A PRELIMINARY SURVEY OF THE SOIL MYCOFLORA OF AN ACACIA KARROO COMMU-NITY IN POTCHEFSTROOM WITH A DESCRIP-TION OF A NEW SPECIES

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C O N T E N T S

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CHAPTER 1

INTRODUCTION

It is a well-known fact that the world of science presents a never-ending line of investigation and that not even a lifetime of dedicated study could satisfy a desire to know the ultimate about the subject under consideration. Regarding the study of mycology, Sir Edwin Butler wrote in his preface to the book by Bisby, Buller and Dearness (Fungi of Manitoba) that knowledge of the fungus flora appeared to him to be the foundation of all mycological work, but that it was not to be gained in a few months. Even in the older countries where the fungi have been studied for centuries the collecting and naming have not been completed. As the study of mycology in South Africa has been undertaken seriously only since the beginning of this century, there is, as can be expected, still very much to be done on the subject.

After the settlement of the pioneers in 1652 on the Southern tip of Africa, conditions could not have been very favourable for any scientific research. It is therefore assumed that little or no study was made of the South African flora. The first knowledge of South Africa's indigenous flora was consequently not acquired by the settlers but by visiting scientists who made collections of flowering plants, lichens and fungi and took these collections to their nativelands for further study.

Carl Thunberg, who came to South Africa in 1772, was probably the first person to undertake a botanical study in South Africa. Although his object was the study of flowering plants, Thunberg listed fourteen fungi and thirty-nine lichens in his book, Flora capensis. These were undoubtedly the first Cryptogams to be recorded in South Africa. (Doidge, 1950)

Another important visiting botanist was William Burchell who collected fifty-six fungi and ninety lichens which were listed in his Catalogus Geographicus. Unlike Thunberg and most of the other collectors whose main interests seemed to be limited to the larger fungi, Burchell listed many microscopic fungi from the leaves and the bark of Angiosperms he had collected.

In the third and fourth decades of the 19th century several botanists became keenly interested in the flora of South Africa and through their studies made valuable contributions towards the accumalation of knowledge about the Cryptogams of Southern Africa. Carl Zeyher, J.F. Drege, J.A. Wahlberg, William Harvey and the Rev. A.E. Eaton were some of the visiting scientists who studied the indigenous flora and contributed to the sum of the knowledge about various species. Most of them sent their collections to Europe for identification.

As far as can be ascertained, Peter MacOwan and John Medley Wood were the first South Africans to make any contribution towards the expansion of knowledge about South African fungi. Until 1905 the collections of

fungi which were made by MacOwan and Wood and consisted of 756 specimens constituted the only fungus herbaria in South Africa.

The establishment of the Transvaal Department of Agriculture in 1903 opended a new chapter in the development of mycological research in South Africa. Dr. Pole Evans was appointed mycologist and under his guidance a national collection of fungi was built up in Pretoria. After the formation of the Union of South Africa in 1910, a separate Division of Plant Pathology and Mycology was formed for the investigation and control of plant diseases throughout the four provinces. The widening of the scope of the work obviously called for an increase of staff and Dr. P.A. van der Byl and Miss. A.M. Bottomley joined the staff in 1911 and 1913 respectively. Under the care of Miss. Bottomley the herbarium developed rapidly and in 1918 the number of fungi registered was 11,369. At the end of 1945, the Cryptogamic Herbarium at Pretoria housed 61,200 specimens, of which 35,000 were fungi.

At Stellenbosch a second fungus herbarium was established by Dr. P.A. van der Byl and after his death his work was continued by Dr. Len Verwoerd. Dr. Duthie, E.L. Stephens and Mrs. R. Brown were also responsible for collections of fungi which were housed in various herbaria in Cape Town and Johannesburg. (Doidge, 1950)

Although the number of fungi recorded in
South Africa increased rapidly, no comprehensive
list of South African fungi was published until 1945.
This task was successfully undertaken by Dr.
Ethel Doidge whose list of all the fungi and lichens
which had been recorded up to 1945, is today re-
garded of fundamental importance for the further
study of the South African mycoflora. (Doidge, 1950)

In 1921 the publication Bothalia, was launched
as a medium for the publication of papers based on
material in the National Herbarium. Since then a great
number of papers on several groups of fungi, including
Phycomyetes, Ascomycetes, Rusts and Fungi Imper-
fecti, have been published by various authors.

As far as is known little or nothing has been
published on soil fungi. The only attempt to study soil
fungi was that of Cohen (1950) who investigated the
occurrence of fungi in the soil, after different burning
and grazing treatments of the veld in the Transvaal. It
would appear therefore that little has been done on this
aspect of mycology and that an unexploited field of re-
search remains to be investigated.

In Europe the knowledge of fungi dates back
to the fundamental studies made by a group of bota-
nists which included DeBary, Brefeld and Ferdinand
Cohn. These investigators were followed by mycolo-
gists and plant pathologists, who, although they came
in contact with soil-inhabiting fungi at an early date,

were not interested in them as participants in soil
processes but regarded them to be of importance
only as causative agents of diseases. Consequently
it seems that although the presence of fungi in soil
was realized, the first scientific study on soil fungi
was undertaken by Adametz in 1886. (According to
Waksman, 1944)

Adametz studied the organisms present in
a sandy and a loamy soil. He took samples from
the surface layer and at a depth of 25 and 30 cm.
Several fungi, which included species such as
Penicillium glaucum, Mucor mucedo, Mucor
racemosus, Mucor stolonifer, Aspergillus glaucus
and Oidium lactis were isolated. According to
Waksman (1944) studies on soil fungi were underta-
ken by Müller in 1883, who noted that humus-rich
soils contain an abundance of fungal hyphae and
that the fungus types in forest soils differ from those
in peat. Ramann followed in 1900 and he empha-
sized the fact that acid forest soils contain an
abundance of fungal species. Most of the earlier
investigators seemed, however, to be studying the
fungus flora in an attempt to elucidate their chemical
activities and the first exact study of soil fungi,
according to Waksman (1916,b.), was that made by
Oudemans and Koning in 1902.

These two scientists inoculated plates of
wort agar or wort gelatine with dilutions of pulverized
fragments of vegetable matter which had been taken
from soil. Forty species of fungi were identified from

the inoculated plates. With their investigations
Oudemans and Koning definitely demonstrated the
presence of moulds in the soil of high organic
content and established the fact that an appreciable
variety of species occurs under such conditions.

Following the preliminary investigation
of Oudemans and Koning, a number of studies were
undertaken by various workers in different countries.
Hagem (Waksman 1916, b.) examined several soil
types in Norway; such as arable, meadow, garden,
forest and other soils for their content of Mucorales.
Another mycologist who also limited his studies to
the Mucorales, was Lendner who worked in
Switzerland. In 1912 Jensen made a fairly complete
study of the fungus flora of soils in New York. He
isolated 35 species, many of which had also been re-
ported by previous investigators. In England the
fungus flora of sandy, chalky, peat and "black earth"
soils were studied by Dale. Waksman (1916, b.)
expressed his surprise on the fact that so many or-
ganisms, such as <u>Trichoderma koningi,</u> several Mu-
cors, Penicillia, Cladosporia and others, which had
been isolated by previous investigators in Norway,
Holland and the U.S.A. were identical to those iso-
lated by Dale. In 1913 Goddard isolated eighteen species
of fungi from American soils. Despite the fact that he
isolated only a limited number of species, he came to the
conclusion that there was a rather constant and charac-
teristic fungus flora in the soil.

A number of investigations were also undertaken by workers in other countries. Waksman (1917) made a fiarly extensive survey of a number of soils from different parts of America. He isolated many species from the soil representing the sampled localities. Many of the species isolated from the different soils had al so been previously isolated by other investigators working on soils from different parts of Europe. This led Waksman to the conclusion that there was a rather distinct fungus flora present in soil.

As a result of the increasing number of studies undertaken, the knowledge about fungi gradually increased and in the following decades a better understanding of these organisms was gained. As the information accumalated the number of problems also tended to increase. The most important problems which mycologists had to deal with and which are relevant to this discussion, were;

(a)   to establish whether fungi live in the soil and produce vegetative mycelium,

(b)   to develop methods for the enumeration of soil fungi in order to gain detailed information concerning the nature of the fungus population, and

(c)   to establish whether each soil type possesses its own distinct population, depending on the nature of the soil, the climate and surface vegetation.

The results of investigations of these and many other problems bearing upon soil fungi were reported in various scientific journals and in special publications.

The problems discussed in this treatise are dealt with in such an order that they present a concise and logical picture of the accumalated knowledge and not necessarily in the sequence in which they were investigated and solved.

