SOME STUDIES OF SOIL FUNGI IN TRINIDAD

By

IVOR D. FIRMAN

1958 - 59

D.T.A. Report.

Submitted in part fulfilment of the requirements for the Diploma in Tropical Agriculture of the Imperial College of Tropical Agriculture

TRINIDAD. W. I.
INTRODUCTION

As recently as 1916 doubt was expressed as to whether fungi were significant in the soil. The soil mycologist Crow (1916) was under the impression that fungi were not of great importance, but despite this unfortunate misunderstanding, several earlier authors have related their work to a knowledge of fungi in the soil. This statement has become increasingly true as more and more fungi have been discovered. The present investigation is an attempt to extend this work to a larger number of soil samples from various parts of Trinidad.

Investigation of St. Augustine Loam Soil

The present investigation dealt with the investigation of the soil from St. Augustine, a loam soil, in order to determine the types of fungi present. The methods employed were similar to those used by previous investigators, but the results were more comprehensive due to the larger number of samples analyzed.

Further Investigations

Further investigations were conducted on the soil from the Caroni Swamp, the River Estate, and Mount St. Benedict. These investigations focused on determining the types of fungi present in these soils and comparing them with those found in St. Augustine loam soil.

Discussion

A discussion of the results of these investigations was presented, highlighting the differences and similarities between the soils and the fungi present in each.

Summary

The summary of the investigation concluded that fungi are significant in the soil and indicated the need for further research to understand their role in soil ecology.

Acknowledgements

The author acknowledges the support and assistance of various individuals and organizations during the course of the investigation.

References

A list of references was provided, including works by previous investigators and additional sources used in the research.

Appendix

The appendix contains a list of fungi isolated during the investigation, along with descriptions and notes on their identification.
SOME STUDIES OF SOIL FUNGI IN TRINIDAD

INTRODUCTION

HISTORICAL

As recently as 1916 doubt was expressed as to whether fungi grew and lived in the soil. The soil bacteriologist Conn (1916) was rather sceptical of their importance, but despite an unfortunate impression gained from the later literature that he refuted their presence altogether, his actual conclusion was that "Although Waksman (1916) was presumably correct in his statement that fungus mycelium is present in the soil, it is doubtful whether it exists there to a significant extent." Following on from the pioneer work of Waksman, however, there has been increased interest in the fungi of the soil and Chesters (1949), in his presidential address to the British Mycological Society, shows clearly that fungi are significant and widespread in soil and indicates many profitable future lines of research into their activities.

Adametz (1886) was responsible for the first isolation of fungi from soil, followed by Oudemans and Koning (1902), Oudemans identifying forty species so obtained. Fungal taxonomy has had to keep pace with this increased knowledge of soil fungi and Gilman (1945) in his Manual of Soil Fungi has provided a useful tool in the hands of the investigator into soil fungi.

Numerous methods of isolating fungi from the soil have been tried. All these methods must be to some extent selective in that they favour the isolation of certain fungi, or groups of fungi, at the expense of others. Waksman placed lumps of soil directly on to an agar plate and isolated them as they grew out into the medium. By comparing the rate of growth of such fungi with the rate of growth of various selected fungi from mycelial and spore inocula he was also able to show that in many cases the first growth from such lumps of soil must have developed from active mycelium in the soil, and not just from spores simply resting in the soil. This provided good evidence that fungal mycelium did, in fact, grow actively in the soil. Soil mycologists also became interested in the actual numbers of fungi per unit of soil and used essentially bacteriological methods for their determinations in that successive dilutions of soil in sterile
media were plated out and the number of colonies developing counted. The validity of such counts, however, is rather doubtful since any given colony may have arisen from a fragment of mycelium or a spore. The heavy sporulation of such fungi as Penicillium will obviously weight the counts considerably and, furthermore, there is the fact that conditions favouring mycelial growth may be different from those favouring sporulation. However, fungi can be isolated by this method quite satisfactorily, but too much importance should not be attached to the numerical aspect.

Warcup (1950) describes a soil-plate method in which a small quantity of soil is spread over the bottom of a Petre-dish and cooled agar poured over it.

Rossi (1928) pressed clean microscope slides against a freshly cut surface of soil and after fixation examined these to give a picture of the positional relationships of fungi, bacteria, and soil particles. Cholodny (1930), Conn (1936) and Jensen (1935) have used various modifications of this method.

The disadvantage of the Rossi-Cholodny slides is that sporulation is only rarely seen and the fungi cannot be identified. This is related to an observation made by Chesters (1948) who suggests that "vigorous sporulation in the soil causes early cessation of vegetative growth". If the substrate encourages sporulation, then sterile hyphae are those likely to extend the furthest from their substrates and thus occur more frequently on Rossi-Cholodny slides. Garrett (1956) sums these difficulties up well by saying "that with the plate count method one identifies what one cannot see (i.e. in situ), whereas with the direct method one sees what one cannot identify."

Kubiena (1932, 1935), using vertical illumination, has, however, been able to some extent to study the fungal population in situ and has shown the active growth of certain species not usually appearing in dilution plates.

THE PRESENT INVESTIGATION

The object of the investigation was to gain some idea of the type and distribution of fungi in the soils of Trinidad. Only a limited amount of information on such a wide field could be gathered
in the time available. In the course of the investigation it was also envisaged that the writer would become more acquainted with the methods of isolation of fungi and so also, of course, with the limitations of such methods, and would gain some experience in the identification of fungi in general.

It was decided to use soils from various localities so as to obtain as varied a selection of fungi as possible, and also so that there may be some possibility of comparison between sites.

For the first investigation a soil type represented in the College grounds was chosen, namely the St. Augustine Loam of Chenery's (1952) classification. As this soil type occupies a considerable area a choice of cropping was also involved. The samples were, in fact, taken from Fields A and B under cultivation with cocoa.

The second soil examined was the alluvial soil of the Caroni Swamp in an area where the dominant plant was mangrove (Avicennia nitida).

The third soil examined was River Estate Loam from the I.C.T.A. cocoa plantation at River Estate. It was not originally intended to use this soil but it was examined with reference to the possible occurrence of Ceratostomella since this fungus is at present causing a disease of the cocoa on the estate.

