horizon, while four of these isolates occurred in both the litter zones.

The distribution of some representatives of Fungi Imperfecti is interesting. From the results it is clear that species of Fusarium were very abundant in the decomposing litter of the A₀ horizon, while only one species, F. oxysporum, was present in the A₀₀₀ layer. Three species of Aspergillus were isolated from the A₀₀₀ horizon of which only one, A. japonicus, also occurred in the A₀ zone. The Penicillia seem to have an even distribution in the different litter layers. Except for the two species Penicillium olsoni and P. pulvillorum, all the species occurring on the fresh litter (A₀₀₀) were also present in

the decomposing litter (Ao). If it is considered that the latter two Penicillium species were common in the underlying mineral soil, it seems that their absence from the Ao horizon might be due to a shortcoming in the isolation technique rather than to their not actually being present in this litter layer. One species, P. casei, was isolated only from decomposed litter and not from fresh litter.

Trichoderma sp. UZ.19 and Cladosporium oxysporum were isolated abundantly from all zones of the litter. Two other species of Trichoderma occurred only in the Aooo zone. This observation is not in accordance with the results obtained by Pugh (1958), for the vertical masses of leaf litter accumulating round plants of Carex paniculata.

Species of Mortierella have been reported to be among the most common fungi of forest litter. Ward (1952), reported on species of this genus as being the commonest Phycomycetes in coniferous forests. Melin (1934) noted that Mortierella was the most frequent phycomycete in profiles of Manitoba soils. On the other hand Macauley and Thrower (1966), found no Mortierella species on Eucalyptus regnans leaf litter in Australia. Two species of Mortierella, M. isabellina and M. ramanniana var. ramanniana, were isolated from gum tree litter in this investigation. They had a rather low occurrence on the isolation plates.

If the fungi isolated from Eucalyptus maculata leaf litter are compared with those which were isolated from the underlying mineral soil (compare Table 25 with Tables 13, 15 and 17), the following conclusions may be drawn:

A total of eighty-eight fungus species were isolated from the soil and leaf litter at site 1, of which thirty-two species were common to both soil and litter. Forty-four of the isolated species were present only in the underlying mineral soil horizons and absent from the litter. Twelve species were isolated only from the litter layer, being either absent from the soil or present in such small numbers that they were not recorded on isolation plates. They are Aspergillus cervinus, Aureobasidium sp. UZ.98, Cephalosporium sp. UZ.121, Chaetomium sp. UZ.97, Melanospora sp. UZ.99, Penicillium pulvillorum, Phoma sp. UZ.94, Sclerotium sp. UZ.155, Sphaeroraema sp. UZ.58, Trichoderma sp. UZ.61, and two sterile mycelial forms, UZ.127 and UZ. 164.

Of the sixteen Phycomycetes isolated from site 1 only nine were present in the litter, while all sixteen were isolated from the mineral soil. A similar scarcity of phycomycetous species in litter has been noted by other workers (Kendrick, 1958; Pugh, 1958; and Yung and Stenton, 1964). Half of the Ascomycetes isolated from this site were also present in the litter. Sixty-one species of Fungi Imperfecti were isolated from site 1, of which thirty were present in the litter.

Although this investigation showed that there are great differences in the composition of the fungous flora of the litter and in that of the soil proper, it is significant to note that thirty-two fungus species were common to both the soil and the litter. This suggests a close agreement between the organic horizons and the mineral horizons of the soil profile with respect to the mycoflora which they support.

CHAPTER 11

DISCUSSION

Apart from contributing to the knowledge concerning the soil-inhabiting fungi of Zululand, the main value of this investigation is that, by making parallel studies of the fungal population of two soils, it has been possible to indicate relationships between soil type, vegetation, litter and active soil fungal population.

Table 26 lists all the fungi isolated during this investigation and gives the site from which each species was isolated. The majority of these species are spore formers. It is possible that many mycelial forms were not recorded owing to the method of isolation employed during this investigation. A total of one hundred and twenty-one species of fungi was isolated. At site 1 a total of seventy-six species was isolated, while the number of species isolated from site 2 was sixty-six. The leaf-litter at site 1 yielded forty-four species.