Waksman (1916, a.) made an attempt to answer the first question, namely whether fungi are present in the soil as vegetative mycelium or as dormant spores. He placed small soil clumps on the surface of suitable agar media and isolated the mycelium growing out of the soil crumbs. He thus succeeded in isolating species of <u>Mucor</u>, <u>Zygorhynchus</u>, <u>Rhizopus</u> and <u>Trichoderma</u>. It was believed that these isolates were present in the form of viable hyphae since they had been isolated from a variety of soils great distances apart, as well as from different dephts of the same soil. The assumption that fungi were present in soil as mycelium, was checked by inoculating agar plates with the mycelium of certain fungi obtained in pure culture and by comparing the rate of growth of the mycelium with that of germinating spores of the same species. It was revealed however, (Warcup, 1960) that the chances were even that the isolated species could have originated from spores because it was found that the rate of growth of germinating spores was equal to that of mycelium and could even surpass that of hyphae. In the course of

years, several other convinding results were ob-
tained to establish the fact that fungi are present
in soil as vegetative mycelium.

In 1922 Conn demonstrated, with the use
of a special staining technique (see following chapter),
that nearly all soils contain an abundance of fungi in
the form of mycelium. Kubiena, (according to
Waksman, 1944) confirmed the findings of the above-
mentioned workers in 1932 when he demonstrated by
direct microscopic examination that a large number
of fungi are capable of producing an extensive myce-
lium and might even sporulate in soil. This can be
accepted as a confirmation of Waksman's (1916, b.)
assumption that soil contains an autochtonous fungus
flora.

The abovementioned investigations as well
as many others which had been undertaken to deter-
mine the role of fungi in different soil processes
(Waksman 1916, b. and others) can lead to but one
general conclusion, i.e. that fungi are to be considered
typical soil organisms playing an active part in many of
the soil processes while in the active vegetative stage.

Regarding the enumeration of fungi in the
soil it remains to be stated that various techniques
have been devised to overcome the numerous problems
which are encountered in the study of soil fungi. This
will be discussed in the following chapter. It can
however, be noted here that no perfect method has yet

been developed by which a complete enumeration of the soil fungus population can be made.

Many contributions concerning the nature of the fungus population have been made. From different studies it appears that many fungi isolated from soil samples are typical soil inhabitants and that they seldom occur in other substrates. On the other hand, some fungi are regarded only as temporary invaders although they are capable of surviving in the soil for a long time. Mycologists therefore had the problem of studying the nature of the fungus flora of the soil and to determine to what extent it is influenced, both quantitatively and qualitatively by nutritional, environmental and ecological soil factors. (Waksman 1944).

In an extensive survey on the fungus population of the soil Waksman (1916, b. and 1917) isolated 31 genera, some of them being represented by many species. He compared his isolates with the representative groups previously isolated by Koning in Holland, by Dale in Great Britain and by Jensen, Goddard, McLean and Wilson in the United States and came to the conclusion that Aspergillus, Penicillium, Mucor and Trichoderma together with eight other genera, also of common occurence, are abundantly present in the soils of the different countries.

Waksman found that a forest soil contained

only a few Mucorales but a large number of
Trichodermae and only a few Penicillia, while in a
well-cultivated garden soil no group was pre-
sent in dominating numbers. The climate also
seemed to have an influence on the groups of
fungi present in dominating numbers because the
Mucorales were found to be more abundant in
colder climates while the Aspergillii were the
dominant group in warmer regions. The
Trichodermae were found to be extensively distri-
buted in acid and water-logged soils. Jensen (1931)
later confirmed these results when he investigated
soils in Denmark and found that forest, moor and
heath soils contain a characteristic flora of Tricho-
dermae whereas the fungus flora of field, marsh
and uncultivated mineral soils consisted largely of
Mucoraceae.

Various research workers found that the
nature of the fungus flora was influenced by nutri-
tional factors. The more fertile soils, which are
rich in organic matter, were found to contain a grea-
ter number and variety of fungi than the less fertile
soils. Singh (1937) substantiated the idea that soil
fertility has a quantitative influence on the occurrence
of microorganisms when he found a direct correla-
tion between soil fertility and the number of fungi
and Actinomycetes present in two soil types at
Rothamsted. Sadasivan (1939) recorded that newly
added plant materials stimulated the growth of fungi.
The chemical composition of the plant materials and

the extent of decomposition appeared to deter-
mine the species which colonize the material.

These abovementioned studies and others,
definitely established the fact that fungi form a very
large and heterogenous group of organisms which
penetrates the surface layer of the soil with its my-
celium and which shows a gradual decrease in
number and type in the lower soil layers. It also
appears that some fungi are widely distributed in va-
rious soils while others seem to be more limited to
certain soil types. The nature of the fungus popula-
tion seems to be influenced by the quantity and na-
ture of the organic matter of the soil, the type of
soil and climatic conditions, surface vegetation and
soil treatment. (Waksman 1944).

Most of the papers on the distribution of
fungi in the soil deal with cultivated soils and less
is known about the occurrence of fungi in natural
communities. Ling-Young (1930) realized this and
emphasized the importance of examining soils from
localities such as forests, peatbogs and mountains
which have remained untouched by man, to obtain a
complete picture of an endemic microflora. Several
papers dealing with the fungus flora of different natu-
ral communities have been published since then.
(Warcup 1951 a, Thornton 1956, Brown 1958, Borut
1960, Griffin 1963 and Chen 1961). Although most of
the results obtained confirmed the assumptions based
on studies of cultivated soils, valuable new information
was obtained.

The generally accepted idea that the same dominant groups of fungi occur in soils with similar characteristics, seems to be still a debatable one. Griffin (1963) found in his study of the activity of fungi in relatively dry soils, that _Penicillium_ and _Aspergillus_ were the dominant species in a soil series with a relative humidity (R.H.) of 81-94 per cent. These findings seem to be a contradiction of Warcup's (1960) conclusions, i.e. that _Penicillium_ and _Aspergillus_ are dominant in damper soils. The explanation for the difference of their results might be due to the fact that they employed different methods in their study of fungi in soil. This accentuates the necessity of evolving a gener ally acceptable scientific method for the study of soil fungi; but it must be stressed however, that whenever such a method is developed, many studies of natural soil mycoflora communities will have to be undertaken to complete the picture of soil fungi in relation with natural plant communities.

CHAPTER 2.

AIMS AND METHODS

(a) Aim.

A research project dealing with the mycoflora
associated with an Acacia karroo community in the
Potchefstroom area was launched by the staff of the My-
cological Section of the Botany Department of the Univer-
sity of Potchefstroom in 1964.   This project deals with
various aspects of the problem, one of which is the com-
position and ecology of the soil mycoflora.

The primary object of this investigation, the re-
sults of which form the basis of this thesis, was a prelimi-
nary survey of the composition of the mycoflora of the
superficial soil layers and the leaf letter of this community.

(b) Methods developed for studying fungi in the soil.

The methods developed for studying the occurrence
of fungi in the soil can be devided into two groups, i.e. di-
rect microscopical observation and indirect cultural methods.

Through direct microscopical observation it is
possible to demonstrate the occurrence of fungi in the form
of vegetative mycelium.   It has however, the disadvantage
that it provides no means of identifying the different species
as the fungi rarely sporulate in soil.   The use of cultural
methods, on the contrary, make the identification of the spe-
cies possible with the disadvantage that no information is
supplied whether the fungus occurs in the soil in the form
of spores or as actively growing mycelium because the iso-
lated species could have arisen from spores or hyphal frag-

ments. It is therefore clear that neither of these methods
of study gives a complete picture of the soil mycoflora and
that the only solution appears to be a combination of both
methods when investigating the occurrence of soil fungi.

Direct microscopical observation methods.

The first direct microscopical study was done
by Conn in 1918. Although he found no fungal hyphae in
the dried and stained soil samples on his microscope
slides, he did not question the presence of fungi in the soil
as has been stated in many mycological papers. Conn
realized that the reason for the failure to demonstrate the
presence of fungi in the soil might be the washing away of
the filaments on the slides when present in but small num-
bers. To offset this disadvantage Conn (1922) attempted to
develop an improved modification of this staining technique,
using wet instead of dry mounts.

In this modification a small crumb of soil was
placed on a microscope slide and was then mixed with
two or three drops of sterile water. A drop of methy-
lyne blue solution was then added and thoroughly mixed
with the soil solution. Microscopic examination was made
with a dry lens and a highpower eyepiece. This improved
modification of the original technique enabled Conn (1922)
to demonstrate the presence of fungal hyphae in the soil.

In the preparing of a soil smear on a micro-
scope slide, the actual arrangement of the soil particles and
micro-organisms as it occurs in the soil, is destroyed.
This loss of detail is avoided in the Rossi-Cholodny slide

technique which gives a picture of the soil microflora in situ.

The Italian microbiologist, Rossi (1928, in Garrett, 1963)pressed a clean glass slide against a freshly exposed soil surface and afterwards fixed and stained the adhering particles. Cholodny (1930 in Garrett, 1963), a Russian colleague, improved on this method by demonstrating that the behaviour of soil organisms can also be studied by inserting a number of slides in the soil and leaving them there for any desired period.