For the fourth soil it was intended to use samples from the slopes of Mount St. Benedict on the foothills of the Northern Range, both under forest and from under fire induced savanna. In fact, there was not time to do the latter but a start was made on the soil under forest.

There are few records of soil fungi from Trinidad; the "Fungi of Trinidad and Tobago" by Baker and Dale (1951) scarcely containing any soil inhabitants except the Basidiomycetes. Most of the fungi found, therefore, will be new records for Trinidad by virtue of the fact that nobody has previously looked for them. In this respect the present investigation may serve as a starting point for a list of Trinidad soil fungi.

Gilman's (1945) "Manual of Soil Fungi" was mainly used for identification of fungi. Penicillium and Fusarium, in particular,
were found to be difficult genera taxonomically. In the case of these two genera identifications are sometimes made to species, but these should be regarded as only tentative, or more usually to the main groups within each genus. Cultures of most of the fungi involved have been preserved. Penicillium species with perfect stages are still referred to in the text as Penicillium. The numbers in brackets after some of the fungi refer to the number of the culture which has been preserved in the Mycology department of I.C.T.A. Fungi preserved as cultures are marked with an asterisk in the complete list of fungi at the end of this paper.

Investigation of St. Augustine Loam Soil

Preliminary Investigation

This was carried out in October. Soil from Field A, I.C.T.A., under cultivation with well established cocoa and with numerous other scattered trees of various species was used. Soil from the base of one of the cocoa trees was collected in a sterile tin. The surface litter was first scraped away and the tin filled as quickly as possible with soil from the top three inches under the litter. The soil on this site was classified by Chenery as St. Augustine Loam and was rather sandy.

This sample of soil was used for the isolation of fungi by several different methods with a view to deciding on methods to be used in the future investigations.

Plain, potato dextrose, malt extract and Czapek Dox agar were used. The Czapek Dox agar contained added yeast extract and was made up to the following formula:-

Yeast extract 5.0 gms.
Sucrose 30.0 gms.
NaNO₃ 2.0 gms.
K₂HPO₄ 1.0 gm.
MgSO₄·7H₂O 0.5 gm.
KCC 0.5 gm.
FeSO₄ a trace
Agar 30.0 gms.
Distilled water 1000 ml.
A small quantity of lactic acid was used with all these media to keep bacterial activity to a minimum. This was put into the petri dishes just prior to pouring the plates.

**Warcup Soil Plates**

Following the method used by Warcup a small quantity of soil picked up on the end of an inoculating loop was spread as evenly as possible over the bottom of a petri dish and cooled but molten agar poured on top. Using Czapek Dox agar fungal growth was visible from soil particles within twenty-four hours and some of the fungi were developed sufficiently for identification within three days. Using plain agar development was somewhat slower but the same species of fungi were eventually found. Similarly with malt agar, which on the whole, however, gave less colonies. The colonies were subcultured onto Czapek Dox for identification.

The following fungi were found:

- *Aspergillus luchuensis*
- *Aspergillus niger*
- *Aspergillus versicolor*
- *Cunninghamella echinulata*
- *Trichoderma viride*

*Aspergillus niger* and *Trichoderma viride* developed first but at a later stage the plates were overgrown with *Cunninghamella echinulata*.

**Waksman Plates**

Soil was spread over the top of the agar in petri dishes.

*Aspergillus niger* quickly developed on all such plates, covering them completely, and was the only fungus isolated.

**Dilution Plates**

25 gms. of soil was shaken up with 250 ccs. of sterile water and the larger particles were allowed to settle. A $\frac{1}{10,000}$ suspension was prepared and 1 cc. of this pipetted into a petri dish. Cooled agar was then added and the petri dish gently rotated to allow mixing. Development was slow on these plates; plain agar seemed to give as good results as Czapek Dox. Fungi isolated were:

- *Penicillium sp.* (Monoverticillata Stricta. *Glabrum* series)
- *Rhizopus nigricans*
Chesters Immersion Tube

One of these tubes was buried in the soil at the time of taking the first soil sample and was left in the soil for a week. Subsequently another was buried and left in this case for two weeks. Czapek Dox agar was used in these tubes, which were prepared in an approximately similar way to that described by Chesters (1940). Ordinary test tubes were used and a spiral of seven capillary orifices was made in them. This was achieved by heating the tubes and pushing a hot needle gently into the glass to make a hole of 1 mm. in diameter. These tubes were then placed inside a boiling tube for sterilisation by autoclaving. They were then filled with agar and buried in the soil, being only removed from the outer tube just prior to burial. The first tube produced *Cunninghamella echiniculata*, while the second produced this fungus and also:

- *Penicillium simplicissimum*
- *Fusarium neoceras*
- *Fusarium sp.*

Partially decayed twig and leaf material

Pieces of twig and leaf were found in the soil sample. These were washed in six changes of sterile water as they were considered to be too far decayed to use a more fungicidal surface sterilant such as mercuric chloride. Pieces so treated were plated out on Czapek Dox agar and the following fungi were isolated:

- from the stem material: *Cunninghamella echiniculata*
- from the leaf material: *Botryodiplodia theobromae*
- *Trichoderma koningi*

Soil Baiting

Turnip seeds were surface sterilised with 1% mercuric chloride and washed in several changes of sterile water. These were sown in thoroughly wet soil in a petri dish and allowed to germinate. The seeds were quickly covered in a weft of mycelium and from this two fungi were identified, namely:

- *Fusarium nivale*
- *Pythium debaryanum*
The following fungi, then, constitute the complete list found in this preliminary investigation:

**PHYCOMYCETES**
- Cunninghamella echinulcata
- Pythium debaryanum
- Rhizopus nigricans

**ASCOMYCETES**
- Botryodiplodia theobromae

**FUNGI IMPERFECTI**
- Aspergillus luchuenis
- Aspergillus niger
- Aspergillus versicolor
- Fusarium neoceras
- Fusarium nivale
- Fusarium sp. (1)
- Penicillium simplicissimum
- Penicillium sp. (Monovermicillata Stricta, Glastrum series) (1)
- Trichoderma koningi
- Trichoderma viride

**Comments on Preliminary Investigation**

The Chesters Immersion Tube is designed to be selective for fungi present as actively growing mycelium in the soil. The presence of Cunninghamella echinulcata in such a state is clearly demonstrated both by its occurrence in these tubes and its rapid growth on the Warcup Plates.