To be able to compare the composition of the mycoflora of the various investigated sites, it is convenient to follow Peyronel's method (Peyronel and Dal Vesco, 1955). In this method the percentage for the separate groups of fungi is presented in the form of a diagram (see Figure 16). This diagram is an adaptation of the so-called polygonal graph or polygon as introduced by Hutchinson (1936), for the simultaneous portrayal of several variables.

The analysis of the two polygons in Figure 16

shows that, with regard to the composition of the mycoflora, the two sites studied reveal some points of similarity. Both sites had a relatively high percentage of Phycomycetes. At site 1, 21.3% of the fungi isolated were Phycomycetes, while the percentage of Phycomycetes at site 2 was 15.1% (see also Table 27). Both these sites had a high Penicillium percentage, but a low percentage of Aspergilli. A similar relationship also exists with regard to the Dematiaceous species. At site 1, 20.0% of the fungi isolated belonged to the Dematiaceae while at site 2 24.3% of the species belonged to this family. On the whole, there exists a close similarity between the composition of the respective mycofloras of these two sites. This is also true if the results summarized in Table 26 are considered. Only twenty-nine of the species of fungi which were isolated from site 2 were not found at site 1, while forty-five of the species isolated from site 1 were not recorded from soil samples of site 2. Thirty-three fungus species were common to both sites. Considering the differences in the composition of the soil and the vegetation (see chapter 3) between the two sites, this similarity in mycoflora composition is a rather unexpected result. Indeed, the only factors common to these two sites are the macro-climate and the fact that both soil types are acid.

If the polygons (Figure 14) for the forest litter and the mineral soil at site 1 are compared, an appreciable difference in the composition of the mycoflora of the litter is noticed. Fewer Dematiaceae were found on the litter than in the soil while the litter had a higher percentage of Tuberculariaceae. The litter also had a

TABLE 26

LIST OF ALL SPECIES ISOLATED FROM SITES 1
AND 2 AND FROM THE FOREST LITTER AT SITE 1.

	SITE 1	LITTER	SITE 2
<u>PHYCOMYCETES</u>			
<u>Mucorales</u>			
Absidia cylindrospora Hagem	+	+	
Circinella simplex van Tieghem	+		
Cunninghamella elegans Lendner	+	+	
C. echinulata Thaxter	+		+
Gongronella butleri (Lendner) Peyronel and Dal Vesco (1)	+	+	
G. butleri (Lendner) Peyronel & Dal Vesco (2)			+
Mortierella isabellina (Oudemans) Zycha	+	+	+
M. ramanniana var. ramanniana (Moeller) Linneman	+	+	
Mortierella sp. UZ.166.			+
Mucor circinelloides van Tieghem	+	+	
M. flavus Bainier	+		
M. fragilis Bainier	+	+	
M. lamprosporus Lendner	+		+
M. strictus Hagem	+	+	
M. vallesiacus Lendner	+		+
M. varians Povah			+
M. silvaticus Hagem	+	+	+
Rhizopus oryzae Went & Gerlings			+

Rhizopus nigricans Ehrenberg	+		
Zygorhynchus moelleri Vuillemin			+
Sterile Phycomycete mycelium UZ.29.	+		
TOTAL NUMBER OF SPECIES	16	9	10
<u>ASCOMYCETES.</u>			
Aspergillus fisheri Wehmer	+		
Chaetomium trilaterale Chivers			+
Chaetomium sp. UZ.97.		+	
Chaetosphaeria sp. UZ.53.	+	+	
Melanospora sp. UZ.99.		+	
Neocosmospora vasinfecta E.F. Smith	+		+
Penicillium asperum (Shear) Raper & Thom	+	+	+
P. javanicum van Beijma	+		+
P. vermiculatum Saccardo	+		+
Pseudeurotium sp. UZ.85.	+		
Thielavia terricola (Gilman and Abbot)			
Emmons var. minor (Rays & Borut) Booth	+	+	
TOTAL NUMBER OF SPECIES	8	5	5
<u>BASIDIOMYCETES</u>			
Peniophora sp. UZ.172.	+		
TOTAL NUMBER OF SPECIES	1	-	-