This method proved to be the most suitable for studying the actual arrangement of the soil parti-cles and the micro-organisms. The Rossi-Cholodny technique is frequently used to study the effect of newly added organic material to the soil. It makes possible the direct observation of the development of fungal hyphae and bacterial colonies in contact with the organic material. Conn (1932) also demonstrated the usefulness of this method for the study of differently treated soils set up in glass jars in a laboratory.

The Rossi-Cholodny technique is mainly used to study the amount of fungal mycelium in different soils at different times or the influence of the addition of acid, lime or fresh organic material to the different soils. Jensen (1934, in Garrett, 1963) determined the frequency of fungal and actinomycete mycelium in his survey of soils in New South Wales by recording the presence or absence of my-celium in a number of microscopic fields taken at random.

The major limitation of this method is the difficulty experienced in the identification of the different species of soil inhabiting fungi. The aseptate mycelium of Phycomycetes can be distinguished from that of Deuteromycetes, Ascomycetes and Basidiomycetes but the fructifications of the different genera are rarely found.

Chesters (1949) mentioned, however, that reflexion on the scope and accuracy of this method raised doubt as to whether in fact it does picture the soil flora as it occurs naturally. Although no nutrients are added to the soil, the glass surface allows accumalation of moisture, permitting the proliferation of bacteria and it may even act as a surface for the transport and passage of hyphae which are not normally present as hyphae in the soil. Furthermore, it certainly alters the aeration of the soil. Dobbs and Hinson (1953) found justification for Chesters doubts about the application of this method in soil studies. Jensen (1935 in Garrett 1963) limited the use of the Rossi-Cholodny technique to primary qualitative work and to quantitative work where a number of microscopic fields are examined.

A direct method which could be employed to obtain quantitative data, was developed by Verplancke (1932 in Burges, 1958). He weighed a soil sample, stained it with a suitable stain and tried to relate the weight of the sample to the number of organisms counted in the stained soil. But as Jensen (1922) pointed out, so much of the soil is lost in the staining and washing processes, that the accuracy of Verplancke's method to obtain quantita-

tive results is questionable.

In 1948 Jones and Mollison introduced a valuable method for the determination of the amount of fungal mycelium in the soil. They made a suspension of a weighed soil sample in cooled melted agar and prepared films of known volume with the aid of a haemocytometer slide. The films were removed when set and dried, then stained and mounted for microscopic observation. The bacteria within a particular area were counted, the total lenght of fungal hyphae measured and the number of bacteria and length of fungal hyphae per gram of soil was calculated. It is clear that this method is of little value for qualitative work.

Kubiena (1938 in Burges, 1958) applied vertical illumination for the observation of the living fungus population in situ and made interesting discoveries about the growth of certain fungi which did not usually appear on dilution plates. Direct microscopic observation in the field is often inconvenient and difficult and various workers have tried to fix and embed the soil so that the examination can be continued in the laboratory. Kubiena introduced the use of thermolabile resins but as these have certain disadvantages various plastics have been used instead for the impregnation of undisturbed blocks of soil of which sections were cut for examination. Alexander and Jackson (1954), Bourbeau and Berger (1947) and Hepple and Burges (1956) made use of polyester resins which greatly simplified the preparation of sections.

Minderman (1956) described a method by which
the soil was embedded in gelatine, the mineral particles
dissolved away with hydrofluoric acid and the spaces left,
filled with more gelatine. Serial microtome sections
were then prepared and stained..

Burges and Nicholas (1961) described the use
of sections prepar ed with Marco and Bakelite polyester
resins in the estimation of the amount of hyphae in
different soil horizons and during different seasons. Their
investigation of a humus-podzol on glacial sands, indicated
that this method of direct observation is capable of contri-
buting valuable information on the number, distribution and
morphological groups of hyphae in soil which can be com-
pared with results obtained from other methods.

The use of fluorescent antibody techniques for the
detection of micro-organisms is a new approach that has
become a valuable tool in the hands of the soil microbiolo-
gist. Coons (1954), Cherry et al. (1960) and Schmidt
and Bankole (1963) undertook valuable research in this
field. In this technique, as devised by Schmidt and
Bankole, the micro-organism under investigation is iso-
lated in pure culture from soil and is used as the antigen
in the preparation of active antiserum. Schmidt and
Bankole (1963) prepared the antiserum of <u>Aspergillus</u>
<u>flavus</u> by the intravenous and subcutaneous injection of a
suspension of the fungal mycelium into rabbits. The an-
tiserum is then labeled with a fluorescent dye, for example
fluorescein isothiocyanate. The labeled antiserum is then
used as a stain for soil samples on a microscope slide.

The presence of the organism concerned causes the antigen-antibody reaction. This reaction is restricted to the growth sites of the specific organism and can be observed by fluorescent microscopy. By employing this technique Schmidt and Bankole (1963) were able to distinguish <u>Aspergillus flavus</u> on buried slides.

Cultural methods.

A wide range of cultural methods have been used in the study of soil fungi; some devised for the investigation of the population as a whole, others to study a single organism or group of organisms with common properties.

The soil-dilution plate method, the most widely used in soil mycology, was originally developed for the isolation of soil bacteria. The method consists of diluting a known amount of soil in sterile water and preparing a progressive series of dilutions. From one of the higher dilutions, 1-ml. samples are placed in Petri dishes and dispersed with cooled melted agar by rotating the dish before the agar solidifies.

The dilutions used are usually between 1/1,000 and 1/100,000 and are chosen so that the degree of interference between developing colonies through competition and antagonism can be minimized. Waksman (1944) recommended dilutions giving 30 to 100 colonies per plate. Brierly et al. (1927) recommended one giving 35 to 45 colonies per plate, but the average of 25 chosen by

Bisby, James and Timonin (1933) appears to be the most suitable dilution.

To reduce the growth of bacteria and Actinomycetes on dilution plates, Waksman (1922,c) recommended the acidification of the medium with sulphuric acid. The acidification of the isolation medium successfully eliminated most of the Actinomycetes and bacteria on the plates, but did not prevent the spreading of certain fungi. Most mycologists, however, prefer the use of streptomycin or aureomycin (Martin, 1950; Johnson 1957) for the suppression of bacteria. Rose bengal (Ludwig & Henry, 1943 and Smith and Dawson, 1944) is used to reduce the spreading of certain fast-growing fungi to a minimum and to act as a bacteriostatic agent.

Mycologists have employed soil-dilution plates for the comparison of the fungus flora in different soils, in the same soil after different treatments and in the same soil at intervals during the year. It has the advantage that the different species can be identified and their frequency in any sample of soil determined. The method has been used for the study of microbial activity in soil and for estimating total populations of fungi, bacteria and Actinomycetes.

Mycologists are aware of the fact that a serious difficulty arises when this method is applied for the estimation of total populations of fungi, as it gives no information whether the fungus colonies have originated from

active hyphae or from dormant spores.

Another disadvantage of the soil-dilution plate method arises directly from the procedure followed in diluting the original suspension of soil. When making the dilution series many of the heavier particles in the soil suspension sediment to the bottom of the shaking vessel before a sample can be taken with a pipet. The actual samples that are finally plated out contain only the lighter parts such as the finer soil particles, bacterial and actinomycete cells, fungal spores and some hyphal fragments. It was discovered that the number of viable fungal spores greatly outnumbered the viable hyphal fragments in the final dilution. Fungi which spore abundantly will consequently be represented on the dilution plate at a frequency out of all proportion to the extent of its activity in the soil. Direct support for this was obtained by Warcup (1955 b, 1957). He found that 75% of the colonies which develop on dilution plates originated from spores and only 5% from hyphal fragments. His comparative studies (1957) indicated that dilution plates not only failed to produce a large number of fungus species but also that many of these fungi were present in the soil as hyphae. These fungi seemed to be discarded with the residue of the original soil suspension which included the heavier organic fragments, the larger mineral particles together with soil crumbs that had not been disaggregated in the shaking of the original soil suspension (Warcup, 1951 a; Saitô, 1955). Chesters and Thornton (1956) made a comparative study of different isolation methods and confirmed the general assumption that the soil-dilution method suffers from the disadvan-

tage of favouring heavily sporing fungi.

McLennan and Ducker (1954 b) on the other
hand found that the suspension technique gave a repre-
sentative picture of the soil mycoflora and did not fa-
vour the isolation of fungi with a high sporing capacity.

The dilution plate method will probably always
be a popular and widely used method in soil studies but
it must be borne in mind that it has the mentioned limi-
tations and reveals only a part of the total number of
species present in the soil.