Of the freely sporing fungi Aspergillus niger and Trichoderma viride are obviously very abundant in this soil.

The list on the whole is rather disappointingly sparse although showing a reasonable range over the various groups of fungi. It is difficult to say at this stage whether the fungal population is poor in numbers of different species, or whether the sparsity is due to the restricted site of soil examined or some other factor.

The Warcup Soil Plate seems a suitable method for obtaining soil fungi quickly but due to the quick development colonies overlap and intermix and identification and isolations are made difficult in...
many cases.

The Dilution Plates at a rather less dilution should give good results and good separation of colonies, while the Chesters Immersion Tube also gives valuable information on the fungi actively growing through the soil.

FURTHER INVESTIGATIONS

Throughout November soil and leaf litter from Field B, I.C.T.A., also under established cocoa was studied. Soil from the top three inches yielded similar species as before with *Aspergillus niger* and *Trichoderma viride* again being very common on all plates. There were, however, more *Penicillium* species isolated and on the dilution plates these far outnumbered other species. A \( \frac{1}{1,000} \) dilution was used this time. From the Warcup Plates *Gliocladium fimbriatum* was the only species not found previously. Chesters Immersion Tubes again yielded *Cunninghamella echinulata*, and also *Botryodiplodia theobromae*.

Dilution plates yielded the following additional species:

- *Geotrichum candidum*
- *Penicillium decumbens*
- *Penicillium humicola*
- *Penicillium oxalicum*
- *Penicillium steckii*
- *Penicillium wortmannii*

Leaf litter was surface sterilised with \( \frac{1}{1,000} \) mercuric chloride solution followed by washing in six changes of sterile water. This yielded *Trichoderma viride*. Leaf litter washed only in six changes of sterile water showed considerable growth of very fine hyphae, probably Actinomycetes, and a *Fusarium* species of the Section Elegans, sub group *orthoceras*. Although a lot of sterile hyphae grew from this material no clamp connections were seen so it is not certain that it was Basidiomycete mycelium as might be expected. Soil at a depth of five inches yielded only *Trichoderma viride* and *Aspergillus flavus* but this is not considered as a true representation of the fungus flora at this depth. The following fungi are therefore added to the previous list:
In view of the fact that the species found are still low in number it was decided to use rose bengal – streptomycin agar in the next investigation. Miller et al (1957) using a wide variety of media in a survey of soils in Georgia found that this medium yielded the greatest number of species and was valuable in limiting colony size and in suppressing bacteria and Actinomycetes. Farrow (1954) gives the formula for IIa rose bengal – streptomycin agar as:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>10.0 gms.</td>
</tr>
<tr>
<td>Peptone</td>
<td>2.0 gms.</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.5 gms.</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.5 gms.</td>
</tr>
<tr>
<td>Rose Bengal</td>
<td>50.0 mgms.</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 gms.</td>
</tr>
<tr>
<td>H₂O distilled</td>
<td>1 litre</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>8.0 ml.</td>
</tr>
</tbody>
</table>

This is the medium to be used in the present investigation, subcultures being made onto Czapek Dox as before. A 500 p.p.m. solution of streptomycin was prepared to be added to the medium just before pouring the plates. Farrow found that the advantages of using this medium were:

1. Species of Aspergillus and Penicillium developed better on this medium than on any other tested.

2. The inclusion of rose bengal made it possible to detect growth early.
3. The use of the dye limited colony size, which in turn allowed an increase in the number of fungi on a plate, and bacterial growth was suppressed.

Martin (1950) also describes the use of this medium. In order to test the use of this medium the following soil plates were set up.

A. Dilution Plate 1. Rose bengal agar + streptomycin. 
B. """" "" "" without streptomycin.
C. Warcup plate + streptomycin.
D. "" "" + lactic acid.
E. Waksman plate.
F. Dilution Plate 1. Plain agar + streptomycin. 
G. "" "" "" + lactic acid.
H. Warcup Plate. Plain agar.
I. Waksman Plate. ""

After twenty-four hours the following observations were recorded on these:

A. Only one bacterial colony. One fungus isolation.
B. Twelve bacterial colonies. Two fungus isolations.
D. Only one fungal colony. No bacteria.
E. Heavily overgrown with bacteria around soil particles.
F. No colonies of any sort. The best fungal growth with regard to growth was observed in this plate.
G. No colonies of any sort.
H. No colonies of any sort. Fortunately, this combination was not used.
I. Some bacterial growth around soil particles.

The plates were examined again after forty-eight hours when the following observations were recorded:

A. Two more bacterial colonies. Fourteen isolations made.
B. Bacterial growth extensive. Sixteen isolations made.
C. Bacterial and fungal growth too widespread for further isolations.
E. Fungal colonies and bacteria round all particles.
F. Colonies only visible with difficulty. Four colonies isolated.
G. No growth yet.
H. Many fungi now visible. Only four isolations taken however.
I. Fungi now visible with the bacteria.

It would have been possible to isolate more fungi from the series D - I but sufficient petri dishes and media were not available.

A. Of the fifteen isolations from A, eight of these showed no further growth after forty-eight hours' incubation. In the case of one of these at least bacterial contamination was the cause. There was some bacterial contamination on the seven remaining plates but this was not serious.

B. Of the eighteen isolations made from B only one failed to grow. Five of the cultures, however, showed severe contamination with a bacterial spreader and subcultures were made onto Rose bengal with lactic acid to try and preserve these fungi.

C. Of the fourteen isolations made from C, seven showed no further growth mostly due to bacterial contamination.

D. Of the ten isolations made from D, only one failed to grow and the remainder were free from bacteria.

Cultures from the remaining plates were all badly contaminated with bacteria except in the case of G, where after seventy-two hours growth was visible and there was absolutely no bacterial contamination at all. Three isolations were made from this plate.

From these records it comes out clearly that the least bacterial contamination and the best fungal growth with regard to isolation was on plate D, where a combination of rose bengal and lactic acid was used. Unfortunately, this combination was not used for the dilution plates. However, rose bengal in these gave early visibility of fungal colonies, while lactic acid in the plain agar dilution plate gave freedom from bacteria. A combination of these two characteristics would seem to be most desirable.