<u>FUNGI IMPERFECTI</u>			
<u>Moniliaceae.</u>			
(a) Aspergilli:			
Aspergillus allahabadii Mehrotra + Agnibotri	+		
A. cervinus Massee emend. Neill		+	
A. flavus Link	+	+	+
A. japonicus Saito	+	+	+
A. tubingensis (Schröder) Mosseray			+
TOTAL NUMBER OF SPECIES	3	3	3
(b) Penicillia:			
Penicillium aurantio-candidum Dierckx	+		
P. casei Loubière	+	+	
P. citrinum Thom	+		+
P. digitatum Saccardo	+		
P. funiculosum Thom	+	+	
P. humuli van Beijma	+	+	
P. jenseni Zaleski			+
P. multicolor Grigorieva-Manoilova & Poradielova	+	+	
P. ochro-chloron Biourge			+
P. olsoni Bainier & Sartory	+	+	+
P. pulvillorum Turfitt		+	
P. simplicissimum (Oudemans) Thom			+

P. soppi Zaleski	+	+	+
P. steckii Zaleski			+
P. velutinum van Beijma	+	+	+
P. verruculosum Peyronel	+	+	+
TOTAL NUMBER OF SPECIES	11	9	9
(c) Other Moniliaceae:			
Cephalosporium sp. UZ. 69.		+	+
Cephalosporium sp. UZ.121.			+
Cylindrocladium scoparium Morgan	+		
Monilia sp. UZ.28.	+	+	
Monilia sp. UZ.108.			+
Paecilomyces elegans (Corda) Mason & Hughes apud Hughes	+		+
Paecilomyces sp. UZ.82.	+		
P. marquandii (Masse) Hughes	+		
Rhinotrichum sp. UZ.33.	+		
Scopulariopsis brumptii Salvanel-Duvat			+
Sphaeroraema sp. UZ.58.		+	
Trichoderma sp. UZ.19.	+	+	+
Trichoderma sp. UZ.35.	+	+	+
Trichoderma sp. UZ.61.		+	+
Trichoderma sp. UZ.115.			+
Trichoderma sp. UZ.122.			+
Trichoderma sp. UZ.125.			+
Verticillium sp. UZ.14.			+
TOTAL NUMBER OF SPECIES	8	6	12

<u>Dematiaceae.</u>			
<i>Alternaria tenuissima</i> (Fr.) Wiltsh.	+		
<i>Alternaria</i> sp. UZ.84.	+		+
<i>Aureobasidium</i> sp. UZ.98.		+	+
<i>Bispora</i> sp. UZ.116.			+
<i>Botryotrichum</i> sp. UZ.110.	+		+
<i>Cladosporium cladosporioides</i> (Frescn.) De Vries	+		
<i>C. oxysporum</i> Berk. & Cart.	+	+	+
<i>Cladosporium</i> sp. UZ.41.	+		
<i>Clathrosphaerina zalewskii</i> van Beverw.	+		
<i>Fumago</i> sp. UZ.95.			+
<i>Helminthosporium</i> sp. UZ.72.	+	+	
<i>Humicola</i> sp. UZ.23.	+		+
<i>Humicola</i> sp. UZ.64.	+		+
<i>Humicola</i> sp. UZ.67.	+		
<i>Humicola</i> sp. UZ.90.			+
<i>Humicola</i> sp. UZ.139.	+		
<i>Humicola</i> sp. UZ.151.			+
<i>Humicola</i> sp. UZ.153.	+		
<i>Humicola</i> sp. UZ.161.			+
<i>Humicola</i> sp. UZ.162.			+
<i>Humicola</i> sp. UZ.167.			+
<i>Lacellinopsis sacchari</i> Subramaniam	+		
<i>Phialophora</i> sp. UZ.165.			+
<i>Septonema</i> sp. UZ.130.			+
Dark sterile mycelium UZ.25.	+		
Dark sterile mycelium UZ.150.			+
TOTAL NUMBER OF SPECIES	15	3	16