Menzies (1957) proposed a modification of the
dilution method in order to eliminate certain difficulties
that are inherent in any method in which a pipet is used
for transferring a certain volume of a suspension to wa-
ter blanks. He made use of a dipper with a capacity of
1-cc. which can be used to agitate the suspension before
the removal of a sample. The sample therefore included
not only the lighter fragments, but also some of the mine-
ral particles, heavier soil crumbs and organic fragments.
After several months of experimenting with the dipper
Menzies and his co-workers concluded that this modifi-
cation was not only more reliable and accurate for quan-
titative work but that it took up less time and was easier
for dilution work in general than the use of a pipet.

In order to overcome some of the disadvantages
of the dilution plate Warcup (1950) developed a technique
known as the soil plate method. This technique is of

considerable value for qualitative and ecological
studies where the numbers of water blanks to be
handled become prohibitive.

In this method samples of approximately
5 - 15mg. are taken from the composite samples by
means of a nichrome needle with a flattened tip,
which is also used to crush and disperse the soil
aggregates in a drop of sterile water in a Petri dish.
Melted and cooled agar is then added and the particles
are distributed evenly by rotating the dish before the
agar solidifies.

This method at least has the possibility of
allowing the isolation of fungi that adhere to soil parti-
cles and are consequently discarded with the residue
when preparing soil-dilution plates. In comparative
studies, investigators using soil plates and dilution
plates found that usually, but not always, more spe-
cies of fungi were recorded from soil than dilution
plates. (Warcup, 1951 a.) In further comparative stu-
dies between soil and dilution plates, Warcup (1957)
came to the conclusion that both methods give essen-
tially the same overall picture of the fungus flora of a
wheat-field soil.

Although it was assumed that incorporation
of soil with the medium should allow any hyphae that
might be present to grow, recent work suggests that
this is rarely the case. Under most conditions, the
number of fast-growing species present as spores com-
pete successfully with viable hyphae. Chesters and

Thornton (1956) substantiated Warcup's results when
they found that isolations by both dilution and soil plates
favour sporulating species. It seems therefore that the
soil plate method reduces but does not eliminate, the
disadvantage of favouring heavily sporulating fungi.

It must be noted that the soil plate method
as devised by Warcup, was initially used in the study of
soils that contained relatively   low numbers of fungi.
It was found in humus-rich soils that 5 - 15mg. of soil
yielded such large numbers of colonies per plate that
it was very difficult to make accurate counts, to identi-
fy or to transfer them.

Johnson and Manka (1961) endeavoured to
eliminate this problem of severe inter-colony competition
without losing the basic advantage of the method, namely
the possibility of isolating fungi from soil particles. They
developed a method by which soil is diluted with sterile
sand.

The sterile sand and the collected soil sam-
ples were sieved prior to each experiment so that no
particles larger than 1.3 mm. were used. Aliquots of
soil, each one gram, were placed in wide-mouthed,
250-ml. Erlenmeyer flasks containing various amounts
of sterilized sand.

The flasks containing the soil-sand mixture
were rotated gently for one minute in order to disperse
the soil particles in the sand. Aliquots were transfered

to sterile Petri dishes with a microspatula. The microspatula was so designed that approximately 9.1 mg. of a 1/500 soil-sand mixture was transferred to each Petri dish. Approximately 15 ml. of agar medium, cooled to just above the solidifying temperature was poured into each dish.

From the results obtained it appeared that the dilution of soil with sand was beneficial whenever the soil to be used supported high populations of fungi. Johnson and Manka (1961) came to the conclusion that this modification of the soil plate reduced the number of colonies per plate to an adequate number of colonies per plate for isolation and counting. Besides this apparent advantage, this modification of Warcup's method is easier and takes up less time than the dilution plate method.

In both the soil-dilution and the soil-plate method, it is impossible to determine whether the fungal colonies originate from spores or hyphae. This can only be done if an analysis of the unit of origin of individual colonies is carried out. Consequently neither of these methods can be used successfully to evaluate the activity of fungi in soil.

McLennan (1928) suggested that it might be possible to distinguish between fungal mycelium and spores in soil by subjecting soil samples to desiccation. McLennan plated out samples of moist soil and soil which had been dried over calcium chloride. The number of

fungal colonies were compared in both cases and a
marked decrease after drying was noted. Sterile
soils into which fragmented mycelium and spores had
been incorporated were in turn plated out before
and after drying. No colonies developed from the
samples containing only mycelium, whereas the sam-
ple containing spores was unaffected. McLennan
suggested that the decrease obtained with natural soils
was due to the desiccation of vegetative mycelium and
that the normal fungus constituents of soil were exten-
sively present as mycelium.

Several investigators doubted, however,
whether McLennan's conclusion that fungi exist mainly
as active mycelium in soil, was generally valid. (Jensen
1934 in Garrett 1963). According to Warcup (1960),
Garrett (1952) considered that it was improbable that this
treatment would affect anything approaching a complete
separation of colonies derived from spores and mycelium
because from general evidence available spores are more
resistant to desiccation than is mycelium. Eastwood (1952)
considered using this technique, but on testing it with fungi
in pure culture found that mycelium was destroyed by de-
siccation and that there was also a reduction of about 25%
in the total number of spores. Harley and Waid (1955)
pointed out that methods such as these which depend on the
dilution-plate technique, were subject to errors because
the colonies isolated from the diluted soil might be so pre-
ponderantly derived from spores that estimates of vegeta-
tively active forms would be greatly affected by variations
in the number of spores. The studies made by Warcup
(1955 b.) on the origin of colonies of fungi developing on

soil dilution plates suggested that this view was probably correct.

Warcup (1957) decided to investigate this method because the results obtained by different workers were contradictory, particularly those relating to the proportion of spores lost in drying. He collected samples of surface soil (one inch deep) in autumn and in winter. From each soil sample dilution plates were prepared immediately after collection and again after drying for three days over calcium chloride as recommended by McLennan. Results obtained showed that drying over calcium chloride caused a marked decrease in the number of colonies and that this reduction was due mainly to the loss of spores.

Watson (1960) developed a soil washing technique to solve the problem of the preponderance of fungal spores in soil studies. The soil washing was done in 500-ml. Erlenmeyer flasks with 1 g. of air dried soil and about 200 ml. water for each wash. The soil was agitated thoroughly several times and allowed to settle down. After this the water was decanted. Dilutions were prepared in the usual way. The soil residue remaining after washing was dispersed in a Waring Blendor, after which it was diluted and plate counts made. It was found that plates from washed soil revealed more of the soil-borne pathogens, more genera of fungi and a large number of fungi other than Penicillium and Aspergillus.

Parkinson and Williams (1961) continued
with this concept and designed a method of serial
washing to make as complete as possible an assess-
ment of the fungus content of the soil. The soil
washing was performed by using perspex boxes
fitted with three stainless steel sieves of different
mesh. A soil sample was serially washed under
aseptic conditions by passing sterile water and com-
pressed air through the boxes. The agitation resulted
in the dislodging of the aggregated particles and the
releasing of spores held by these aggregates. On
completion of washing, discrete soil particles were
distributed on the sieves according to their size. When
such particles are plated, the possibility of obtaining i-
solates from hyphae is considerable greater than when
unwashed soil is plated. The spore content of a soil
sample can also be assessed by plating the collected
drainage water after washing.

Williams (1963) undertook an investigation of
the distribution of fungi in the horizons of a podsolised
soil by making use of the soil washing technique. More
species were obtained than from the drainage water
from which a dilution series was made and plates poured.
All species isolated from drainage water also appeared
on washed particles and twenty one types isolated from
washed soil were absent from the drainage water. These
included many sterile forms, together with a few
Basidiomycetes and Ascomycetes.

Another method in which soil is incorporated
with agar is the direct inoculation method of

Waksman (1916 a.)   This method was devised by
Waksman to answer the question then very contro-
versial, viz, whether fungi actually lived and pro-
duced mycelium in the soil, or whether they were
deposited as spores and remained viable but inert.
Waksman transferred lumps of soil, about 1 cm.
in diameter, to sterile agar plates.   After an in-
cubation period of 24 hours, hyphal tips of myce-
lium radiating out from the lumps of soil, were
removed.   By inoculating similar agar media with
mycelium and spores of various species and com-
paring the results, Waksman concluded that the
development of mycelium from lumps of soil was
too rapid to have been derived from germinating
spores and must have originated from pre-existing
mycelium within the soil.   Various workers who
used this method (Waksman 1927; Jensen 1931;
Park 1954; Chesters and Thornton 1956) also noted
that the method was selective for fast-growing fungi.