Unfortunately, all the isolations from D turned out to be *Trichoderma viride* and also many isolations from other plates were also this fungus. This undoubtedly indicates a very high population of Trichoderma in the soil concerned but probably also reflects the ability of this fungus to grow on these media better than other fungi.
Rose bengal medium seems to be selective for *Trichoderma viride* especially in acidified plates. This is not surprising as the tolerance of *Trichoderma* to acid conditions and its suppression of other fungi under these conditions is well known.

Many of the other isolates in this investigation were lost due to the activity of a number of insects which invaded the cultures and spread bacterial contamination and spores of *Trichoderma* on nearly all the plates. The insect was a member of the Diptera, Family Phoridae, possibly a *Diploneura* specie, and was apparently strongly attracted to agar. Eventually all petri dish cultures had to be destroyed due to its depredations.

Five more fungi were added to the previous lists following this investigation and the complete list now stands as follows:-

**PHYCOMYCETES** :-

- Cunninghamella echinulata
- Pythium debaryanum
- Rhizopus nigricans

**ASCOMYCETES** :-

- Botryodiplodia theobromae
- Penicillium wortmannii
- Phaeopeltis sp.

**FUNGI IMPERFECTI** :-

- Aspergillus flavus
- Aspergillus luchuensis
- Aspergillus niger
- Aspergillus versicolor
- Fusarium neoceras
- Fusarium nivale
- Fusarium sp. (Sect. Elegans)
- Fusarium sp. (1)
- Geotrichum candidum
- Gliocladium fimbriatum
- Monilia sitophila
- Penicillium biiforme
- Penicillium decumbens
Penicillium humicola
Penicillium oxalicum
Penicillium simplicissimum
Penicillium steckii
Penicillium sp. (Biverticillata Asymmetrica. Velutina) (2)
Penicillium sp. (Monoverticillata stricta. Glabrum series) (1)
Trichoderma koningi
Trichoderma viride

Discussion

It is interesting to note the occurrence of Botryodiplodia theobromae in the Immersion tubes, indicating that it is capable of free mycelial growth through the soil. This fungus is a common saprophyte of woody materials in the tropics and a well-known wound parasite of cocoa. This record of its possible status as a soil fungus is, therefore, of particular interest.

The isolation identified as Penicillium wortmannii produced abundant yellow masses of asci but conidial production was very sparse. Basidiomycetes were very common on the leaf litter at the time of this investigation, especially Marasmius species. Of these Basidiomycetes the following were identified:-

Collybia dealbata
Collybia nivea var. cohortalis
Crinipellis septotricha
Lepiota micropholis
Marasmius aciculaeformis
Marasmius ferrugineus
Marasmius haedinus var. minor
Marasmius leoninus
Marasmius polyporoider
Marasmius tageticolor

A closer examination of the leaf litter also led to the discovery of a fungus which defied identification and could not be placed satisfactorily into any of the groups of fungi. Specimens of this fungus accompanied by slides and drawings were sent to the Commonwealth
Mycological Institute at Kew where it was suggested that it may well belong to a hitherto undescribed genus. It was, therefore, intended to investigate the life history of the fungus and describe it, as suggested by the C.M.I., but unfortunately no further specimens could be found.

The fungus was originally found in Field B, I.C.T.A., on a fallen but only slightly decayed cocoa leaf. It was rather less than one millimetre in height and just visible to the naked eye. The fungus was colourless and on closer examination was seen to consist of a basal vesicle partially buried in bacterial slime on the surface of the leaf, and a short mycelial filament bearing at its apex a sporing structure. The basal vesicle was ovoid in shape being about 190 x 85 µ in size. What appeared to be a longitudinal furrow on the vesicle was clearly visible in all preparations. From the apex of the vesicle a vertical mycelial filament with four septa grew up and supported the sporing structure. This consisted of a central column with a fringe of hairs at its base and apex. These hairs were bent upwards and downwards respectively to produce a more or less symmetrical "cage" around the central column. Some of the hairs, however, were longer than the rest and stood out from the "cage". The central column bore short almost rectangular sterigmata all over its surface and the spores were borne on these sterigmata. The spores themselves were thick walled and irregularly globose but with a stalk-like basal portion where they were attached to the sterigmata. The spore size was about 16 x 10 µ.

The whole fungus therefore would seem to consist of a basal vesicle bearing a single comdiophore. This makes the life history of the fungus an interesting problem in that it is difficult to imagine how the spores give rise to the large basal vesicle and what, if any, are the intermediate stages.

The accompanying drawing illustrates the main features of the fungus (Plate 1).

Although the preceding account obviously does not give a full list of the fungi of the soil concerned it was decided to stop work on this soil and continue with an investigation of soil from the Caroni swamp. By doing this a preliminary examination of both of these soils
should be completed before the onset of the dry season, thus making it possible to compare the fungal floras under comparable climatic conditions.

INVESTIGATION OF SOIL FROM THE CARONI SWAMP

The soil for this investigation was collected from near the sluice gates at the south of the swamp. The actual sample used was collected in the vicinity of a mangrove tree, \textit{Avicennia nitida}. The top layer of this soil is a greyish-brown colour while at a depth of four to five inches it is a deep blue mud. The soil is more or less permanently waterlogged and contains numerous crab burrows.

Soil from the top layer and from the blue mud layer was collected in the manner previously described. Isolations were made by the Warcup plate and Dilution plate methods using Rose bengal and plain agar acidified with lactic acid. This time sub-cultures were made onto potato dextrose agar as this supports growth of most fungi as well as Czapek Dox and is always at hand in the department.

The first set of isolations carried out in December were rather disappointing in that few of the fungi developed after sub-culturing. This was believed to be due to the media being too acid and in a second attempt the lactic acid was diluted by half and good results were then obtained.