Tuberculariaceae and Stilbaceae:			
Epicocœum nigrum Link (1)	+		
E. nigrum Link (2)			+
Fusarium avenaceum (Fr.) Saccardo	+	+	
F. culmorum (W.G. Smith) Saccardo			+
F. moniliiforme Sheld			+
F. oxysporum Schlesht. ex Fr.	+	+	+
F. semitectum Berk. and Rav.	+	+	
Fusarium sp. UZ.54.	+	+	
Stysanus medius Saccardo	+		
TOTAL NUMBER OF SPECIES	6	4	4
Other Fungi Imperfecti:			
Candida albicans (Robin) Berkhout	+		+
Ceuthospora sp. UZ.135.	+		
Endomyces sp. UZ.46.	+		
Pestalotia pauciseta Saccardo			+
P. sorbi Pat.	+	+	
Phoma sp. UZ.94.		+	
Sclerophoma sp. UZ.118.			+
Sclerotium sp. UZ.155.		+	
Trichosporon sp. UZ.45:	+		+
Trichosporon sp. UZ.50.	+		+
Sterile colourless mycelium UZ.27.	+		
Sterile colourless mycelium UZ.42.	+		+
Sterile colourless mycelium UZ.127.		+	

Sterile colourless mycelium UZ.164.		+	+
TOTAL NUMBER OF SPECIES	8	5	7
TOTAL NUMBER OF SPECIES AT EACH SITE	76	44	66
TOTAL NUMBER OF SPECIES ISOLATED	121		

TABLE 27
COMPOSITION OF THE FUNGUS FLORAS OF SITES 1
AND 2 AND THE FOREST LITTER OF
SITE 1.

PEYRONEL'S GROUPS	POLYGON AXES NUMBER	SITE 1.		LITTER.		SITE 2.	
		NUMBER OF SPECIES	PERCENTAGE OF TOTAL	NUMBER OF SPECIES	PERCENTAGE OF TOTAL	NUMBER OF SPECIES	PERCENTAGE OF TOTAL.
Phycomycetes	1.	16	21.3	9	20.4	10	15.1
Ascomycetes	2.	8	10.6	5	11.4	5	7.6
Aspergilli	3.	3	4.0	3	6.8	3	4.5
Penicillia	4.	11	14.6	9	20.4	9	13.7
Other Moniliaceae	5.	8	10.6	6	13.6	12	18.2
Dematiaceae	6.	15	20.0	3	6.8	16	24.3
Tuberculariaceae and Stilbaceae	7.	6	8.0	4	9.1	4	6.0
Other Fungi Imperfecti	8.	8	10.6	5	11.4	7	10.6
TOTALS		75	99.7	44	99.9	66	100