Saitô (1955), isolated Mucor, Rhizopus
and Zygorhynchus from garden soil by using
Waksman's direct inoculation method.   He examined
the lumps of soil microscopically and found that "apart
from septate fine hyphae, none of the none-septate,
broad hyphae characteristic of the mucoraceous fungi
could be found even by careful direct microscopic ex-
amination;   therefore, the mycelia developed from the
lumps of soil should be derived from spores."
Warcup (1955, a.) supported Saitô's results.   He
examined lumps of soil which would invariably give rise
to Rhizopus if placed on agar, by means of the hyphal

isolation method but could not reveal any aseptate
hyphae of this fungus. Warcup's experience with
spores and hyphae of Rhizopus obtained from soil,
showed that mycelial hyphae do not invariably grow
faster than germinating spores which may, as a
single hyphae, grow to surprising lengths within
24 hours. These results obtained by Saitô (1955)
and Warcup (1955 a.) indicated that in some cases
the fungal growth obtained by direct inoculation
could have been derived from spores.

All the techniques already mentioned
consist basically of the incorporation of a soil sus-
pension or a portion of a soil sample in an agar me-
dium. In the soil immersion-tube and screened
immersion plate methods this procedure is reversed
and instead the agar is exposed to the soil for possi-
ble fungal growth.

The immersion-tube method was designed
by Chesters for the purpose of isolating actively growing
fungi from the soil. As described by Chesters (1940 &
1948), the method consists of the introduction into the
soil of a glass tube, with four to six spirally arranged
invaginated capillaries, filled with nutrient agar. A soil
core is removed in the field to be studied and an im-
mersion tube inserted in its place. After a specific in-
cubation period, varying from seven to fourteen days, it
is removed and the fungi then isolated from it by remo-
ving the core of agar from the length of the tube with a
stainless-steel corer and cutting it into portions which are
plated out.

Chester's (1948) view of the limits of
the immersion-tube method was, that it easily isola-
ted active spreading mycelium or active localized my-
celium which happended to come into contact with the
capillaries. The fungi so isolated must obviously be
capable of growth into and through the medium in the
tube.

Chesters and Thornton (1956) made a
comparison of the fungi isolated from a forest soil by
immersion tubes and by screened immersion plates.
From the results obtained they came to the conclu-
sion that colonization of the immersion tubes depended
upon the ability of individuals to compete successfully
with other members of the soil population for entry
through capillary orifices. Because of the low oxygen
tension resulting from sterilization of the agar during
preparation, the fungi must also be capable of tolerating
a low oxygen tension to succeed in growing into the
agar in order to be isolated from the central core of
isolation medium. The predominance of Mortierella
humilis and Mucor hiemalis in immersion tubes, which
was only partially confirmed by the screened immersion
plate method, led to the consideration that fast-growing
fungi once established would likely colonize the bulk of
the agar within the tubes. These fast-growing fungi
would therefore be recorded with a high frequency of
isolation. The excretion of metabolic products by such
colonizing organisms might also prevent or retard the
establishment of other species and consequently prevent
their isolation.

The immersion-tube method has been par-
ticularly successful in isolating mycelia of soil fungi
that are either non-sporing (e.g. Rhizoctonia solani)
or sparsely sporing (e.g. Pythium spp.) (Chesters,
1948.) It must be pointed out however, that many of
the species recorded are sporing species that are,
to a certain extent found on dilution plates. The con-
densation of water in the orifices of the immersion
tubes could provide a habitat for spore germination
and consequently some fungi that are not present as
mycelium in the soil, could be isolated. Observations
by Hinson (Dobbs and Hinson, 1953) with La Touche
traps and 'gap traps' substantiated this when they
found that spores germinated in water condensed be-
tween the slides and provided origin of the hyphae
seen in the traps after burial in the soil.

In order to reduce the selective action
of the immersion tube towards rapidly growing fungi
which can tolerate low oxygen tensions, a modifica-
tion was devised by Gochenauer (1964). The immer-
sion tubes were smaller (10 mm. by 76 mm.) and
only 2 to 4 capillaries were made. Instead of a nu-
trient agar substrate, 1 g. of dried soil taken from the
forest to be studied, was added to each tube. The
tubes were immersed in water to moisten the soil,
capped with aluminum foil and placed in a carrying
container which was fitted with a plastic closure, the
whole being sterilized. Although it has been shown that
heat sterilization alters the chemical composition of the
soil (Coleman et al, 1916), the texture apparently re-

mained unchanged with this treatment. The tubes were placed in the soil for 7 days,according to Chesters (1948) directions and after removal the contents were removed and plated out.

The use of soil instead of agar provided a well-aerated medium in which the moisture content approximated that of the surrounding soil.. This modification is obviously an improvement on the original method because it greatly reduces the selectivity towards species tolerant of low oxygen tension and it eliminates many of the technical problems resulting from the use of agar while at the same time it permits the isolation of a wider range of species, including Ascomycets. Unfortunately this method still seems to favour the isolation of mucoraceous forms. (Gochenauer, 1964).

Another method based on the exposing of agar to the soil, is the screened immersion plate method. This method is a development of the Rossi-Cholodny technique and was devised by Thornton in 1952. The agar is carried on a microscope slide which is enclosed in a Perspex box with 10 evenly spaced holes for entrance of fungal growth. The slides are examined microscopically after a sufficiently long period of burial and the fungal colonies on the agar transferred for subculturing on potato dextrose agar. The reason for this subculturing is that fungi growing on water agar, which is used as isolation medium, do not readily produce their characteristic fruiting bodies by which they can be identified.

Parallel studies with different techniques (Chesters and Thornton, 1956) showed that isolations made from screened immersion plates showed close agreement with those from immersion tubes and direct inoculation. When comparing the results of the screened immersion plate with those of the immersion tube, it is clear that the screened immersion plate reduces the advantage of species tolerant of low oxygen tension. The use of plain water agar as isolation medium is at least partly the reason for the success of the wider range of species isolated. The intensity of competition between isolates is much reduced by the use of water agar which is less selective than nutrient agar media because mycelia do not grow strongly enough to produce excretory products in such concentrations that they inhibit the growth of adjacent mycelia.

When experiments with the screened immersion plate method were carried out for the purpose of this treatise, practical difficulties were encountered. After an incubation period of three days (Thornton kept his immersion plates in soil for seven to fourteen days.) there was already interference between colonies arising below adjacent holes on the screen. This intensified the problem of removing inocula of single colonies to potato dextrose agar for further study. If more time had been available the isolation might have been done successfully. The time and labour seemed to be important limitations in the application of this method in soil studies.

Besides the abovementioned studies, which

were devised for the study of soil fungi in general, a
wide range of specially designed techniques have been
employed for the study of fungal colonization of selec-
ted substrata and for the study of a certain specimen
or groups of organisms which may be present in a
particular substratum.

Although Warcup's hyphal isolation method
(1955 a, 1957) is not concerned with fungi colonizing
any particular substrate, it is a selective method and
hence appropriately considered here.

Warcup reported this simple method for
extracting and plating hyphae from soil after he made
the observation that when a soil suspension is prepa-
red many of the fungal hyphae remain with the heavier
soil particles of the residue. Removal of the fine,
suspended material from the residue permits visual
examination of the latter for the presence of individual
hyphae or hyphal masses, which may then be removed
and cultivated on agar media. This method was found
to permit the isolation of species that rarely appeared on
dilution plates. (Warcup 1957)

In this method a soil crumb is allowed to be-
come saturated with water and is then broken up by fil-
ling the beaker with a rapid jet of tap water. The hea-
vier particles are allowed to settle and the finer particles
are poured off in suspension. The process of washing
is repeated, until a residue consisting of heavy particles
alone remains. These residual particles of soil are then

distributed in small quantities in Petri dishes. The ma-
terial is examined by means of a binocular dissecting
microscope for the presence of fungal hyphae, which are
picked up with sterile forceps and plated out on nutrient
agar.

Warcup (1957) concluded that hyphal isola-
tion may be selective for hyphae of large diameter, for
mycelia that do not fragment readily and fungi with dark-
coloured hyphae. Hyphal isolation may tend to neglect
hyphae that fragment easily, fine single hyphae, hyphae
in large humus particles and hyphae closely adhered to
or occuring in plant roots. Another selective factor is
that not all fungi present as hyphae in soil may be able
to grow on the isolation medium i.e. a weak Dox plus
yeast agar. A further question is whether the actual
isolation of hyphae from soil may damage or kill them.
Circumstantial evidence indicates that many hyphae are
extracted alive while some are probably damaged.

In the wide range of selective methods which
have been used to isolate fungi from soil, there seems
to be an infinite number of substrates that may be added
to soil to encourage specific organisms or group of or-
ganisms. Several, so-called 'baiting' methods have
been employed and these studies seemed to be especially
valuable when a particular organism is present in soil in
very small numbers.

Bait added to water covering a soil sample
is useful for those organisms that produce zoospores.

A great variety of baits, such as cellophane, boiled
grass leaves, pine pollen, hemp seed, shrimp chi-
tin, insect wings, hair, and cast snakeskin have been
used for the isolation of soil chytrids. (Sparrow,
1957). Another example is hemp seed which is the
usual bait used for the isolation of Pythium and water
moulds.