From the top soil the following fungi were isolated:

**PHYCOMYCETES:**
- \textit{Mucor hiemalis}
- \textit{Rhizopus nigricans}

**ASCOMYCETES:**
- \textit{Botryodiplodia theobromae}

**FUNGI IMPERFECTI:**
- \textit{Aspergillus flavus}
- \textit{Aspergillus versicolor}
- \textit{Fusarium avenaceum}
- \textit{Fusarium sp. (Section Elegans. Subgroup Orthoceras)} (2)
- \textit{Monilia sitophila}
- \textit{Penicillium digitatum}
**Penicillium restrictum**

**Penicillium sp. (Bivert. Asymmetrica. Velutina)** (11)

**Penicillium sp. (Bivert. Asymmetrica. Velutina)** (4)

**Phoma humicola**

**Trichoderma viride**

From the blue sub soil the following:

**ASCOMYCETES:**

**Erysiphales** sp. (unidentifiable genus)

**Penicillium wortmannii**

**Penicillium** sp. ) species producing (6)

**Penicillium** sp. ) yellow asci (7)

**FUNGI IMPERFECTI:**

**Curvularia lunata**

**Monilia geophila**

**Penicillium pinophilum**

**Penicillium westlingi**

**Penicillium** sp. (Bivert. Symmetrica) (3)

**Penicillium** sp. (Bivert. Asymmetrica. Velutina) (5)

**Trichoderma viride**

*Rhizopus nigricans* was the most frequently occurring fungus in the top soil and was also found in the Chesters Immersion tubes so it would seem that it occupies the niche occupied by *Cunninghamella echiniculata* in the previous soil type. Actually the isolations of *Rhizopus* from the immersion tubes were slightly atypical in that the rhizoids were only weakly developed.

In the sub soil *Monilia geophila* was a frequent isolate but the most conspicuous feature was the presence of three distinct species of *Penicillium* producing asci. *Penicillium wortmannii* was present as in the previous soil, of the other two one produced only asci, while the other produced both asci and green conidial areas. The yellow asci of these three species were a distinct feature on all the isolation plates.

A fungus apparently belonging to the *Erysiphales* was found in the sub soil. On potato dextrose agar this produced a characteristic tufted white aerial mycelium. The medium showed a bright red colour.
at first, gradually becoming darker until almost black in old cultures. On plain agar it produced dark perithecia bearing stiff, septate, serrate hairs. These hairs were dark yellow and were spread evenly over the perithecia although in older perithecia they curled upwards to produce an apical tuft of hairs. Some of the hairs had slightly swollen bases but no movements in response to moisture were noted. The perithecia were non-ostiolate and contained eight hyaline lenticular ascospores. The perithecia were up to 100 μ in diameter, the asci approximately 22 x 10 μ and the ascospores 8 - 10 x 6 - 8 μ. Perithecia were not formed so readily on potato dextrose as on plain agar. Beyond the fact that the fungus appears to be a member of the Erysiphales no further identification could be made.

The accompanying drawings illustrate the main features of the fungus (Plate 2).

Several fungi isolated from the top soil failed to make any appreciable growth on any of the usual media while some grew but did not produce any sporing structures so could not be identified. One such fungus produced a very characteristic ropy arachnoid growth on potato dextrose agar.

Mucor, Rhizopus, Trichoderma, and surprisingly Monilia species occurred mainly on the Warcup Soil plates while the Penicillium species were isolated mainly from the dilution plates.

The fungal florlas of the top and sub soils are quite distinct probably due to considerable differences in these two layers when considered as a microhabitat for fungi, the subsoil being waterlogged and conditions almost certainly being practically anaerobic. The ascomsporic Penicillia seem to be a very distinct feature of this soil. Trichoderma viride is common to both layers.

Considered as a whole this soil also shows a different fungus flora from the previous soil type. There are, however, several fungi which are found in both these soils, namely: Trichoderma viride, Rhizopus nigricans, Botryodiplodia theobromae, Monilia sitophila, Aspergillus flavus and Aspergillus versicolor. In the case of the first three especially, this is not at all surprising as these are probably of very common occurrence in all tropical soils. As already
mentioned a very striking difference is the absence of *Cunninghamamella* from the swamp soil. This fungus was of very common occurrence in the cocoa plantation soil, and in the swamp soil *Rhizopus nigricans* seems to replace it as the commonest Phycomycete.

An attempt was also made to isolate aquatic Phycomycetes from this soil. A small quantity of soil was placed in a sterile petri dish and covered with sterile water, and pieces of boiled Jack Fruit seed (*Artocarpus integer*) added as a "bait". This is the method used by Wolf (1939) in isolating such fungi from Mexican soils except that Wolf used the standard bait of boiled hemp seed. Unfortunately hemp seed is not available in Trinidad so Jack Fruit was used since it belongs to the same family as hemp. This bait, however, was rapidly colonised by several fungi including *Curvularia*, *Rhizopus*, *Monilia* and *Penicillium* species, and the surface of the water covered by a bacterial pellicle. No aquatic Phycomycetes were found. A *Fusarium* specie (Section Eupionnotes) (5) not previously found was, however, isolated from such cultures and *Curvularia* was found in one of the cultures prepared from the top layer of soil whereas previously it was only reported from the blue sub-soil.

A further attempt was made by suspending pieces of this seed material and also pieces of potato in flasks of sterile water at the bottom of which a small quantity of soil was placed. This method largely prevented the contamination of the bait with other fungi but nevertheless no Phycomycetes were observed. The water in these flasks rapidly turned blue and oily due to bacterial activity.

**INVESTIGATION OF SOIL FROM THE RIVER ESTATE**

Many of the cocoa trees on River Estate have succumbed to a wilt disease caused by *Ceratostomella fimbriata* in conjunction with the wood boring beetle *Xyleborus*. This investigation was instigated primarily with the object of seeing if *Ceratostomella* could be isolated from the soil in the neighbourhood of these trees.

Soil samples were collected at the end of January from the base of infected trees. These samples contained a large proportion of the wood dust and detritus from the borings of the beetle.
Ceratostomella was not isolated from any of these samples. In pure culture on potato dextrose and rose bengal agar this fungus makes very slow growth; on P.D.A. for example a colony attained two centimeters diameter in nine days while on rose bengal a colony attained only half a centimeter diameter in the same period. This slow growth combined with its probable low saprophyte competitiveness probably accounts for it not being isolated by the methods used. To determine whether, in fact, this fungus is a soil inhabitant would require more critical experiments but it is nevertheless unlikely that it can live actively in the soil.