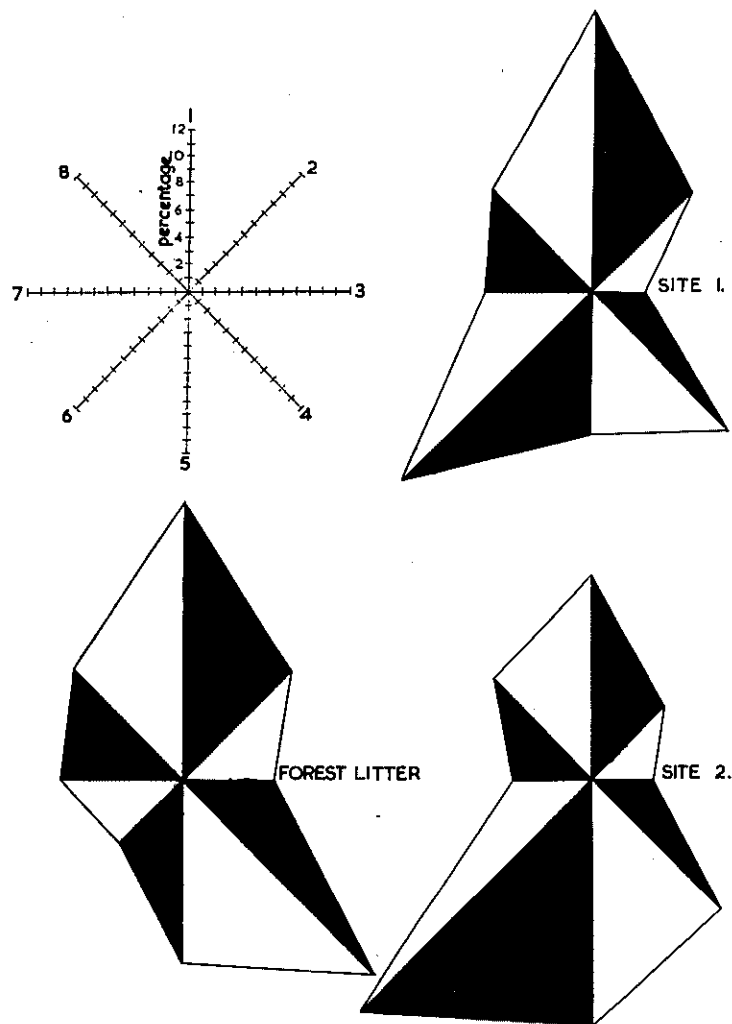


Figure 16. Percentage of species belonging to Peyronel's groups isolated from site 1, forest litter of site 1 and site 2. The axes of the diagrams correspond to the following groups:

- 1 - Phycomycetes,
- 2 - Ascomycetes,
- 3 - Aspergilli,
- 4 - Penicillia,
- 5 - Other Moniliaceae,
- 6 - Dematiaceae,
- 7 - Tuberculariaceae and Stilbaceae,
- 8 - Other Fungi Imperfecti.

much higher percentage of *Penicillia* and *Aspergilli* than the mineral soil. The *Moniliaceae* were also relatively more numerous on litter than in the soil. A noticeable difference between the mycoflora of the litter and that of the mineral soil is the complete absence of any *Humicola* species or yeasts from the former habitat. In the mineral soil of site 1 yeasts were numerous.

The sharp decrease in the numbers of fungi with increasing soil depth, as found in this study, has been noted in the reports of many investigators. Waksman (1916), found that the number of fungi, but not the number of species, decreased with depth. Miller, Giddons and Foster (1957), reported the same phenomenon in soils of Georgia, U.S.A. They found that the same species occurred at soil depths of 2, 12, 16 and 24 inches, with the exception of *Trichoderma viride*, which they isolated chiefly from the top six inches of soil. The conclusions of these workers are not supported by the results of this investigation. A sharp decrease in the number of fungi with increasing depth was accompanied by a similar decrease in the number of species. More recent investigators have also reported this decrease of specific fungi with increasing depth (Warcup, 1951, a; Sewell, 1959; Yung and Stenton, 1964). Site 2 differed from site 1 with regard to the vertical distribution of fungi. At the former site the initial decrease in the number of fungi with increasing depth was followed by an increase in the colony numbers in deeper soil layers. This increase in colony numbers correlated with an increase in soil organic matter content at these depths. Coupled with this increase in colony numbers an increase in the number of species of fungi was also found at these deeper

soil horizons of site 2. The close correlation between soil organic matter content and the number of colonies and number of species found at site 2 could not be demonstrated for site 1. At the latter site, with its sandy soil and relatively low moisture content, the soil moisture factor seemed to have a greater influence on the vertical distribution of the fungi than did the organic-matter.