According to some other methods soil is
placed on or in plant tissues. An example of this
type of isolation is the carrot method for the isolation
of Thielaviopsis basicola. (Yarwood, 1946). Yarwood
spread soil from field collections over the surface of
5 mm. thick carrot root discs in Petri dishes and added
enough water to make the soil quite moist but with no
free water present. After 2 or 4 days the discs were
washed to free them of soil and incubated in moist
chambers. When soils containing Thielaviopsis were
used as an inoculum, greyish colonies appeared in about
6 days after inoculation. In most cases transfers direct-
ly from the aerial mycelium to potato-dextrose agar gave
pure cultures of Thielaviopsis.

A different approach to the isolation of a par-
ticular group of organisms is the treatment of soil with
heat or chemicals before plating it out. Warcup (1951 b.)
subjected the soil to steam and found that the number of
certain Ascomycetes isolated from soil increased greatly.
Warcup (1952) and Evans (1955) demonstrated that cer-
tain dosages of fumigants such as formalin and carbon
disulphide might act selectively by killing most, but not

those fungi tolerant towards these fumigants. It was found by Warcup (1952) that Pythium did not survive the treatment by steam or formalin and that this species recolonized the soil very slowly while Trichoderma viride was often the dominant recolonizer of formalin-treated soil. Evans (1955) studied the survival and recolonization of fungi treated with formalin and carbon disulphide. He found that Trichoderma viride established itself as the dominant fungus and that this dominance persisted during a 6 months period of sampling. Trichoderma viride also became dominant after treatment of the soil with carbon disulphide. After treatment with higher dosages than 0.5 ml./250 g. of moist soil, the dominant fungi were Penicillium luteum and Asperigillus fischerii, which were found to be highly tolerant of carbon disulphide in comparison with other fungi tested.

The selective methods discussed are, however, only of value when a particular specimen or group of organisms are to be studied and are not of great importance in the studies of soil fungal communities.

(c) Methods and materials used in this investigation,

Sampling procedure.

A typical Acacia karroo community, situated in the northern part of the grounds of the University, was selected as the area for investigation.

In order to eliminate errors of selection
and to obtain a representative sample of the myco-
flora of the community, two strips crossing the area
at right angles, were marked off.   Forty samples of
2 spoonfulls each, were taken from these strips at in-
tervals of 12 ft.   For this purpose a sterile dessert
spoon was used.

The soil samples, which included the leaf
litter and first inch of soil, were transferred to the
laboratory in sterile bottles.   The samples were tho-
roughly mixed in a large sterilized glass jar and then
twice passed through a stainless steel sieve of 0.039
in. mesh.   The composite sample was divided into
5 equal parts.   From each of these 5 dilution plates
and 3 soil plates were prepared.

Dilution plate method.

A modification of the dilution method, as
devised by Menzies (1957), was employed and follo-
wing his instructions the plates were prepared in the
following way.

Aliquots of 25 g. of each subdivided soil
mass, were placed in 5 sterile flasks containing 250 ml.
sterilized water.

The suspensions were stirred and poured
into 1-litre Erlenmeyer flasks.   The flasks, containing
the suspensions, were shaken on a mechanical shaker
for 30 min.

The dipper of 1-ml. capacity, was dipped
into the suspension several times and 1 ml. was quickly
transferred to the first water blank of 9 ml. of sterile
water. The dipper was used for agitation to ensure
an even distribution of the suspended particles.

Five dilution series, (one from each soil
mass) were prepared with the same dipper which was
rinsed in alcohol and flamed after each dilution prepa-
ration. The procedure of mixing and transferring was
repeated in the five series until the desired dilutions of
1/10,000 were obtained. The dilution of 1/10,000 was
chosen because it gave an average number of 18-20
colonies per plate.

From each final dilution, five 1-ml. portions
were transferred to sterile Petri dishes by means of
the dipper: The total number of soil-dilution plates was
25.

Peptore-dextrose agar plus rose bengal and
streptomycin (Martin, 1950; Johnson, 1957) was used as
isolating medium. The medium was melted and cooled to
just above the solidifying temperature and 12 to 15 ml.
were added to each Petri dish. By gentle rotation of the
Petri dishes the suspension was evenly dispersed in the
agar medium.

Soil plate method.

For the preparation of soil plates the method
of Warcup (1950), as modified by Johnson and Manka (1961)

was employed.

The sand used for the dilution of the soil was also passed through the 0.039 inch-mesh sieve.

Five aliquots (70 gram each) of sand were placed in wide-mouthed, 250-ml. Erlenmeyer flasks and sterilized in an autoclave at 15 pounds pressure for 30 min.

One gram samples of each soil mass were added to the Erlenmeyer flasks containing the sterilized sand. The flasks were rotated gently for 1 min. to effect an even distribution of the soil in the sand.

Three microspatula samples, with an average weight of 55 mg. each, were taken from the 5 Erlenmeyer flasks and transferred to sterile Petri dishes. Fifty-five milligramme were used because it had been previously determined that an average of 18-20 colonies per plate were obtained when using this quantity of the mixture.

Approximately 15-ml. agar medium (Martin, 1950; Johnson, 1957) melted and cooled to just above the solidifying temperature, were added to each dish. The particles were dispersed by gentle rotation of the Petri dishes before the agar medium had solidified.

All the Petri dishes were incubated for 3 weeks at a temperature of 25°. During this period all colonies were subcultured for identification as they appeared on the plates. A total number of 726 specimens

was isolated.

Identification of cultures.

For the diagnosis of the cultural and morphological characters of the various isolates, standard mycological procedures were followed. In a few instances special techniques had to be employed to elucidate certain morphological details.

The identification of the cultures was accomplished by making us of the available literature. Where local library facilities were found to be inadequate, use was made of more elaborate and comprehensive collections of mycological works in larger centres.

Most of the identifications could be verified by checking against specimens from an existing collection of fungi isolated and identified during earlier stages of the research undertaken by this department on the mycoflora of the Acacia karroo communities in the Potchefstroom area.

In a few instances it was found necessary to consult recognised authorities outside the university and even abroad. In this connection the author would like to mention the names of Mr. G.C.A. van der Westhuizen, Plant Protection Research Institute, Department of Agricultural Technical Services, Pretoria; Dr. J.A. von Arx, Director, Centraalbureau voor Schimmelcultures, Baarn, Netherlands and Dr. G.L. Hennebert, Mycological Institute, University of Lovain, Belgium.

Media used.

(1)   <u>Peptone-dextrose agar plus rose bengal and streptomycin</u>. (Martin, 1950) (Johnson, 1957).

Agar	20.0 g.
KH_2PO_4	1.0 g.
$MgSO_4.7H_2O$	0.5 g.
Peptone	5.0 g.
Dextrose	10.0 g.
Distilled H_2O	1000.0 ml.
Rose bengal	1:30,000
Streptomycin	30 µg/ml.

Preparation:

All the materials except rose bengal and streptomycin are dissolved in water. The mixture is heated slowly while stirring until it starts to boil. It is removed from heat and a 1:3000 dilution of rose bengal is added at a rate of 1 ml/100 ml. of medium. After bottling and autoclaving and before pouring plates. Streptomycin is added to the cooled liquid medium or in the dishes just before the medium is added.

(2)   <u>Potato-dextrose agar</u> (Riker and Riker, 1936 in Johnson et al. 1959).

Used as culture medium and for the identification of species.

Agar	17 g.
Potatoes (peeled and sliced)	200 g.
Dextrose	20 g.
Water	1,000 ml.

Preparation:

The potatoes are cooked for one hour in a steamer or for 40 minutes in an autoclave, in 500 ml. of water. At the same time the agar is melted in 500 ml. of water. The potato juice is strained or decanted into the melted agar and the volume is adjusted with water. Dextrose is then added.

(3)   ### Oatmeal agar.

Quaker's oats	30 g.
Water	1,000 ml.
Agar	15 g.

Preparation:

The water and oats are brought to boiling temperature, then allowed to simmer over a low flame for one hour. After the simmering period it is strained through a cloth.
The filtrate is filled up to 1 litre and sterilized for 1 hour at 120 degrees C. The agar is then added and the medium sterilized at 120 degrees C. for 20 min.

(4)   <u>Hay-infusion agar</u>:

Hay	50 g.
Water	1,000 ml.
K_2HPO_4	0.2 percent
Agar	15 g.

<u>Preparation</u>:

50 g. of partially decomposed hay
is infused for half an hour in 1,000 ml.
of tap water in an autoclave at 120 degrees C.
The infusion is filtered and the filtrate made
up to 1,000 ml.
0.2 percent K_2HPO_4 and 1.5 percent agar is
then added and the pH adjusted to approximate-
ly 6.2.
The medium is sterilized at 15 pounds pressure
for 30 min.