The sampling site for this soil was rather restricted and the other fungi isolated were few in number although many of them proved of considerable interest.

The commonest fungus, and one which was particularly abundant on the dilution plates, was a *Penicillium* species of the *Biverticillata - Symmetrica* group having the characteristic feature of a green conidial area overgrown with yellow hyphae. This feature, however, seems to be rather dependent on cultural conditions or sectoring because some subsequent sub-cultures did not show it.

*Aspergillus niger*, *Mucor hiemalis*, and *Trichoderma viride* were frequent isolates from the Warcup soil plates. Two species of *Penicillium* which have been reported from previous soils examined, namely *P.westlingi* and *P.wortmannii* were also found in this soil.

*Cladosporium herbarum* and *Spicaria simplicissimum* were among species not previously found. The other fungi found were of especial interest and are described separately below.

*Aspergillus* species:

This species, perhaps related to *A.wenti* or the *A.terreus-ustus* group of Thom and Church (1926), showed a sparsely growing white aerial mycelium bearing conidiophores of five to six millimeters in length. The conidial heads were radiate and fawn coloured. Large round bodies resembling cleistothecia and composed of parenchymatous mycelium developed on the surface of the medium. These were a darker brown than the conidial heads. They contained no asci so were probably sclerotia as they contained hyphae bearing terminal cells resembling
the Hülle cells described by Thom and Church for several members of 
this genus. 

**Candida species:**

Colonies of this species had a largely submerged mycelium, 
white in colour but with a greenish tinge. The reverse of these 
colonies was slightly yellow. The mycelium was quite well developed 
for a member of this genus but at times became up to 6 μ thick and broke 
up easily. Conidia were produced terminally and laterally and fre-
quently budded to produce further conidia in the usual way. The 
conidia were often globose in which case they were about 12 μ in 
diameter, but larger ovoid and irregular spores were abundant. Some 
of the hyphae tapered to a point and were rather sinuous in a manner 
rather reminiscent of Actinomycete mycelium. Some of these features 
are illustrated in the accompanying drawings (Plate 3). 

**Fusarium species:**

This species produced a very bright red colour in the medium, 
the colony sometimes having a yellow fringe. Microconidia were present 
in chains and also in lenticular shaped slime masses, these latter being 
a conspicuous feature of the fungus when viewed with the binocular 
microscope. Macroconidia were present in pionnotes of yellowish slime. 
Some of these macroconidia had terminal cells of a most unusual turbi-
nate shape. These turbinate cells being present at either one or 
both ends of the conidium. Some of the conidia however had normal 
more or less blunt or slightly pedicillate terminal cells. The 
accompanying drawing shows some of the macroconidia (Plate 4). 

**Botrytis species:**

This species had a grey-green mycelium and was very slow growing 
on P.D.A. The species was unusual in that some of the conidiophores 
seemed to produce a few terminal chains of conidia. This peculiarity 
together with the fact that Botrytis is predominately a fungus of 
temperate regions makes this record of particular interest. 

**Penicillium species (9):**

This was a slow growing fungus with a penicillus of the Biverti-
cillata assymetrica lanata divaricata type, but with pale red conidia. 
This colouring suggests the genus Gliocladium rather than Penicillium,
but there was certainly no mucilage present and the penicillus was
definitely of the Penicillium type. Thom however describes a species, Penicillium vermoesini, which he believes illustrates a transition form between Gliocladium and Penicillium and which has red conidia. The conidia of the fungus in question though are smaller than those of P. vermoesini and are globose to very slightly elliptical rather than elliptical.

One other fungus was found having a white mycelium with erect bodies similar to young Clavaria fructifications. No spores were observed and the nature of this fungus remains undetermined as yet.

The following fungi then were recorded:

**PHYCOMYCETES** :-

*Mucor hiemalis*

**ASCOMYCETES** :-

*Penicillium wortmannii*

**FUNGI IMPERFECTI** :-

*Aspergillus niger*  
Aspergillus species (1)  
Botrytis species  
Candida species  
Cladosporium herbarum  
Fusarium species (3)  
Penicillium westlingi  
Penicillium species (Bivert.Symm.) (8)  
Penicillium species (9)  
Spicoria simplicissimum  
Trichoderma viride

**INVESTIGATION OF SOIL FROM MOUNT ST. BENEDICT**

A comment on the choice of this soil has been made in the introduction. The soil was a very sandy loam under permanent forest. There was insufficient time for a full investigation of this soil but despite this it proved of interest in that several rather unusual fungi were found.

The first set of isolations was ruined by severe contamination
of all the petri dish isolations with *Monilia sitophila*. As a result of this it was decided to attempt to fumigate the laboratory in the manner described by Smith (1954) using formaldehyde. The windows of the room were closed, ventilators sealed and formalin was poured onto a dish of potassium permanganate, placed in the middle of the room, in sufficient quantity to wet it thoroughly. The room was then kept closed overnight. This method gives rise to a rapid evolution of formaldehyde gas.

To test the efficacy of this treatment two petri dishes of P.D.A. were exposed in the laboratory for five and thirty minutes respectively on the following morning. The plate exposed for five minutes developed one colony of *Monilia sitophila* while that exposed for thirty minutes showed none. A Penicillium colony and a few bacterial colonies were also present but it was considered that the fumigation had been reasonably successful and work was continued.

Warcup soil plates made from this soil were rapidly swamped by *Trichoderma viride* which also appeared very frequently on the dilution plates. This is to be expected on a very sandy acid soil.

As would also be expected from a forest soil *Mucor* species were common. Two of these belonged to the *Hiemalis* group but the third was unusual and deserves special mention.

*Mucor* species (3) :-

This species had a white mycelium and the turf was less than three millimeters high. The sporangia and spores were hyaline, the sporangium wall not dissolving very rapidly in water. The sporangia were from 18 to 20 in diameter and the peculiar feature of this fungus was that beneath the sporangium was a spherical to subspherical apophysis about half the diameter of the sporangium. A very small columella was present and after dehiscence a collar was left at its base. Details of this fungus are shown in the accompanying drawings (Plate 5).