The investigations at site 1 yielded no positive results as to whether there is a clear seasonal variation in the numbers of soil fungi. It is true that during the period of sampling some irregularity in the rainfall pattern occurred, and it seems very likely that this influenced the results. During the very warm summer months of January and February, the rainfall was comparatively low. On the whole a slightly higher occurrence of the fungi was noticed during the winter and early spring than during the rest of the year. Tresner, Backus and Curtis (1954), also established that the greatest number of micro-organisms occurred during winter and spring in soils of Wisconsin. Timonin (1935), reported more bacteria in Manitoba soils during spring, while more fungi were present in autumn. Smith and Gall (1944), found that in Florida soils the number of micro-organisms decreased in warmer summer months when the temperature rose above 70°. At site 1 a very close relationship between soil moisture content and the number of fungi developing on dilution-plates was demonstrated.

If the list of species isolated from Zululand soils is compared with the list for acid Georgia soils (Miller,

Giddons and Foster, 1957), a striking similarity is apparent. These widely separated areas have many species in common. The order of dominance of particular genera, however, was quite different for the two areas. These investigators found that the sequence of the genera in order of dominance was Penicillium, Aspergillus, Cunninghamella, Trichoderma, Rhizopus, Gliocladium and Fusarium. At site 1 the genera could be arranged in order of dominance as follows: Penicillium, Trichoderma, Gongronella, Absidia, Cunninghamella, Fusarium, Mucor and Humicola. The order of dominance at site 2 was found to be Penicillium, Candida, Gongronella, Trichoderma, Fusarium, Paecilomyces, Mucor and Humicola. Aspergillus was reported to be the dominant genus for many soils. Abbott (1926), found it dominant in Louisiana soils while Werkinthin (1916), found Aspergillus species dominant in Texas soils. At both Zululand sites studied, Aspergilli were not common, either in number of colonies on isolation plates or in number of species. This result was somewhat unexpected. From many reports on soil fungi it appears that Aspergillus species are the main constituent of the soil fungi in warm climates, while Penicillium species predominate in cold regions. The results of this investigation appear to be just the opposite. Zululand has a warm, subtropical climate, but at both sites studied, species of Penicillium dominated the mycoflora of these sites, both in number of species and in number of colonies. Aspergilli were relatively rare at both sites.

The fungous flora of the soil is essentially a cosmopolitan one (Waksman, 1916). A pronounced similarity exists between floristic lists compiled in different regions

of the world. The soil fungus flora is comparatively constant as compared to the more obvious taxonomic variety of higher plants in different regions of the world. According to Garrett (1955), this similarity in soil fungus floras can be attributed partly to the fact that the soil environment is physically better buffered than the sub-aerial environment, so that organisms do not suffer such extremes as higher plants do.

From the results of this investigation it is possible to draw certain general conclusions regarding the fungus flora which inhabits the litter and soil of certain Zululand forest communities.

1. Phycomycetes:

All the Phycomycetes isolated during this investigation belonged to the Mucorales. Even with the application of special baiting methods, no Oomycetes could be recorded for these soils. The members of the Mucorales form an important component of the fungus flora of the sites studied. This is especially applicable to the genera Gongronella, Absidia, Mucor and Cunninghamella. These genera were particularly abundant in the gum tree forest at site 1. Absidia cylindrospora have a very interesting distribution in these soils. At site 1 it was found to be one of the most abundant fungi but at site 2 it was completely absent from all isolation-plates. Christenberry (1940), found A. cylindrospora to be very common in Carolina soils. Gongronella butleri was common at both sites. It must be pointed out, however, that the two isolates

of this organisms differed morphologically from each other and that each type was confined to one site.

The genus Mucor was well represented in both sites although more species were isolated from the plantation soil of site 1. A total of eight Mucor species was isolated. Paine (1927), compared natural soil with cultivated woodland soil and found Mucor species more abundant in the forest areas. Only three species of Mortierella were isolated and their percentage frequency of occurrence was low.