(5)   <u>Corn meal agar</u>: (McLean and Cook, 1941)

Corn meal	50 g.
Water	1,000 ml.
Agar	15 g.

<u>Preparation</u>:

The corn meal is suspended in the
water and warmed at a temperature of 60
degrees C for 1 hour. The suspension is
filtered, 15 g. agar added and steamed for
90 min. The medium is filtered again and
sterilized in an autoclave in the usual way.

Czapek-Dox agar (Thom & Raper 1945, in Johnson et al. 1959) and Malt extract agar (Thom & Raper, 1949 in Johnson et al. 1959) were also used for the identification of certain specimens.

CHAPTER 3.

SPECIES LIST AND A DESCRIPTION OF A NEW SPECIES

(a)   Species list:

Zygomycetes.	Number of isolates.
Absidia sp.	5
Absidia cylindrospora Hagem	21
Cunninghamella echinulata Thaxter	4
Gongronella butleri (Lindner) Picci	3
Mortierella sp.	3
Mucor circinelloides v. Tieghem	6
Rhizopus arrhizus Fischer	7
Total	49

Deuteromycetes. (Fungi Imperfecti)	
Acremonium album Preuss	2
Acrospeira levis Wiltshire	1
Alternaria tenuis Nees	5
Amerosporium atrum (Fück) v. Hohn ex Fr.	5
Arxiella terrestris Papendorf	1
Aspergillus carneus (v. Tiegh) Blochwitz	4
Aspergillus flavus var. columnaris	1
Aspergillus fumigatus Fresenius	3
Aspergillus ochraceus Wilhelm	3
Aspergillus puniceus Kwon & Fennell	5

Aspergillus versicolor (Vuill) Tiraboschi	1
Beauveria bassiana (Bals) Vuill	4
Beauveria densa (Link) Vuill	2
Beltrania rhombica O. Penzig	4
Cephalosporium acremonium Corda	1
Cephalosporium spinosum Negroni	1
Cephalosporium sp.	1
Chrysosporium pannorum (Link) Hughes	1
Cicinnobella sp.	1
Cirrhomyces flavovirens v. Hohn	1
Cladosporium cladosporioides (Fres) de Vries	2
Coniothyrium fückelii Sacc.	2
Cytospora sp.	2
Diplodia sp.	1
Discosia arctocreas (Tode) Fr.	1
Dothichiza sp.	1
Fusarium equiseti (Corda) Sacc.	5
Fusarium moniliforme Sheldon	2
Fusarium oxysporum Schlechtendahl	1
Fusarium solani (Mart.) App. & Wr.	4
Fusarium lunatum (Ellis & Ev.) von Arx	1
Gliocladium penicelloides Corda	2
Gliocladium roseum (Link) Thom	4
Gliomastix sp.	1
Haplosporella bakeriana Sacc.	1
Helminthosporium dematioideum	1
Helminthosporium sativusm.	3
Helminthosporium speciferum (Bain)	1
Humicola sp.	19

Humicola fuscoatra Traaen	2
Hyalotiella transvalensis Papendorf	1
Monocilium humicola Barron	3
Myrothecium roridum Tode ex Fr.	2
Myrothecium verrucaria (Alb. et Schw.) Ditmar	5
Myrothecium sp.	1
Paecilomyces farinosus (Dicks ex Fr.) Brown & Smith	1
Penicillium adametzi Zaleski	8
Penicillium frequentans Westling	3
Penicillium lanosum Westling	1
Penicillium lilacinum Thom	31
Penicillium multicolor Grigorieva- Manoilova & Poradielova	1
Penicillium paraherquei	87
Penicillium spinilosum Thom	1
Periconia igniaria Mason et. M.B. Ellis	3
Periconia atra Corda	1
Pestalotia sp.	1
Phialophora aurantiaca v. Beyma	2
Phialophora mustea Neergaard	1
Phoma herbarum Westend	1
Phoma sp.	2
Pithomyces quadratus (Atk.) M.B. Ellis	1
Robillarda sessiles Sacc.	2
Scolecobasidium constrictum Abbott	2
Scolecobasidium humicola Barron et Busch	6
Scopulariopsis humicola Barron	10
Stagnospora sp.	2
Sympodina sp. nov.	2

Trichoderma viride Pers ex. Fr.	27
Trichoderma koningi (Oud.)	2
Verticillium chlamydosporum Goddard	5
Verticillium cinnebarium	2
Verticillium sp.	2
Volutella ciliata	1
Total	323

Ascomycetes:

Auxarthron umbrinum (Boudier) Orr et Plunkett	1
Chaetomium bainierii Munk	1
Chaetomium pachypodioides Ames	1
Chaetomium sp.	1
Endothia sp.	1
Herpotrichia striatispora Pap. & von Arx	1
Sordaria fimicola (Rob.) Ces. De.& Not.	1
Total	7

Grand Total: 379.

(b) Description of a new species.

At least 6 of the fungi isolated in the course of this investigation were found to be new and undescribed species with 4 representing new unknown genera.

One of these fungi which will be described as a new species, shows a close resemblance to

Scolecobasidium and even more to an unidentified isolate (No. 8816) mentioned by Barron and Busch (1962) in their paper on Scolecobasidium. A detailed diagnosis revealed that this isolate is generically similar to Sympodina which was erected by Subramanian and Lodha (1964).

In this study the fungus was cultured on a variety of agar media including potato-dextrose, potato-carrot, hay-infusion and malt agar.

Observations made:

The growth is similar and uniform on all the media used and the colonies develop fairly slowly reaching a diameter of plus minus 2.5 cm. in 10 days. The aerial mycelium is fluffy or flocculent, not more than 50 mm. high and brownish in colour. The reverse of the colony is grey-brown. Individual hyphae are slightly pigmented and straw-coloured, occasionally practically without pigment and hyaline. They are smooth, thin-walled, septate and inclined to be aggregated into ropy bundles.

The conidiophores arise laterally from the vegetative hyphae, are of indeterminate growth and show prominent geniculate scars where the conidia are attached. They are simple or branched and of variable length with the colour usually similar to that of the hyphae.

The conidia are typical aleuriospores and are produced singly at the tip of the conidiophore and of its

branches and successively at the growing points which arise sympodially and more or less alternately on the ĩdeterminate conidiophore. The two-celled conidia, which are hyaline or faintly straw-coloured are not uniform in shape and size though generally oblong-ellitical to short cylindrical and frequently more or less constricted at the septum. The lower cell is somewhat tapered and bears a broad truncate basal scar at the point where the conidium was attached to the conidiophore.

Sympodina simplex. sp. nov. (fig. 1, 2 and 3)

Coloniae tarde crescentes, velut flocci, velut brunneae. Hyphae leves, colore stramenti vel brunneae, 1 - 2.5 µ latae. Conidiophora se a latere levant ex hyphis, simplicia vel ramosa incremento incerto, ostendentia vestigia prominentia colore stramenti velut brunnaea 4 - 60x1.5 - 2.5 µ. Conidia genita singillatim in apice conidiophori vel ramorum eius et successive ad apices sympodialiter formata, oblongata-elliptica, cylindrica brevis 6.5 - 11.00 x 2.5 - 3.5 µ, hyalina vel colore stramenti, bicellula, constricta in septo, cellutaria nonnumquam irregularia, cellula inforiora fastigata in basem planam latam.

Habitat in humo silvatico sub Acacia karroo, Potchefstroom, Transvaal, Africa australi. Typus in Herbario Cryptogamense Academiae Potchefstroom, Africa australi, no. 306; et CBS, Baarn, Hollandia.

Colonies on potato-dextrose agar slow-growing,

flocculent, brown. Hyphae smooth, straw-coloured to brownish, 1 - 2.5 μ in diameter. Conidiophores arising laterally from hyphae, simple or branched of indeterminate growth and showing prominent geniculate scars, straw-coloured to light brown, 4 - 60x1.5 - 2.5 μ. Conidia produced singly at the tip of the conidiophore and its branches and successively at the sympodially formed growing points, oblong-elliptical to short cylindrical, 6.5 - 11.00x2.5 - 3.5 μ, hyaline to faintly straw-coloured, 2-celled with cells often unequal and frequently constricted at the septum, the lower cell slightly tapering to a broad truncate basal hilum.

Isolated from leaf-litter and top soil of an Acacia karroo community, Potchefstroom, Transvaal, Republic of South Africa. April 1966. J.W. du Toit.

Transfers of the holotype have been deposited in the Centraalbureau voor Schimmelcultures, Baarn, Netherlands and in the Cryptogamic Herbarium, University of Potchefstroom, South Africa.

Sympodina simplex differs from Sympodina coprophila mainly in conidiophore and conidium characters. In Sympodina coprohila the conidiophores can be readily distinguished from the vegetative hyphae on colour differences, the former being distinctly pigmented in contrast to the hyaline hyphae. In Sympodina simplex the conidiophores and hyphae have similar pigmentation.