Two other fungi were particularly common in this soil. One of these was a *Fusarium* species, (4), producing microconidia only, these being produced in dirty white powdery masses. No macroconidia were seen. The other was a *Penicillium* species, (10), which produced numerous yellow sclerotia giving the colony a granular appearance.
Conidial areas were sparse or late in developing and were light green and velvety. The penicillus was menoverticillate or occasionally asymmetrically biverticillate.

One other fungus deserves special mention, this being the one tentatively assigned to the genus Papulospora since it seems to correspond to the description of this genus given by Smith (1954). Unfortunately Hotson's (1917) monograph on this genus was unavailable.

**Papulospora ? species:**

The mycelium of this species was white to yellow the reverse of the colony going brown with age. Structures resembling the spores of Sarcinella or Stemphyllium in their muriform nature were abundant on the mycelium. These were borne on short lateral branches of the mycelium but unlike the spores of the above-mentioned fungi were hyaline and not dark. It is suggested, therefore, that they may be the vegetative propagules, or bulbils, described for Papulospora. The true spores for this genus have not been described but this culture showed conidiophores of the Verticillium type, these developing from the same mycelium as the bulbils. The accompanying drawing (Plate 6) illustrates the details of the fungus.

The following fungi were isolated from this soil:

**PHYCOMYCETES:**

- Mucor sp. (Hiemalis group) (1)
- Mucor sp. (Hiemalis group) (2)
- Mucor sp. (3)
- Rhizopus nigricans

**ASCOMYCETES:**

- Phaeopeltis sp.

**FUNGI IMPERFECTI:**

- Aspergillus clavatus
- Aspergillus niger
- Aspergillus ustus
- Cladosporium herbarum
- Fusarium sp. (4)
- Monilia sitophila
- Papulospora ? sp.
- Penicillium steckii
There would seem to be two possible extreme approaches to the problem of investigating the soil fungus flora of any particular area. One is a close study of a series of well defined sub-divisions within the area, giving due attention to season, soil horizons, vegetative cover, associated micro-organisms, ecology and nutritional requirements and all the many and varied factors affecting fungal growth in the soil. The other is the indiscriminate collection of soils with a view to isolating as many fungi as possible irrespective of the knowledge gained of their ecological status. There is no doubt that the first approach is the ideal to be aimed at.

The present investigation set out to study several soils and consequently insufficient data was collected on any one type. However a start was made on the compilation of a list of Trinidad soil fungi and some sixty species have been isolated. Limited comparisons can also be made between the various soil types studied.

Considering the number of species of fungi which must be present in the soil it is clear that only a small fraction of these were isolated. This is to a large extent due to the limitations of the investigation. One of these limitations was the time factor, in that four soils were studied in a period of little over six months. Also since many soil fungi are of widespread occurrence the commoner ones appeared repeatedly in each successive set of isolations. Since it is advisable to isolate from soil plates as soon as mycelium appears, at a stage when it is virtually unidentifiable, in order to get pure cultures, a fungus such as Trichoderma will account for a large number of the isolations due to its very common occurrence in these particular soils. This in turn leads on to the limitations imposed with respect to numbers of petri dishes and quantity of agar available at any one time. However, even when these were available...
in reasonably sufficient quantity it was not found possible to run more than about fifty plates simultaneously due to the difficulty of keeping pace with identification of the isolates. In fact, with experience, it was found possible to identify colonies of Trichoderma at an early stage so that this particular problem was to some extent avoided. It is obvious, however, that with a limited number of isolations being made there was a bias towards the isolation of quick growing fungi.

The methods of isolation used were somewhat restricted, the majority of isolates coming from Warcup soil plates and dilution plates. Other methods were also used with varying success but here again the time factor became all important. A technique which at first proves unrewarding would normally be further investigated and modified until it either proved successful or was discarded. It was not thought justifiable in the present study to spend too long on any one technique. This problem is exemplified by the attempt at baiting for aquatic phycomycetes, a method which would almost certainly have proved rewarding if further baits and techniques had been tried. Attempts at isolating cellulose decomposers from 'filter paper soil plates' also had to be discarded early on for similar reasons. The methods used then can be said to be selective for certain groups of fungi. The Warcup plates were rapidly swamped by Trichoderma, Aspergillus, or the rapidly growing phycomycetes such as Mucor, Rhizopus and Cunninghamella, while the dilution plates favoured heavily sporing fungi, Trichoderma and Penicillium species in particular.

The use of Rose Bengal agar, and lactic acid to acidify the media also had a selective affect. When lactic acid was not used it was found that severe contamination from bacteria occurred. Rose Bengal was useful in restricting colony size so preventing adjacent colonies coalescing and consequent mixed cultures. The acidification of the media obviously had a considerable effect on the type of fungi isolated and Trichoderma, already extremely abundant, was favoured even more. The use of more varied media would presumably have increased the variety of fungi. Various media are known which are selective for particular groups of fungi. Potato
Dextrose agar was prepared by the laboratory staff and so was readily available. It was found worthwhile to prepare Rose Bengal agar for the above-mentioned reason but the time spent on preparing Czopek Dox agar was not considered worthwhile as it seemed to differ little from P.D.A. in its effectiveness as a medium either for soil and dilution plates or for sub-culturing. Although this and other media such as soil extract agar, maize meal agar and many others would undoubtedly have been useful, the making of them would have been too time-consuming under the circumstances.

Because of all the above factors only limited comparisons can be made between the soils examined. Due to the relatively small numbers of fungi isolated from each, too many generalisations about what is and what is not present must be avoided. However, using the methods and media described certain differences did appear. In the I.C.T.A. cocoa fields Cunninghamella echinulata was present as a fungus showing active mycelial growth in the soil, while in the Caroni swamp this role was found to be filled by Rhizopus nigricans. Mucor hiemalis was not recorded where Cunninghamella was present but Mucor species were present in the Caroni swamp and River Estate soils, and especially common in the Mount St. Benedict soil where three species were recorded. In the Ascomycetes Botryodiplodia theobromae is of common occurrence in the first two soils examined and there is a suggestion that it exhibits active mycelial growth through the soil. One striking feature in this group is the very common occurrence of Penicillium species producing their perfect stage, this being especially marked in the subsoil of the Caroni swamp but also occurring in two of the other soils. Passing on to the Fungi Imperfecti, Monilia geophila was also found to be a characteristic inhabitant of the Caroni swamp subsoil. Monilia sitophila is also very common but its occurrence as a laboratory contaminant may give cause for suspicion with regard to some of the isolations of this fungus. Aspergillus species were frequent isolates, Aspergillus niger being very common in all the soils except that it was not isolated from the swamp. Penicillium species were very common but due to taxonomic difficulties it would be unwise to
attempt any comparison of their occurrence as between different soils. The same applies to the genus Fusarium. Trichoderma was far and away the commonest fungus in all of these soils. Its rapid growth, high sporulation, competitive saprophytic ability and antibiotic production and its tolerance of acid conditions are all too well known to require further stressing. Other fungi found can be seen in the appropriate section or in the complete list. Several species were of particular interest and have been described in detail.