2. Ascomycetes:

Bisby, James and Timonin (1933), reported that Ascomycetes, producing asci in culture, are rare in soil isolations. In this investigation, however, eleven species of ascomycetes, producing asci and ascospores, were recorded. The chief species isolated were Neocosmospora vasinfecta, Thielavia terricola, and several ascomycete stages of Penicillium and Aspergillus. Thielavia species have been recorded from many soils, particularly in the U.S.A. and China, but Neocosmospora vasinfecta have been recorded on only two occasions, once from soil isolations in China (Gilman, 1957) and once from Georgian soils (Miller, Giddons and Foster, 1957). Both these isolates were common on isolation-plates and produced ascospores in culture. The Penicillium species which produced abundant cleistothecia and very few conidia should probably be placed in the Carpenteles series. They include the species Penicillium asperum, P. javanicum

and P. vermiculatum. An isolate, which remained sterile on the original isolation-plates and as sub-cultures on potato dextrose agar, proved to be a species of Pseudeurotium, after it had been transferred to cellulose agar (Eggins and Pugh, 1962), where it produced abundant cleistothecia. Two species of Chaetonium and one of Melanospora were also isolated.

3. Basidiomycetes:

Only one Basidiomycete species was isolated during this investigation. It was isolated from soil-plates prepared from soil samples from site 1 which were treated with steam for six minutes. It is a species of Peniophora. The methods which were employed during this investigation did not prove to be successful in the isolation of Basidiomycetes. Many investigators in soil mycology reported no Basidiomycetes (Waksman, 1916; Werkinthin, 1916; Jensen, 1931; Chandhuri and Sachar, 1934; Paine, 1927; etc.) Warcup (1951 a), was successful in isolating many Basidiomycetes by isolating them directly from mycelium in the soil. His method was not employed during this investigation.

4. Fungi Imperfecti:

Most of the fungus species isolated from Zululand soils were Fungi Imperfecti. The genus with the largest number of species was Penicillium. Twenty species of this genus were recorded from soil and forest litter. Three of these species were ascosporic. The Penicillium species recorded during this investigation were, on the

whole, very similar to those commonly found in many lists of soil fungi. Only six species of Aspergillus, of which one had a perfect ascosporic stage, were recorded.

Fusarium species were very common in both soils studied and six species were isolated. Fusarium oxysporum was the most common and it was isolated from soil samples of both the investigated sites, and also from forest litter. Gordon (1954) recorded F. oxysporum as the most abundant fungus of wheat plots in Canada.

Three species of Paecilomyces were recorded. P. elegans occurred at both sites. A culture, UZ.82, which resembles P. elegans very closely, but which differs in some respects from the original description of this species by Hughes (1951), was isolated from site 1.

The genera Cladosporium, Humicola and Trichosporon were well represented and formed an important constituent of the mycoflora of these Zululand soils. Gilman (1957) describes many species of Cladosporium and Trichoderma which were isolated from soil. Warcup (1955), noted that these and some other genera were generally isolated from passive survival structures in the soil. On the few occasions he isolated species of these genera from hyphae (excluding Trichosporon), they were present either on plant débris or on decomposing residues which had been ploughed into the soil. Tribe (1957), found few species of these three genera on buried cellulose. From this evidence it seems that these fungi are largely confined to certain micro-habitats in the soil.

Three pycnidial members of the Phomaceae were isolated from the soils studied. They are Ceuthospora sp., Phoma sp. and Sclerophoma sp. The Phoma isolate was only recorded on forest litter from site 1 and not from mineral soil samples.

During this investigation a large number of sterile forms was isolated. In Table 26 only a few of the more commonly occurring isolates are listed. Two dark-coloured hyphomycetes and four colourless forms were often encountered. In subsequent studies these isolates were subjected to a variety of treatments to induce sporulation. Various media (see appendix) were employed. They were cultured on a variety of plant débris and incubated at different temperatures and under different lighting conditions, viz in the dark, in daylight and in ultra-violet light (Leach, 1962 a and b), but without success. These isolates should in all probability be regarded as true sterile forms.