Sympodina coprophila commences to sporulate only
when the conidiophore reaches a length of 50-100 μ
and by continued growth and sporulation it can reach
a length of up to 350 μ with the fertile region
80 - 200 μ long. The conidiophore of Sympodina
simplex is relatively short and the length never
exceed 60 μ while sporulation commences at about
5 μ.

Finally the conidiophores of Sympodina
coprohila are much wider than those of Sympodina
simplex being 3.2 - 4.4 μ and 1.5 - 2.5 μ broad,
respectively. The conidia of Sympodina simplex are
also more slender than those of Sympodina coprophila,
their width being only 2.5 - 3.5 (3.8) μ in contrast
to 3.2 - 4.8 μ in the case of Sympodina coprophila.

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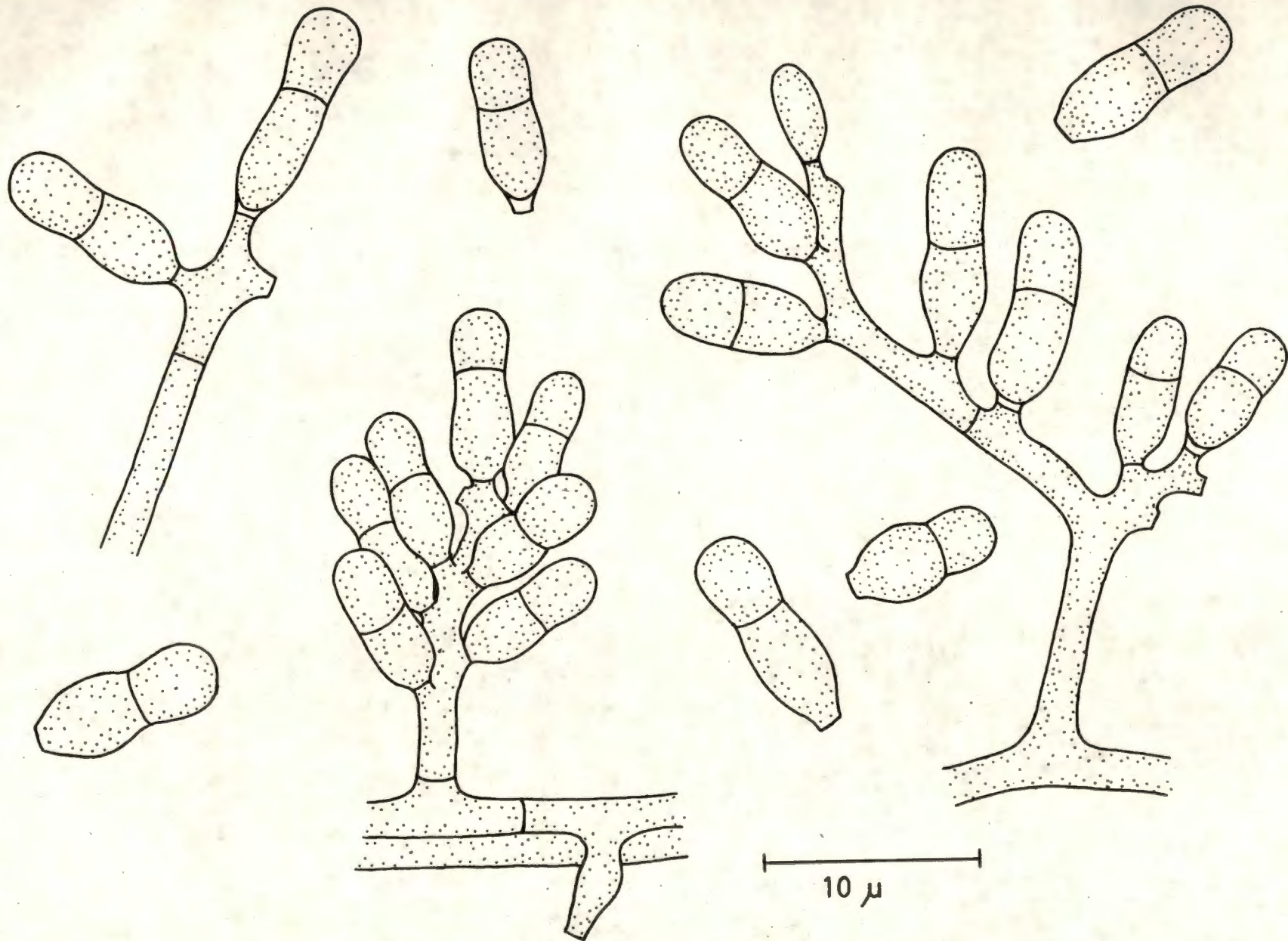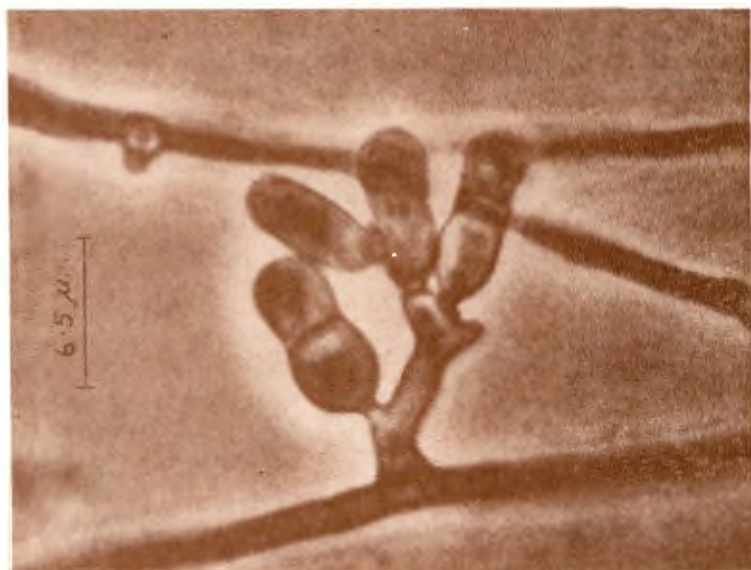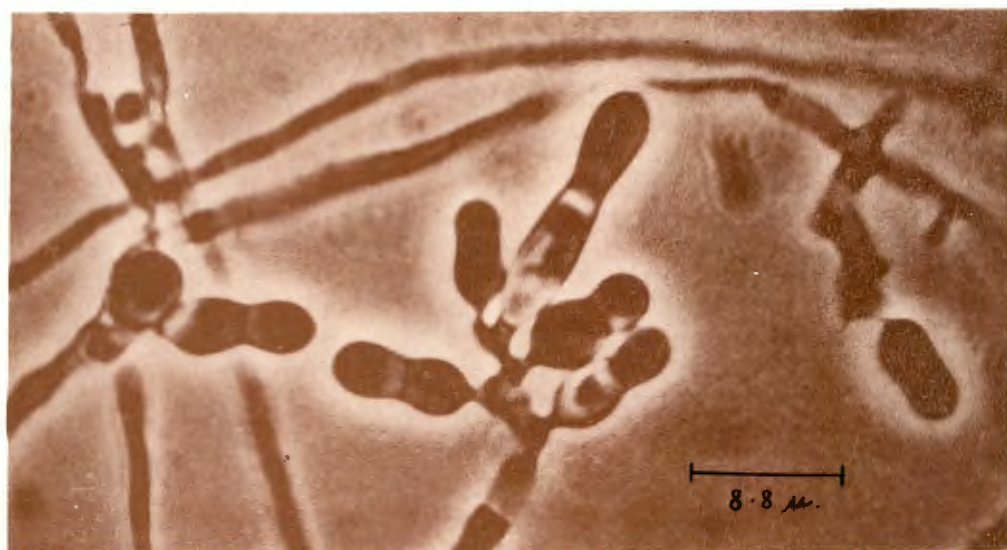   Antonie van Leeuwenhoek 30:317-330.

Fig. I. <u>Sympodina simplex</u>, hyphae, conidiophores and conidia.

(Fig. 2)

Sympodina simplex, hypha, conidiophore
and conidia.

(Fig. 3)

Sympodina simplex, hyphae, conidiophores and conidia.

S U M M A R Y

A preliminary survey of the mycoflora of an
Acacia karroo community in the vicinity of Potchef-
stroom, was made. Only the leaf litter and first inch
of soil were investigated.

Two different methods were employed for
the isolation of the fungi, viz. the dilution method as
modified by Menzies (1957) and the soil plate method
of Warcup as devised by Johnson and Manka. (1961)

Seven hundred and twenty six pure culture
isolations were made and 379 of these were identi-
fied.   The identified specimens
included 49 Zygomycetes, 323 Fungi Imperfecti and 7
Ascomycetes.

Several of the specimens were found to be
new and undescribed species with 4 representing new
genera.   One of the new species is described as
Sympodina simplex.

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