SUMMARY

1. Some fungi have been isolated from Trinidad soils and a list of these is appended.

2. The methods used have been discussed.

3. Limited comparisons have been made between the fungus floras of the soils examined.

4. Fungi of particular interest, including a possible new genus, have been described in detail.
ACKNOWLEDGEMENTS

My thanks are due to Mr. R. F. Barnes for his help, advice and encouragement throughout, and to the laboratory staff of the Botany department.

Aspergillus elegans
Aspergillus flavus
Aspergillus niger
Aspergillus usutus
Aspergillus versicolor
Botrytis sp. (1)
Candida sp. (1)
Gladosporium herbarum
Curvularia lunata
Fusariumavenaceum
Fusarium avenaceum
Fusarium nivale
Fusarium sp. (Section Flavus)
COMPLETE LIST OF FUNGUS

* indicates that a culture of the fungus has been preserved in the Mycology Department of I.C.T.A.

Numbers in brackets refer to culture numbers in this collection.

+ - see Appendix.

PHYCOMYCETES :-

- Cunninghamella echinulata *
- Mucor hiemalis
- Mucor sp. (Hiemalis group) (1) *
- Mucor sp. (Hiemalis group) (2) *
- Mucor sp. (3) *
- Pythium debaryanum
- Rhizopus nigricans *

ASCOMYCETES :-

- Botryodiplodia theobromae *
- Erysiphales (unidentified genus) *
- Penicillium wortmannii *
- Penicillium sp. (6) *
- Penicillium sp. (7) *
- Phaeopeltis sp. *

FUNGI IMPERFECTI :-

- Aspergillus clavatus *
- Aspergillus flavus *
- Aspergillus luchuensis *
- Aspergillus niger *
- Aspergillus ustus *
- Aspergillus versicolor *
- Aspergillus sp. (1) *
- Botrytis sp. (1) *
- Candida sp. (1) *
- Cladosporium herbarum *
- Curvulavia lunata *
- Fusarium avenaceum *
- Fusarium neoceras *
- Fusarium nivale *
- Fusarium sp. (Section Elegans)
Fusarium sp. (1) *
Fusarium sp. (Section Elegans. Sub group Orthoceras) (2) *
Fusarium sp. (3) *
Fusarium sp. (4) *
Fusarium sp. (Eupionnotes) (5) *
Geotrichum candidum *
Gliocladium fimbriatum *
Monilia geophila *
Monilia sitophila *
Papulospora ? sp. +
Penicillium biforme *
Penicillium decumbens *
Penicillium digitatum *
Penicillium humicola *
Penicillium oxalicum *
Penicillium pinophilum *
Penicillium restrictum *
Penicillium simplicissimum *
Penicillium steckii *
Penicillium sublateralitum *
Penicillium westlingi *
Penicillium sp. (Monoverticillata Stricta - Glabrum series) (7) *
Penicillium sp. (Biverticillata Asymmetrica - Velutina) (2) *
Penicillium sp. (Biverticillata Symmetrica) (3) *
Penicillium sp. (Biverticillata Asymmetrica. Velutina) (4) *
Penicillium sp. (Biverticillata Asymmetrica. Velutina) (5) *
Penicillium sp. (Biverticillata Symmetrica) (8) *
Penicillium sp. (9) *
Penicillium sp. (10) *
Penicillium sp. (Biverticillata Asymmetrica. Velutina) (11) *
Phoma humicola
**Spicaria simplicissimum**

**Trichoderma koningi**

**Trichoderma viride**

**REFERENCES**


Contributions to the Microbiology of Australian Soil. III. The Rossi-Cholodny method as a quantitative index of the growth of fungi, with some preliminary observations on the influence of organic matter on the soil microflora. Proc. Linn. Soc., N.S.W. 60. 145-54.


Miller et al. (1957) A survey of the fungi of forest and cultivated soils of Georgia. Mycologia Vol.44 No.6 p.779


Rossi, G.M. (1928) Il terreno agrario nella teoria e nelle realte. L'italia Agric. No.4.


Wolf, T. (1939) A study of some Aquatic Phycomycetes isolated from Mexican Soils. Mycologia. 31. 376-
APPENDIX

Cultures of the four fungi marked + in the Complete List of Fungi were sent to the Commonwealth Mycological Institute for identification.

The fungus described on page 20, line 32 was identified as *Paecilomyces marquandii* (Massee) Hughes, (= *Spicaria violacea*)

The fungus described on page 20, line 26, was identified as *Oidiodendron priseum* Robak

The fungus described on page 22, line 22, was identified as *Absidia butleri* Lendner

The fungus described on page 23, line 8, was described as *Verticillium chlamydosporum* Goddard

The latter is of special interest as it is only the second isolate of this fungus to reach C.M.I. and appears to be not at all common.
A: Appearance of fungus on leaf litter
B: Spores on stipe
C: Detail of basal vesicle from slide preparation

vesicle: 140 x 85μ
vesicle to base of hairs: 320μ
spores: 16 x 10μ

A: Penthosarium
B: Detail of pentedolam hair
C: Ani and ascospores

Penthosarium: approx. 100μ diameter
Pent. hair: 2μ wide
asci: approx. 22 x 10μ
ascospores: 8-10 x 6-8μ.
A. Peritheciun
B. Detail of peritheciun hair
C. Asci and ascospores

Peritheciun approx 100μ diameter
Peritheciun hair 2μ wide
Asci approx 22 x 10μ
Ascospores 8-10 x 6-8μ.
Candida sp.
Plate 6.

Verticillium chlamydosporum