SUMMARY

The mycoflora of the soil and litter of two areas in Zululand with markedly different soils and vegetational cover was studied over a period of one year. This was a first attempt to investigate the nature and composition of micro-fungi in the soil of Zululand. The seasonal and vertical distribution of the mycoflora was investigated by means of both the dilution-plate and the soil-plate methods. Data were obtained on the occurrence and frequency of occurrence of the more common members of the mycoflora. The relationship between the fungi of the litter of one forest community and of the soil proper was investigated.

One hundred and twenty-one species representing forty-nine genera, were isolated from the two sites. These include twenty-one Phycomycetes, eleven Ascomycetes, one Basidiomycete and eighty-eight Fungi Imperfecti. The most common genera encountered were Penicillium and Trichoderma, followed by Gongronella, Candida, Absidia, Fusarium, Cunninghamella, Mucor, Humicola and Paecilomyces.

No indication of any seasonal periodicity in the occurrence of either the fungus population as a whole or any of the more common species could be established. Although higher plate counts were recorded during the warmer summer months, the occurrence of fungi appeared to depend on the moisture content of the soil rather than on the time of the year.

It was found that the number of fungus species and colonies in a profile decreased with depth, the rate of reduction being dependent on a number of factors, of which

the soil moisture content was the most important. In one profile an increase in colony numbers and number of species at deeper horizons was probably the result of a marked increase in the carbon content of these horizons. The vertical distribution of the more important species was investigated in detail.

A considerable number of species were common to both the forest litter and the soil proper. The composition of the litter fungous flora however, differed considerably from that of the underlying mineral soil.

APPENDIX

MEDIA EMPLOYED:

In the course of this investigation various media were employed in addition to those mentioned in chapter 2 on methods. The composition and preparation of these media is given below.

1. Potato dextrose agar:

Potato	200 g
Dextrose	20 g
Agar	20 g
Distilled water	1000 ml

Preparation:

- (a) 200 g of washed and sliced potato was covered with 500 ml of distilled water and simmered on an electric plate for 30 minutes.
- (b) 20 g of agar was dissolved in 500 ml distilled water by bringing the water to boiling point.

After filtering (a) through cheese cloth, (b) was added to the filtrate, 20 g of dextrose was added and the medium made up to 1 litre by the addition of distilled water prior to sterilization at 15 lbs. steam pressure for 15 minutes.

2. Potato carrot agar:

Potato	20 g
Carrot	20 g
Agar	20 g
Distilled water	1000 ml

Preparation:

20 grams of potato and 20 grams of carrot were washed and sliced, then mixed and steamed for 1 hour in a litre of distilled water. After filtration through cheese cloth 20 grams of agar were added to the filtrate, made up to 1 litre by the addition of distilled water and brought to boiling point to melt and dissolve the agar. The medium was then sterilized at 15 lbs. steam pressure for 15 minutes.

3. Czapek-Dox agar:

This medium was prepared according to the same recipe given for soil-plates in chapter 2, with the exclusion of the "Difco" yeast extract and the anti-bacterial agents.

4. Malt extract agar:

Malt extract ("Difco")	20 g
Dextrose	20 g
Peptone	1.0 g
Agar	25 g
Distilled water	1000 ml

All the ingredients were added to the distilled water and steamed in a steamer until they were dissolved. The medium was then sterilized at 15 lbs. steam pressure for 15 minutes.

5. Soil extract agar:

Soil extract (see below)	5 litre
KH_2PO_4	1.0 g
Glucose	5.0 g
Yeast extract ("Difco")	0.5 g
Agar	90 g

All the ingredients were added to the soil extract and steamed for 30 minutes to dissolve them. The medium was then sterilized at 15 lbs. steam pressure for 20 minutes.

Preparation of soil extract:

2½ Kg of good, air-dried garden loam was placed in a 5 litre flask and 2½ litre of distilled water was added to the soil. The soil suspension was autoclaved for 20 minutes at one atmosphere. After the suspension was cooled, the soil extract was decanted and gypsum (calcium sulphate) was added to it. The gypsum aided in clearing the extract.

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